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5-8 October 1993
Hamburg, Germany

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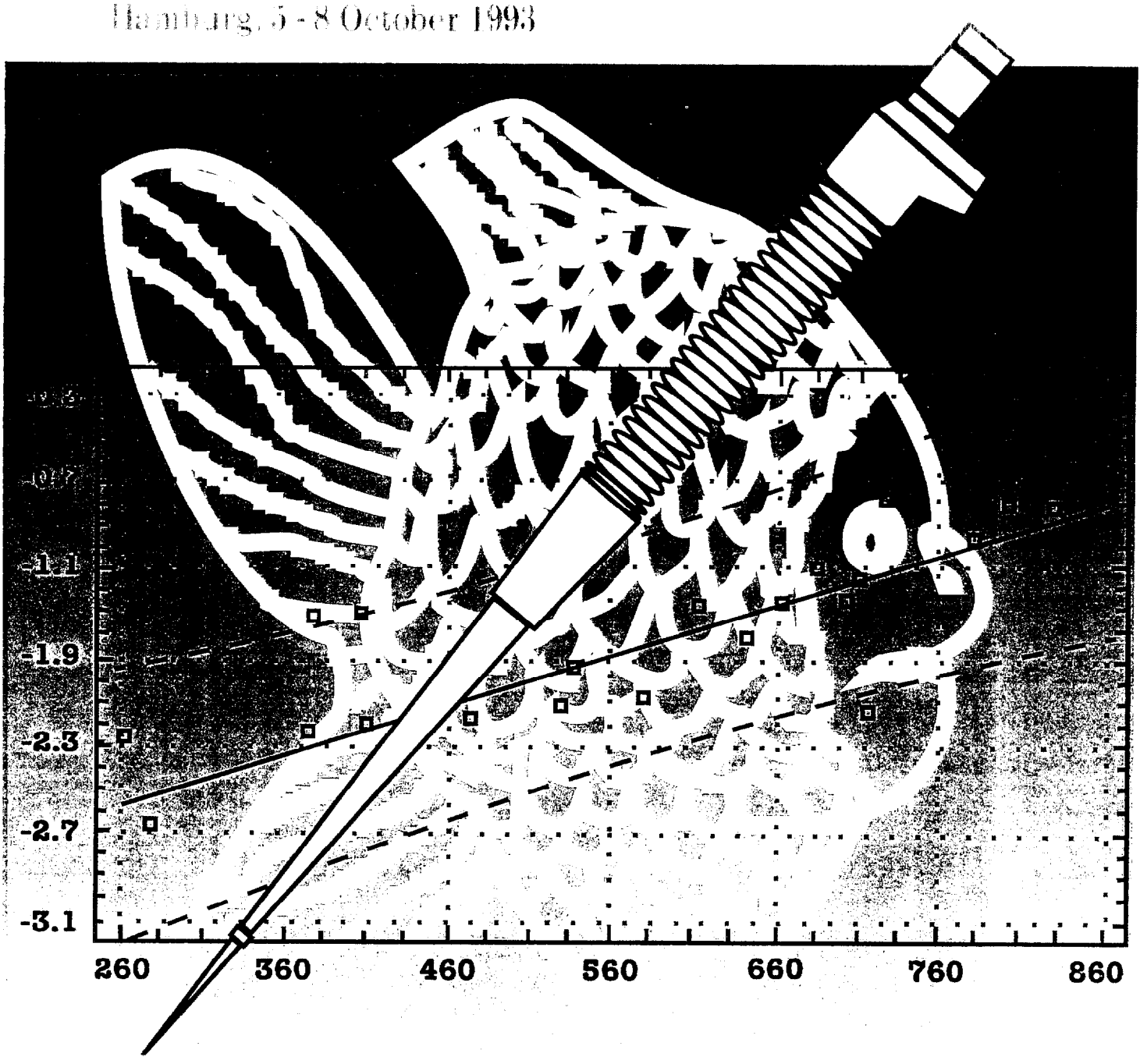

Bundesforschungsanstalt
für Fischerei

Bundesamt für Seeschiffahrt
und Hydrographie

ICES/HELCOM WORKSHOP ON QUALITY ASSURANCE OF CHEMICAL ANALYTICAL PROCEDURES FOR THE BALTIC MONITORING PROGRAMME, 5-8 October 1993, Hamburg, Germany

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Preface

The Workshop on Quality Assurance of Chemical Analytical Procedures for the Baltic Monitoring Programme was held on 5-8 October 1993 in Hamburg, Germany, as a joint effort between the International Council for the Exploration of the Sea (ICES) and the Helsinki Commission. Experts from all Baltic Sea riparian states attended the workshop.

At the workshop the main emphasis was given to quality assurance aspects of nutrients in sea water and trace metals in biota. Other mandatory chemical determinants of the Baltic Monitoring Programme were covered by state-of-art presentations on analytical procedures. This publication contains the manuscripts of the presentations given at the workshop.

The editors, Dr. Graham Topping and Dr. Uwe Harms, and the authors are considered to be responsible for the contents of the publication.

Introduction

Under the auspices of the Baltic Marine Environment Protection Commission - Helsinki Commission - marine environmental monitoring data have been collected since 1979 within the frame of the Baltic Monitoring Programme (BMP). The guidelines for the programme are reviewed in intervals of 5 years by the Commission. The third Stage of the BMP started in 1989.

The monitoring data provided by the Contracting Parties to the HELSINKI Convention serve as a source of information for the preparation of periodic assessments of the state of the marine environment of the Baltic Sea.

On the basis of the results from the monitoring work to date, the Group of Experts for the Preparation of the Second Periodic Assessment of the State of the Baltic Sea (GESPA), have expressed concern over the unsatisfactory degree of interlaboratory comparability of data. They have agreed that a programme of Quality Assurance (QA) should be developed to allow experts to specify the limits of uncertainty of measurements and which would help them to decide objectively whether the analytical information provided is of acceptable quality. In particular, GESPA recommended

"the Helsinki Commission should ensure that certified reference materials are made available for laboratories involved in the Baltic Monitoring Programme in order to improve the quality and the quality control of the data. Further elaboration of methods agreed upon and participation in intercalibration exercises is necessary" (HELCOM, 1990).

Accordingly, the Helsinki Commission

"urged the Contracting Parties to participate in Quality Assurance exercises whenever possible and decided that the participation in QA-exercises is mandatory for the laboratories providing BMP data on parameters included in the mandatory part of the Baltic Monitoring Programme" (Helsinki Commission, 1993a).

Scientific Justification for Quality Assurance of Chemical Analytical Procedures in Marine Monitoring

In a recent paper Topping (1992) concluded that although there had been considerable improvement in analytical chemistry over the past two decades, there was a large number of European laboratories which still had difficulties in providing reliable data in routine work. The author based his conclusion on the results of a series of external quality assessments of analysis (generally referred to as intercomparison exercises), organized over the last 20 years by the International Council for the Exploration of the Sea (ICES), and which have shown that there are large interlaboratory differences in the measurements of contaminants in marine samples.

As a consequence of lacking measures to assure the quality of analytical data, information about variations of contaminant levels both in space and time are often uncertain or misleading, and the effects of political measures to improve the quality of the marine environment cannot be adequately assessed. Therefore, the acquisition of relevant and reliable data is an essential component of any research and monitoring programme associated with marine environmental protection. To obtain such data, the whole analytical process must proceed under a well established Quality Assurance (QA) programme.

Organization of a Workshop on Chemical Quality Assurance

In order to stimulate communication and cooperation between scientists from laboratories involved in the Baltic Monitoring Programme (BMP) and to provide guidance on Quality Assurance (QA) of measurements of chemical determinands in marine media, the organization of a Workshop was regarded as of prime importance.

With reference to a Resolution of the International Council of the Sea (ICES, 1992) and in accordance with a proposal of the HELCOM Environment Committee, subsequently accepted by the Helsinki Commission (Helsinki Commission, 1993 b) a "Workshop on Quality Assurance of Chemical Analytical Procedures for the Baltic Monitoring Programme" was organized in Hamburg, 5 - 8 October 1993, as a joint effort between HELCOM and ICES.

The Workshop encompassed lectures by invited experts, case studies (short presentations) followed by round table discussions and practical demonstrations in the laboratories of the Bundesamt für Seeschiffahrt und Hydrographie (Federal Maritime and Hydrographic Agency) and the Bundesforschungsanstaalt für Fischerei (Federal Research Centre for Fisheries). Emphasis was given to QA aspects of nutrients in sea water and trace metals in biota. Matters relating to measurements of other mandatory determinands were restricted to presentations on the state-of-the-art of analytical procedures.

Dr. Graham Topping, SOAFD Marine Laboratory, Aberdeen, United Kingdom and Dr. Uwe Harms, Bundesforschungsanstaalt für Fischerei, Hamburg, Germany, acted as Co-chairmen for this workshop. A total of 42 scientists (including representatives from all Baltic States and invited experts from non-Baltic countries) took part in this meeting. The Helsinki Commission was represented by the Environment Secretary, Ms. Eeva-Liisa Poutanen .

The Helsinki Commission at its 15th meeting (Helsinki Commission, 1994) decided to publish the manuscripts of the presentations given at the ICES/HELCOM Workshop.

The manuscripts, which were edited by the co-chairmen of the Workshop, are presented in the present volume.

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Quality Assurance of Nutrient Data with Special Respect to the HELCOM Baltic Monitoring Programme

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Summary

The collection, storage, and pretreatment of samples as well as the analytical procedures for nutrients in sea water are the factors dominating the data quality. The principles of quality assurance of chemical data also include training and motivation of personnel, control checks and calibration of methods, and interlaboratory comparisons.

The activities of the Baltic Oceanographers are summarized with respect to the quality assurance of nutrient data and monitoring programmes in the Baltic Sea area. Finally, results of investigations on long-term variations of nutrients are represented for the Baltic Sea.

1. Introduction

All branches of marine sciences have largely expanded in recent decades, including chemical investigations in both oceans and coastal waters. Interlaboratory comparisons and multiship intercomparisons have shown, however, that the chemical data are often not sufficiently comparable. This problem is even more evident, when chemical observations are to be compared over longer periods. Thus, improvement in data quality requires particular attention.

The main reason for the insufficient comparability of chemical data are the very low concentrations of most of the elements and compounds to be studied in the marine environment. Consequently, the analysis of these parameters is very susceptible to contamination. Since the precision and accuracy of analytical procedures have strongly improved in recent times, the collection, storage and pretreatment of the samples are the factors dominating the quality of the results in chemical sea water analysis. However, it should be noticed that the equipment for trace element analysis, including special laboratory conditions, increasingly becomes a financial problem.

The pretreatment and the analysis of many chemical parameters in sea water is frequently performed immediately after sampling and therefore in ship's laboratories. However, the results of the chemical investigations on board of a research vessel, in restricted laboratory space and under unfriendly weather conditions, may naturally differ from those obtained in a well-equipped land laboratory. These factors must be considered in the assessment of the quality of marine chemical data. Good results in

intercomparisons performed in coastal laboratories may not automatically be transferred to field measurements.

Clear and well-written instructions for the quality assurance of chemical analytical procedures can contribute to the improvement of the comparability of marine chemical data. Intercomparisons and intercalibrations of existing methods and techniques will indicate how accurate chemical measurements really are. Furthermore, the replacement of an existing method by a new one should be done after the necessary intercalibration tests have been carried out. In this connection the quality of historical data often measured with less sensitive methods and techniques must be checked before the data may be used in studies on long-term changes of chemical parameters in the marine environment. In principle, every set of data should be accompanied by information on the precision and accuracy of the method used for the analysis.

2. Principle elements of quality assurance

Quality assurance of chemical data is important for both basic research and environmental monitoring programmes. Topping (1992; see also Topping et al., 1993) identified 13 principle elements for the quality assurance of marine environmental data. Although it is not necessary to discuss these principles again in detail, the most important ones can be summarized as follows:

Training and motivation of personal responsible for sample collection and analysis.

Collection, storage, and pretreatment of the samples.

Appropriate chemical analytical methods.

Regular control checks and calibration of methods and instruments.

Participation in external quality assessments (intercomparison and intercalibration exercises).

These principles are valid for all analytical laboratories. However, some of them are of special relevance for the marine environment due to the often very low concentrations of the determinands in sea water on the one hand and the sampling and working conditions at sea on the other hand.

3. Special instructions for the quality assurance of nutrient data

The inorganic nitrogen compounds (NH,+, NO_2^-, NO,), phosphate (PO_4^{3-}) , and reactive silicate (SiO_4^{4-}) are commonly designated as nutrient salts in the true sense in sea water. Their concentrations influence directly the phytoplankton development in the euphotic layer.

Concentrations of total phosphorus (Tot P) and total nitrogen (Tot N), which include inorganic nutrient salts and organic nutrient compounds, are of interest in connection with ecosystem analysis and budgets.

Nutrients belong to the trace elements, because their concentrations are below 1 mg/dm^3 in sea water. Higher concentrations are sometimes observed in contaminated coastal waters. Nutrient concentrations normally measured in sea water are in the range of $\mu g/dm^3$ and thus in the uppermost level of trace element concentrations. In general, enrichment procedures are not necessary in nutrient analysis. However, for measurements of extremely low phosphate concentrations, the blue molybdenum complex formed by the reduction of phosphomolybdenum acid can be enriched by extraction with organic solvents (Strickland, Parson, 1968).

Quality assurance of nutrient data begins with the collection of the samples. Platforms commonly used in sea water collection are research vessels or ships of opportunity (ferry boats in the Baltic Sea). In connection with monitoring programmes, continuous measurements of nutrients at moored platforms are under preparation in shallow waters.

All kinds of samplers, glass, metallic or plastic design, and hydrographic wires, stainless steel or plastic coated, are suitable for the collection of sea water samples for nutrient analysis. Specific cleaning operations are not necessary for the sampling equipment.

Special attention must be paid to ship generated pollution of nutrient samples. Waste water discharges from wash basins, showers, and toilets contain significant amounts of phosphorus and nitrogen compounds and therefore can contaminate the samples from the surface layer. The location for lowering the water samplers into the sea must therefore avoid positions near the waste water outlets, even if no sewage is discharged at the time of sampling. Mixing by the ship's propeller can also disturb the natural distribution of the determinands in the surface layer.

Since phosphorus and nitrogen compounds are secreted through the skin, the samplers and water bottles should not directly be touched by hand during the sampling procedure. Persons responsible for the collection of sea water samples for nutrient analysis should therefore protect their hands by clean plastic gloves avoiding contamination of the samples.

Dark plastic bottles are recommended for the storage of silicate samples, whereas dark glass bottles should be used for other nutrients. New sample bottles sometimes adsorb nutrients on their walls. Before use, they should therefore be filled and stored for few days with sea water containing nutrients. The bottles should than be rinsed with a small volume of sea water from the sampler immediately before they are filled with the sample under investigation.

Since nutrients, especially ammonia and nitrite, undergo biological conversion, their analysis must be performed as soon as possible, at least within 6 hours after collection of the sample. If this is not possible, they should be deep frozen immediately after collection and stored in the dark. Filtration is recommended in this case, because plankton cells can be destroyed by freezing releasing nutrients to the water sample.

Kirkwood (1992) critically reviewed the reagents (acidification, chloroform, mercuric chloride) used for stabilization of nutrient salts in sea water samples. In contrast to Kremling and Wenck (1986), he got good results with mercuric chloride for nitrate, phosphate and silicate. It must be mentioned in this connection that mercury compounds are highly toxic and require special attention during waste discharge.

Most of the methods for nutrient analysis are well tried and tested and yield accurate and precise results, if they are used correctly. Colorimetric procedures are commonly applied for the investigation of nutrient concentrations in sea water samples. They combine the appropriate precision, accuracy, and limits of detection with analytical instrumentation which are suitable for use under ship-board conditions.

Nutrient analysis is performed in the Baltic Sea area following Grasshoff's (1976) Methods of Seawater Analysis. Beside this manual, special instructions often exist in the separate laboratories taking into consideration their own experiences.

Photometric methods are simple analytical procedures allowing the measurement of a great number of samples within a short time period. This is of significance in field investigations which often require a high sampling frequency.

Manual methods and autoanalyzers are applied in marine nutrient analysis. In combination with pumping systems, autoanalyzers allow continuous registrations.

Autoanalyzers require a small sample volume. Therefore, replicated measurements are common with this method, whereas they are an exception in routine manual work with its rather high water volume demand. Moreover, manual nutrient analysis requires more personnel and time.

In common with all advanced analytical routine work, training of personnel, regular control checks, and calibration of methods are also of high significance in sea water analysis. Up to 30 % of the time for monitoring and research programmes are normally spent for this purpose.

For the determination of reagent blanks and the calibration procedures, it is necessary to use a sample of sea water with no detectable levels of nutrients. Offshore sea water from the surface layer collected in summer and stored in a transparent plastic carboy at room temperature in daylight is recommended as such a matrix. This procedure reduces the nutrient concentrations to undetectable levels after about two to three months exposure to light. Water redistilled over sulphuric acid is also suitable for the blank determination and calibration procedure of phosphate and nitrite, whereas artificial sea water is recommended for the reagent blank and calibration solution of silicate (Grasshoff, 1976).

Ammonia analysis is subject to airborne pollution, which contaminates samples, reagents, and labware. Glassware should always be cleaned with diluted hydrochloric acid to ensure low ammonia blanks.

The preparation of inorganic nutrient standards using analytical grade reagents is no problem for an experienced chemist. Stock standard solutions containing more than 1 $\mu mol/cm^3$ may be stored for a long time if kept in DURAN glass ampoules (HELCOM, 1986). A few drops of chloroform can contribute to the stabilization of these standards. The check of new stock standard solutions against old ones is recommended before the latter are discarded.

Diluted standard solutions for the calibration procedure should be analyzed within two hours after preparation. Reagent and nutrient blanks of the water matrix used for the preparation of diluted standard solutions must be subtracted from the total absorbance

of the calibration solutions before the slope (calibration factor) of the regression line is calculated. The calibration should cover the range of concentrations expected in the samples.

The number of control checks depends on the nutrient under investigation and the method to be used. With automated analysis the run should commence with blanks and a full calibration which is repeated after 25 - 30 samples. Additional calibration checks are recommended within this batch of samples.

Manual methods for phosphate, nitrate, nitrite and silicate require blank determinations and calibrations at the beginning of the investigations. We have observed very constant calibration factors for these nutrients for several years, independent of the analyst and the working conditions, provided that the analytical procedure is performed correctly. Therefore, weekly control checks seem to be sufficient in this case. Blanks and a full calibration are of course necessary for every new set of reagents. Since the analysis of ammonia is very sensitive to contamination and changes in reaction conditions, all batches of samples of this nutrient must be accompanied by regular control checks of blanks and calibration.

In order to check the individual standards, the need for a set of certified standards for inorganic nutrients in sea water was discussed in the light of global and regional research and monitoring programmes (Anonymus, 1992). The major problem is to ensure the stability of the certified nutrient standards for a long enough period. Autoclaved sea water acidified to pH 7.2 by hydrochloric acid is recommended for the preparation of stable and reliable reference materials for nitrate and phosphate (Aminot and Kerouel, 1991).

Sagami nutrient standards are not certified reference materials, because they are made up in sodium chloride solution. But comparisons with these standards can be used as a quality control check for the individual standards made up by laboratories.

Since no organic phosphorus compound with a constant composition is available, phosphate standard solutions are used for the calibration of the total phosphorus method. Disodium-EDTA is recommended for the calibration of the total nitrogen method.

The turbidity of the water sample caused by suspended particulate matter can lead to errors in measurements and thus should be removed by filtration. A broad choice of membrane filters can be used for this purpose. The pore size of 0.45 μm has been defined to distinguish between dissolved and particulate matter. Glass fibre filters are suitable for certain investigations as well, although they have no defined pore size. Centrifugation of samples is also recommended, especially for ammonia analysis (Grasshoff, 1976).

If the sample is not filtered a turbidity blank should be carried out by measuring the light absorption of the sample before adding all reagents. However, the dissolution of suspended particulate matter and the excretion of phosphorus and nitrogen compounds by organisms and thus the contamination of the sample in the course of the analytical procedure cannot be excluded in this case.

4. Hydrographic features and nutrient distribution in the Baltic Sea

The Baltic Sea is a semi-enclosed basin containing brackish water due to its restricted horizontal water exchange and positive water balance. One of the most important features in the Baltic Sea is the permanent halocline restricting the vertical exchange of energy and matter and dividing the water column into a surface layer, with the lower salinity, and a deeper layer with higher salinity. Below the halocline, stagnant conditions prevail in central Baltic deep waters, in which oxygen is consumed and hydrogen sulphide is produced under these conditions by microbial processes. The stagnant deep water is exchanged by irregular inflows of highly saline water from the North Sea, which occur under certain meteorological and hydrographic conditions. These deep water renewals are accompanied by the improvement of the oxygen conditions often characterized by a strong change in the redox potential.

The range of nutrient concentrations summarized in Table 1 exclude contaminated coastal waters. In the period of high biological productivity, the nutrient concentrations are near the limit of detection in the surface layer of the Baltic Sea. Only silicate remains in higher concentrations.

Hydrogen sulphide is responsible for the negative redox potential and the remobilization of phosphate and ammonia from the sediments and interstitial waters. Concentrations of up to 11 μmol/dm^3 phosphate and 40 μmol/dm^3 ammonia are measured in anoxic Baltic deep waters.

Table 1. Nutrient concentrations (μmol/dm^3) in the Baltic Sea.

Surface layer

	Winter	Summer	Remarks
NO_3	5 - 7	limit of detection	
NO_2	0.3 - 0.7	< 0.1	max: autumn
NH_4	0.3 - 2	< 0.1	max: autumn
Tot N	20 - 30	15 - 25	
PO_4	0.5 - 0.7	limit of detection	
Tot P	1 - 1.5	1 - 1.5	
SiO_4	8 - 12	5 - 8 (< 1)	

Deep water below the halocline

	Oxic	Anoxic
NO_3	8 - 10 (16)	0
NO_2	0 - 1	0
NH_4	0.2 - 2	5 - 40
Tot N	20 - 30	40 - 50
PO_4	3 - 4	4 - 11
Tot P	3 - 4	4 - 11
SiO_4	40 - 60	60 - 100

Nitrate concentrations do not exceed 13 $\mu mol/dm^3$ in the Baltic Sea. Denitrification eliminates this nitrogen compound from the anoxic deep waters.

Silicate does not belong to the nutrients limiting the primary productivity in the Baltic Proper. Its concentration ranges between 5 $\mu mol/dm^3$ in the surface layer and 100 $\mu mol/dm^3$ in stagnant deep waters.

Concentrations of total phosphorus reach up to 1.5 $\mu mol/dm^3$ in the surface layer of the Baltic Sea, and seasonal variations are relatively small. This implies that strong seasonal variations exist in the ratio between organic and inorganic phosphorus concentrations. Phosphate dominates the concentrations of total phosphorus in the deep water.

High concentrations of humic substances (Gelbstoffe) belong to the chemical features in the Baltic Sea. Although the destruction of these organic nitrogen compounds by the peroxo-disulphate method recommended in the Baltic Monitoring Programme (BMP) of the HELCOM (1986) is incomplete, they form the basis for a considerable background level in the concentrations of total nitrogen, which vary between 15 and 50 $\mu mol/dm^3$. It is therefore stressed that total nitrogen data published for the Baltic Sea should be handled with caution because of analytical problems.

The great range of concentrations covered by phosphate, ammonia, silicate, and total phosphorus and nitrogen in the Baltic Sea must be considered in the analysis of these nutrients. In the manual method, this is dealt with by changing the cell length in the photometer. Exceptionally, the dilution of the coloured solution is necessary before the measurement.

However. because autoanalyzers have a fixed cell length, the sea water samples with high nutrient concentrations must be diluted before they are analyzed.

High concentrations of hydrogen sulphide disturb the analysis of phosphate and ammonia. The reduction of the phosphomolybdenum complex to the blue coloured complex is catalyzed by antimonyions. Hydrogen sulphide reacts with these ions forming a yellow-greenish turbidity, disturbing the photometric measurement. Aeration, for instance by mixing with air taking 4 - 6 hours eliminates this disturbance by oxidization and removal of the hydrogen sulphide.

The analysis of ammonia in the presence of high sulphide concentrations requires a doubling of the strength of hypochlorite concentration, because the oxidizing reagent for the development of indophenolblue is partly consumed by the sulphide oxidization.

Poor data are also produced, when the sampler does not close at the required depth. This source of error is of special significance in the Baltic Sea with its strong vertical parameter gradients. Small deviations in depth, for instance by wire angle, can lead to misleading nutrient data.

5. Quality assurance of nutrient data in the Baltic Sea area

Quality assurance of data is also under discussion in connection with the BMP of HELCOM. Recently, the respective activities are combined with the revision of the BMP for the fourth stage.

The Baltic Oceanographers have made great efforts to prove the comparability of marine environmental data. The first interlaboratory comparisons of hydrographic and nutrient data were performed between marine research institutes surrounding the Baltic Sea as early as in the mid 1960s. In the very beginning, these exercises were initiated by activities of marine senior chemists. Later on, they were organized by ICES and since 1977 also by HELCOM. Biological intercalibration workshops including nutrient intercomparisons have been performed on a regular base in connection with the revision of the BMP guidelines.

Recently, the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) has also included marine chemical laboratories in the Baltic Sea area. This project is supported by the European Community. Table 2 summarizes the most important nutrient intercomparisons including laboratories in the Baltic Sea area. Other chemical determinands and hydrographic parameters not mentioned in the Table are often studied in these intercomparisons as well.

Intercomparisons for hydrographic parameters and nutrients have also been organized between the Baltic countries belonging to the former East Block in the frame of the RGW[1] Programme 'World Ocean". Since these activities were of lower significance, they are not considered in the Table.

The intercomparison exercises in the Baltic Sea area include investigations in both land laboratories and under field conditions on board of research vessels. In the first case, the organizer of the intercomparison distributes the samples to the different laboratories. Most of the intercomparisons in the Baltic Sea area are, however, field exercises sometimes combined with international multi-ship research programmes like PEX-86 and SKAGEX-90. Samples are collected by one research vessel and divided in subsamples for nutrient analysis on board of the other ships. Spiked samples are also distributed. Occasionally, the individual standard solutions were exchanged in these exercises as well. The same procedure is utilized in the regular Biological Intercalibration Workshops of HELCOM in the Baltic Sea.

The consistency of the results of intercomparisons in the Baltic Sea area varies between the different exercises indicating no clear trend in improvement. Phosphate and nitrate (+ nitrite) yield the best results. This agrees with a recent ICES interlaboratory comparison (Kirkwood et al., 1991) showing that 70 % of the participants are able to produce consistent results for both nutrients (Topping, 1992). The deviations in the Baltic Sea intercomparisons are highest for ammonia and total phosphorus and nitrogen.

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Table 2. **Intercomparison exercises for nutrients including laboratories in the Baltic Sea area.**

Place or Name of Exercise	Year	Nutrients	Kind	References
Copenhagen/Denmark	1965	PO_4	Ship	Koroleff, 1966
Leningrad/USSR	1966	PO_4	Ship	Fonselius, 1968
Copenhagen/Denmark	1966	PO,, SiO_4	Ship	Jones and Folkard, 1968 Palmork, 1968
ICES/SCOR	1969/70	PO,, NO,, NO,, SiO_4	Lab	Koroleff et al., 1977
Baltic Intercalibration Workshop Kiel/FRG	1977	PO,, Tot P, NO,, NO,, NH,, Tot N, SiO_4	Lab	Anonymus, 1977
First Biological Intercalibration Workshop, Stralsund/GDR	1979	PO_4, NO_3, NO_2, NH_4	Ship	None
Second Biological Intercalibration Workshop, Rønne/Denmark	1982	PO,, Tot P, NO,, NO,, NH,, Tot N, SiO_4	Ship	HELCOM, 1983
PEX '86	1986	PO_4, NO_3, NO_2, SiO_4	Ship	Dybem and Hansen, 1989
ICES Fourth Intercomparison Exercise for Nutrients in Sea Water	1989/90	PO,, Tot P, NO,, NO,, NH,, Tot N, SiO_4	Lab	Kirkwood et al.,1991
SKAGEX '90	1990	PO,, NO,, NO,, SiO_4	Ship	Olsson, 1990
Third Biological Intercalibration Workshop, Visby/Sweden	1990	PO,, Tot P, NO,, NO,, NH,, Tot N, SiO_4	Ship	HELCOM, 1991
ICES Fifth Intercomparison Exercise for Nutrients in Sea Water	1993/94	PO,, NO,, NO,, NH_4	Lab	Aminot, Kirkwood (under preparation)
QUASIMEME	1993 (start)	PO_4, NO_3, NO_2, NH,, SiO_4	Lab (ctd)	Toppingetal., 1993

In one intercomparison exercise, 7 research vessels met in the Baltic Sea anchoring within 0.7 nm of a fixed position (Anonymous, 1983), collecting samples from fixed depths at the same time. The great inconsistency of the results in this intercomparison was attributed to patchiness in the distribution of hydrographic and chemical parameters.

Standardization of methods and techniques as well as control checks of analytical procedures are necessary for the improvement of the comparability of nutrient data in Baltic research and monitoring programmes. Special instructions for the quality assurance of chemical analytical procedures do not exist for the Baltic Sea area, but are under discussion in connection with the revision of the BMP Guidelines.

6. Monitoring programmes and long-term variations of nutrients in the Baltic Sea

Environmental monitoring programmes have a good tradition in the Baltic Sea. Regular hydrographic measurements in connection with fishery research were initiated by ICES soon after its foundation in 1902. Immediately after this time, national monitoring activities were developed.

At the First Conference of the Baltic Oceanographers in 1957, the Senior Scientists of the Baltic States recommended seasonal cruises covering the international monitoring activities in the following period. In the International Baltic Year (IBY) 1969/70, organized by the Baltic Oceanographers on behalf of ICES, the previous cross sections were replaced by longitudinal sections along the deep water pathway through the central parts of the Baltic Sea.

Since 1979, the BMP of HELCOM has provided a leading function in Baltic monitoring activities, including hydrographic, chemical, biological, and geological investigations as well as studies on contaminants at international agreed stations in every subregion of the Baltic Sea. The BMP also includes Coastal Monitoring Programmes (CMP)which are in the responsibility of the individual coastal states. Fig. 1 shows monitoring stations of the Baltic Sea Research Institute Warnemünde including BMP and CMP stations and areas with unfavourable oxygen conditions and hydrogen sulphide in central Baltic deep waters.

Monitoring data are available for phosphate since 1958 and for nitrate/nitrite since 1969. The so-called historical nutrient data cover the period up to the beginning of the BMP.

In addition to the hydrographic parameters, the phosphate and nitrate distributions in the surface layer of the Baltic Sea are characterized by seasonal variations depending on the phytoplankton development. This is demonstrated in Fig. 2.

The winter concentrations measured before the beginning of the spring development of the phytoplankton are recommended for trend studies of nutrients in this layer (Nehring, 1979). This method fails in the transition area between the Baltic Sea and the North Sea due to strong short-term variations and the absence of a pronounced steady state in the nutrient accumulation.

Figure 1. Map with monitoring stations and areas characterized by unfavourable oxygen conditions and hydrogen sulphide in central Baltic deep waters.

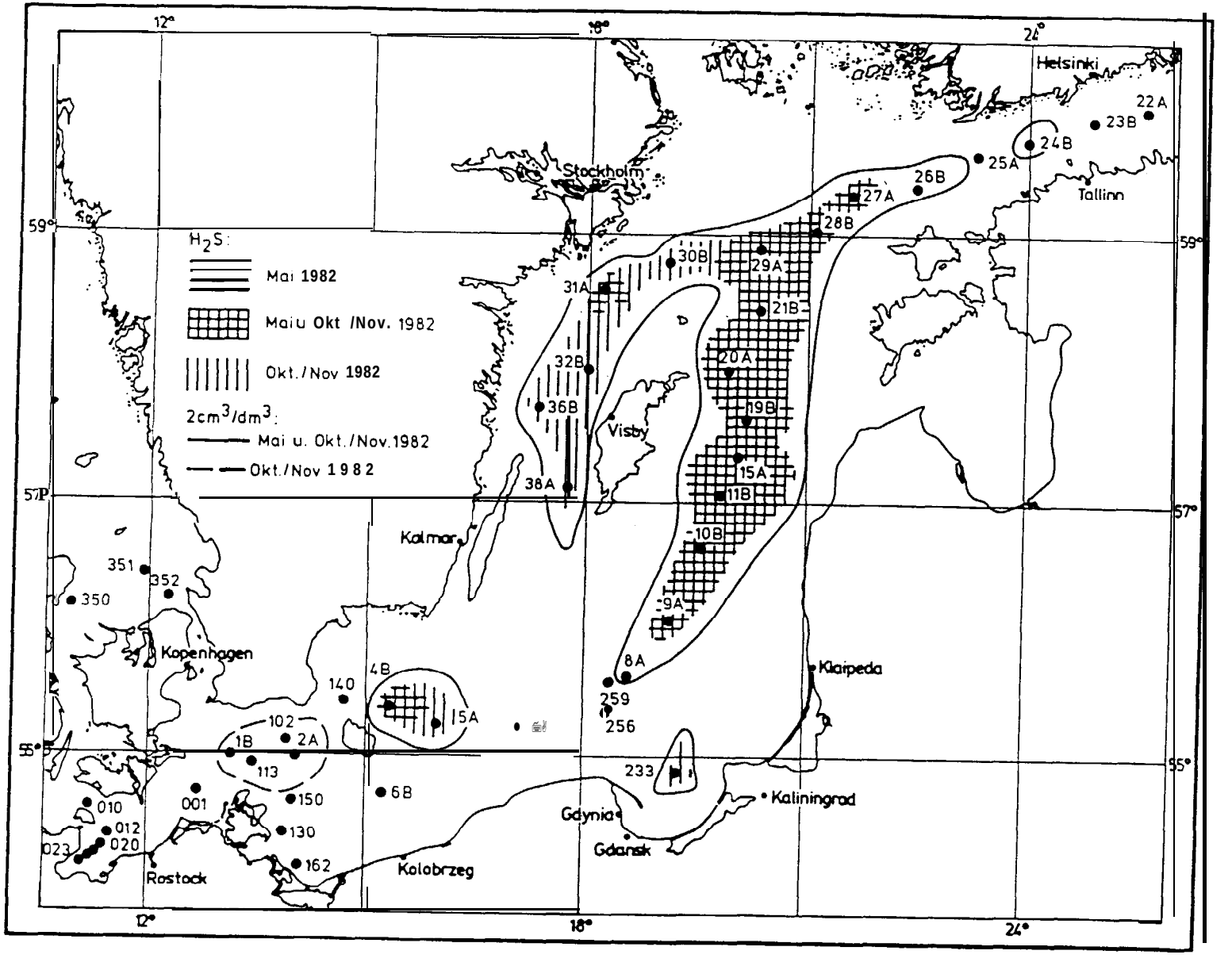


Figure 2. Seasonal variations of hydrographic parameters and nutrients in the surface layer of the Baltic Sea (Stat. 5A).

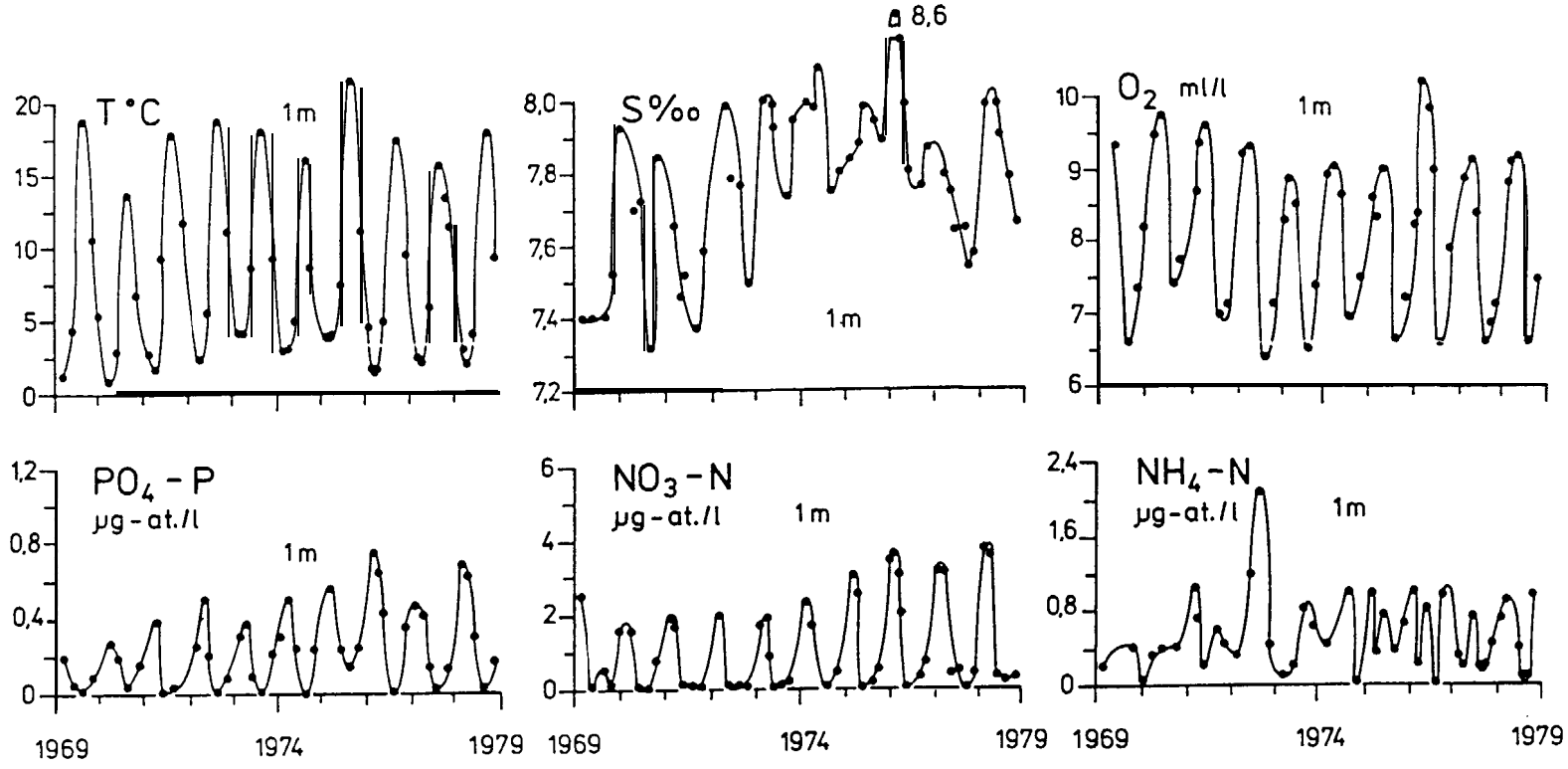
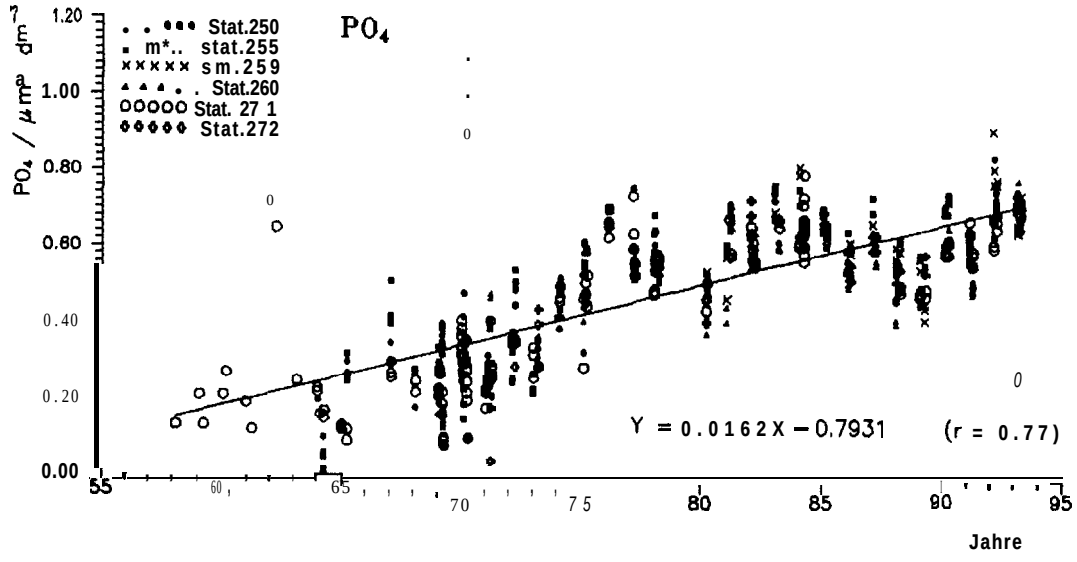


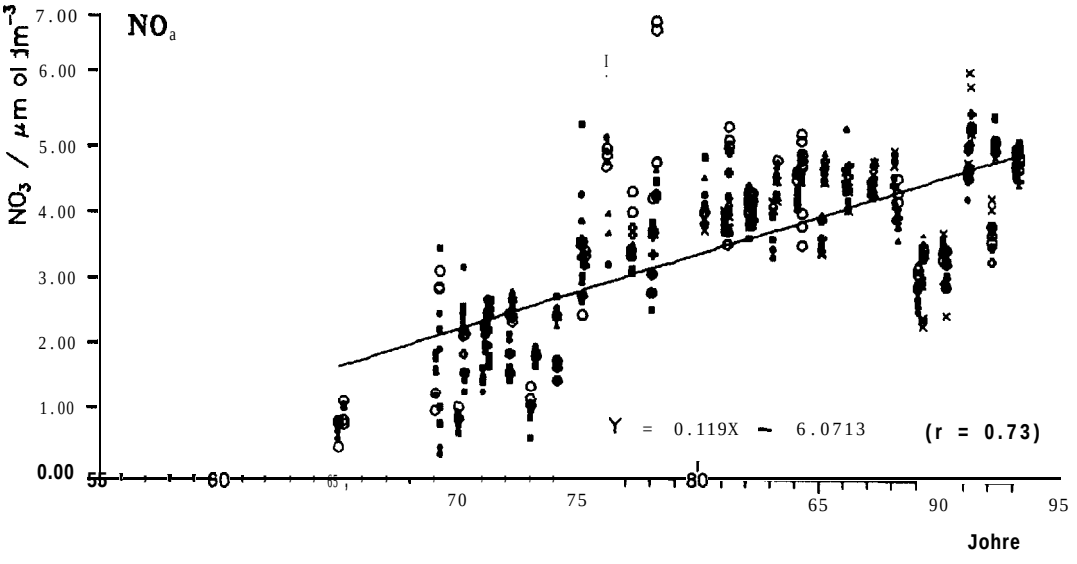
Fig. 3 shows the winter concentrations of phosphate and nitrate in the surface layer at stations in the central Baltic Sea. Positive overall trends are evident for both nutrients in the period under investigation. Sub-trends with a strong increase in the 1970s. followed by a weaker increase mainly caused by the low concentrations in the late 1980s can be identified as well. Positive, but often less pronounced nutrient trends were also observed in the surface layer of all other subregions of the Baltic Sea area (cf. HELCOM, 1990, Nehring, 1991).

Lateral water exchange and stagnation periods determine the phosphate and nitrate distributions below the permanent halocline and mask trends in Baltic deep waters. This is reflected in the Fig. 4 and 5 by measurements in the Gotland Deep, the most central station in the Baltic Sea. Concentrations of both nutrients show on average a clear increase at the 100 m depth. Since nitrate is eliminated by denitrification at low oxygen concentrations, the investigations are restricted to phosphate in the bottom water layer. The variations of phosphate are dominated by deep water renewals and stagnation periods in this layer. Phosphate is remobilized from the sediments, when hydrogen sulphide develops, and precipitated, when conditions become oxic (cf. Nehring, 1987). This was very pronounced in central Baltic deep waters during the longest stagnation period till now which lasted from 1977 - 1993 (Fig. 5). At the end of this period, phosphate concentrations remain at their high level but are no longer increasing due to the exhaustion of the phosphate pool remobilized from the sediments (Nehring, 1989). Recently, it was observed that phosphate concentrations were decreasing in the Gotland Deep due to the renewal and oxygen supply of its bottom water layer in spring 1993 (Nausch, 1993).

The input of phosphorus and nitrogen compounds of anthropogenic origin is the most important reason for eutrophication in the hydrosphere. In connection with the deterioration of the oxygen conditions in stagnant deep waters, eutrophication is the most serious environmental problem in the Baltic Sea. Reliable nutrient measurements and the comparability of nutrient data independent from the originator are therefore of highest priority in the Baltic Monitoring Programme.

Figure 3. Long-term variatons in the winter concentrations (Jan - Apr) of nutrients and salinity in the surface layer (0 - 10 m) of the central Baltic Sea (Stat. 8A,9A, 10B,11B, 15A).





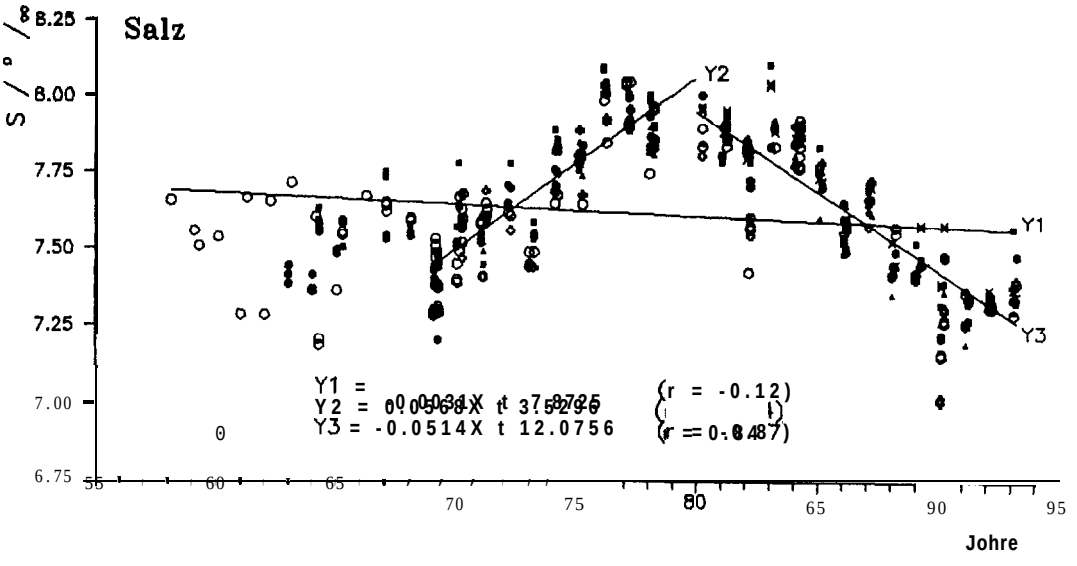


Figure 4. Long-term variations of nutrients and hydrographic parameters in 100 m depth of the Gotland Deep (Stat. 15A).

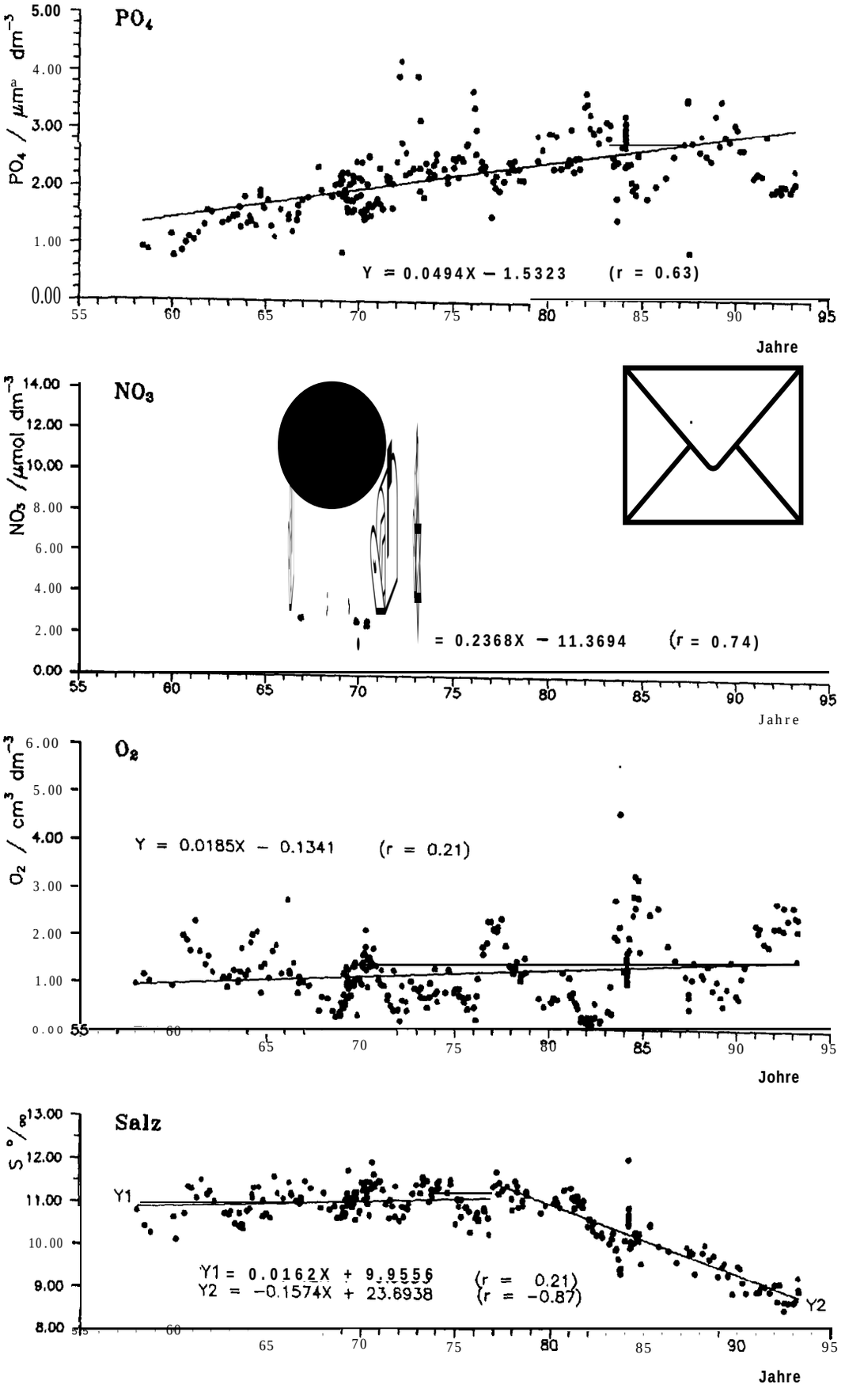
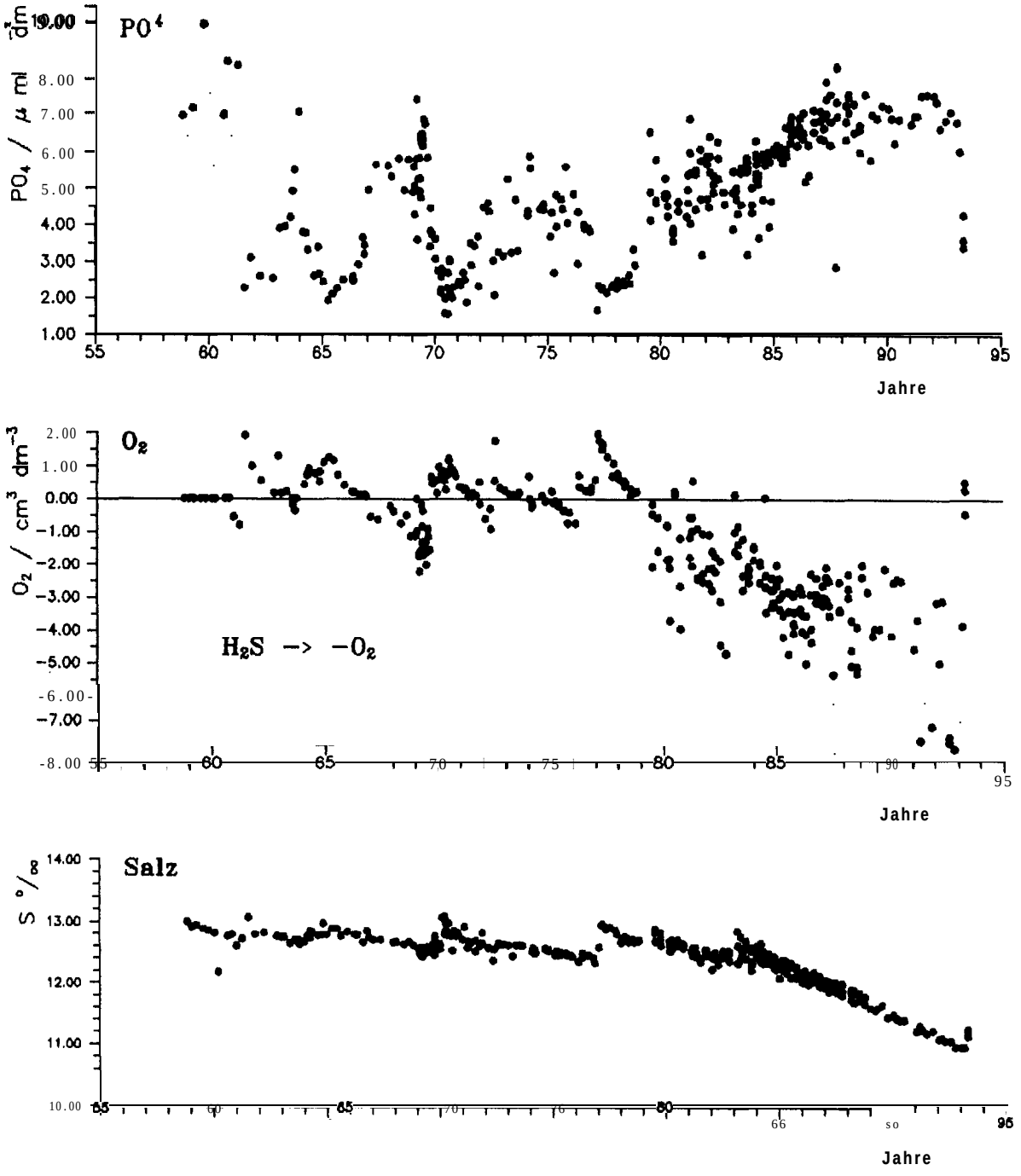


Figure 5. Long-term variations of phosphate and hydrographic parameters in 200 m depth of the Gotland Deep (Stat. 15A).



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Nutrients: Practical Notes on their Determination in Seawater

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1. Introduction

These notes are aimed primarily at the freshwater chemist venturing for the first time into saline waters in search of nutrients, but they are also intended to be of some use to the complete newcomer to the application of automated colorimetric techniques to natural waters. The term "nutrients" is a little difficult to define precisely, but from the point of view of the analytical chemist, in a seawater context, nitrate, phosphate, nitrite, silicate and ammonia are the most frequently encountered of those determinands that can be loosely termed "nutrients". For a fuller discussion and definitions of terms, see Grasshoff et *al,* (1983).

2. Analytical Techniques-General

Some of these nutrients have been measured for several decades by manual colorimetric techniques but the introduction of segmented continuous-flow analysis (CFA) brought about an upsurge of activity in marine chemistry in the 1960s as a result of the increase in sample handling capacity. The main advantage of automated techniques over manual techniques is not so much their speed of operation but their ability to handle large numbers of samples, treating each in an exactly similar manner within strictly prescribed and maintained operating conditions. Despite such advantages, a substantial number of laboratories continue to use manual techniques as was shown in a recent ICES nutrients intercomparison exercise, (Kirkwood et *al.*, (1991)) where 28 out of 68 laboratories indicated that they used manual techniques for phosphate. Economic constraints probably play their part in this choice, as good quality CFA instrumentation is expensive.

Flow-injection (unsegmented) techniques are superseding segmented CFA techniques for some determinations, but as these generally have to trade off sensitivity in order to increase the speed of analysis, flow-injection analysis (FIA) is not readily applicable where lack of colorimetric sensitivity is the major limitation.

This work dwells on problems encountered in segmented CFA as it is anticipated that this technique will continue to be widely used for several more years, including its latest form, micro-continuous flow analysis, a narrow-bore version with improved dispersion characteristics which generally permit higher sampling rates.

Modern systems are supplied complete with dedicated computer and printer, which greatly simplifies the specific tasks of data acquisition, calibration, and calculation and presentation of results. However, results can only be as good as the chemistry that produced them; that is to say, data systems can produce very precise looking numbers from poor-quality analytical chemistry, and unskilled operators are known for their readiness to believe the printed output.

3. Concentration Ranges

Suppliers of CFA equipment generally understate the difficulties of seawater analysis to prospective customers, quite probably unknowingly, because they often lack personal hands-on experience of, for example, the determination of phosphate in seawater at sub-micromole per litre concentrations, or, in units more familiar to them, < 0.03 mg P per litre.

Their methods are generally compromises and may claim, for example, to be "suitable for the concentration range O-10 μmol/l but a typical supplier's configuration is usually substantially different from that which would extract the maximum analytical sensitivity from the measurement system, a condition required for working exclusively in the O-l μmol/l concentration range. Users should be prepared to alter manufacturers' methods when the occasion demands but it is important that changes be made in a systematic manner, keeping intact the chemistry of the original manual method on which the CFA version is based.

Calibration should be undertaken in the range most appropriate to the concentrations of the samples under test. Failure to do so may not produce serious errors in less demanding situations but at other times it may be of utmost importance (see examples a and b below).

a. A sample of potable water with a true nitrate content of 8.0 mg/l (the customary freshwater units) is analyzed by a control laboratory and reported as 9.0 mg/l. Given that the EC 'MAC' (Maximum Admissible Concentration) for nitrate is 50 mg/l the water is evidently acceptable, irrespective of whether the analytical error is due to a calibration slope error, or a baseline bias error, or some combination of these (or other) influences. In the worst possible case, that is where the entire error is the result of a wrongly defined baseline, a sample containing 1.0 mg/l will be reported as 2.0 mg/l but this still won't represent a problem in a regulatory context if the only criterion is whether the concentration is above or below the EC-MAC.

b. Now consider the significance of this 1 .O mg/l bias when a typical North Sea water sample is offered to the same system. If the sample's true nitrate concentration is 5.0 μmol/l (the customary sea-water units), this translates into ≈ 0.3 mg/l but it would be reported as 1.3 mg/l which is seriously in error, a factor of ≈ 4 too high. This is exactly the kind of situation that can result from the uncritical use of a data-system. A calibration curve generated from standards with nitrate concentrations of 25, 50 75 and 100 mg/l could have a respectably high correlation coefficient yet still be capable of producing misleading results in this way. For credible results, standards are required at concentrations both above and below those of typical samples. The conversion factor between these freshwater and seawater units 1000/62 is in itself an indication that typical nitrate concentrations in these media can be expected to be more than an order of magnitude apart.

Much of the current effort devoted to measuring nutrients in seawater is in a trend monitoring context where high accuracy and precision are vital to the detection of trends, given that year-to-year changes of less than 5 % may well be real and meaningful. Accurate and precise measurement of seawater concentrations necessarily means operating CFA instrumentation uncomfortably near to the limits of its

performance capabilities, where problems have to be expected, but it is nevertheless possible if all of the known variables are controlled and optimised. While data-systems have greatly simplified certain operations, there is a continuing need for analysts to be highly critical of their own results and particularly of the manner by which they are obtained.

4. Flow-cell Characteristics

Measurement in the O-l.0 μmol/l concentration range generally requires substantial amplification of the basic electrical signal, and this reveals an important optical effect which can contribute substantial bias if not taken into account. This problem, first described by Atlas et al., (1971), pertains only to CFA (not to manual techniques using conventional cuvettes) and is related to the geometry of individual flow-cells, and consequent refraction of the light-beam.

In the interests of good hydraulic performance, CFA flow-cells require to have rounded ends and low dead-volume, but this produces refractive effects that masquerade as Absorbance signals. (These effects do not occur in the corresponding manual techniques where the optical surfaces of the cuvettes are aligned at precisely 90 ° to the incident light-beam.) These signals are unrelated to determinand concentrations, and they can be demonstrated both in the presence and absence of reagents. They are the results of gross changes in the refractive index of the flow-cell contents such as occur when saline samples are separated by a distilled or demineralised water wash. The disruptive effect of these signals ranges from tolerable, in good quality flow-cells, to catastrophic in those with unfavourable internal geometry, and, in the latter case, the use of artificial or natural low-nutrients seawater as an inter-sample wash is the normal recourse.

5. The Inter-Sample Wash

5.1. Artificial Seawater (ASW)

One litre of a typical ASW formulation containing $NaCl$ (3 1.0 g) and $MgSO_4 7H_20$ (10.0 g) has a nominal salinity of 35 “parts per thousand” which approximates to 35 on the Practical Salinity Scale. (Instructions for preparation can be found in Grasshoff et al. (1983) p. 375-376). Further dilution is required where the salinity of samples differs markedly from 35.

While ASW can be used to overcome the optical problem described in 4.0, it may introduce serious contamination problems (see 7.2).

5.2. Natural “Low Nutrients” Seawater (LNSW)

Low-nutrients seawater is a useful commodity. It can be conveniently collected in bulk in late spring after the main plankton bloom. If stored in a polyethylene carboy in daylight and at room temperature, internal biological activity will cause its nutrients concentrations to continue to deplete naturally and after a few weeks the supernatant can be siphoned off, without filtration, into containers of more convenient size for laboratory use, There is no need for filtration if these operations are carried out carefully. LNSW with nitrate < 0.1 μmol/l and phosphate < 0.02 μmol/l is readily obtained. A residual

silicate concentration of $\approx 1\ \mu mol/l$ can be expected. If supplies are plentiful, LNSW is in every respect preferable to ASW.

5.3. Other Preparations

Mostert (1988) has described the addition of ethanol to pure water to produce a 9.7 % (v) solution which has a refractive index similar to that of sea water. Variations on this theme are mentioned by other workers but these do not appear to be widely used.

5.4. "Pure" Water

There is no substitute for a "zero-nutrients" inter-sample wash which can also serve as a concentration reference. This is more readily achieved using "pure" water rather than ASW or some alternative preparation. Irrespective of the method of purification, a useful safeguard is a small replaceable mixed ion-exchange resin cartridge placed in the pumped supply line immediately upstream of the auto-sampler's wash-point. A suitable device can be readily made using the body of a 20 ml disposable syringe filled with a self-indicating resin such as Duolite MB6113 (MERCK),

5.4.1. Demineralised Water (DMIN)

The demineralisation process is one of ion-exchange and its effectiveness is monitored in terms of electrical conductivity or resistivity. Modern small scale purification systems specially designed for laboratory use generally employ reverse osmosis prior to ion exchange and such systems, according to Mellor (1990) can produce water close to the theoretical resistivity of 18.15 megohms/cm at 25 "C, highly suitable for most purposes in nutrients work.

5.4.2. Distilled Water (DW)

Users must beware of making assumptions about the purity of distilled water. The distilled product is likely to be purer than the feedstock, but its suitability for nutrients work has to be demonstrated, not assumed. This warning applies equally to double-distilled water.

6. Sampling Techniques

A full description of sampling techniques is outside the scope of this work but the arrival on board of a rosette sampler containing one or more filled "Niskin" or similar sampling bottles is taken as the starting point for sample pre-treatment (8.0), but before commencing sub-sampling, some appreciation of contamination is necessary.

There is a "pecking-order" for the removal of sub-samples from the rosette bottles, on the basis of perceived contamination risk from the atmosphere, or the propensity of the sample to change its composition in some way. The nutrients sub-sample is generally some way down the queue behind such determinands as trace-organics, dissolved oxygen and high precision salinity.

7. Contamination

In general, nutrient concentrations in unpolluted seawater are seldom greater than a few micromoles per litre, consequently, from the analytical chemist's viewpoint they should be regarded as trace components, and the potential for contamination of samples (and reagents) should be appreciated.

7.1. Contamination of Samples

Kerouel and Aminot's (1987) systematic study of contamination appears to be the only one of its kind, and their findings are worth re-stating here; they ranked nutrients in decreasing order of contamination risk, thus - urea >> nitrate > ammonia > phosphate > nitrite.

Possible sources of contamination include the ship, equipment, the atmosphere, the analyst, internal (within the sample), or any combination of these.

a. *The ship* - Sampling must not take place while the ship is discharging sewage or any other wastes, and, where possible, sampling should be carried out from the opposite side of the ship from such discharge points. De-rusting operations generally involve phosphoric acid and should not be undertaken immediately before or during a nutrients cruise.

b. *Equipment* - Surfaces that come into contact with the sample may contaminate it if not sufficiently clean. Glass containers present an additional problem. Glass is attacked by seawater and the dissolution rate is appreciable. For example, a 200 ml borosilicate volumetric flask filled with LNSW will contribute silicate (≈ 0.5 μmol/l) to the contents after standing at room temperature for 8 hours. The dissolution rate for soda-glass is 2-4 times higher (see Aminot *et al.* (1992)).

c. *The atmosphere* - Laboratory air is a notorious source of ammonia contamination, even in the absence of smoking, stored ammonia solutions, and ammonia-producing chemical operations. Exhaust gases from ships' engines are a source of oxides of nitrogen. In ammonia and nitrite determinations, samples awaiting analysis on an autosampler carousel can be protected to some extent from airborne contaminants by using a long-reach probe and long (> 10 cm), narrow, partly filled sample tubes. A substantial head-space acts as a buffer-volume by minimising air circulation at the interface.

d. *The analyst* - Direct finger contact with the sample or indirect via equipment surfaces and/or the atmosphere (particularly ammonia and urea).

e. *Internal* - Suspended material, organic or inorganic, small enough to survive filtration, may contribute physically or chemically (or both) to the Absorbance signal.

physical - Turbidity causes light-scatter which is indistinguishable from absorption in simple single-beam colorimetry.

chemical - Reaction conditions, e.g. elevated temperature and/or extremes of pH may cause otherwise insoluble N, P, Si etc to be brought into solution during the actual analysis.

Information of this kind generally cannot be found in text-books but tends to be accumulated slowly and painfully from practical experience.

7.2. Contamination of Reagents

(Most aspects of the discussion of external contamination of samples (7.1) necessarily pertain also to reagent solutions.)

While low level contamination in constantly flowing reagents may be tolerable, high concentrations will give rise to an excessively noisy signal, the noise being in phase with and characteristic of the action of the pumprollers.

Artificial sea water (ASW), though not strictly speaking a reagent when used as an inter-sample wash, has its own special contamination problems which need to be considered in some detail.

Consider a typical supplier's specifications for good quality (analytical reagent grade) sodium chloride and magnesium sulphate.

Maximum concentration limits for specified impurities

	Phosphate (as PO,)	N compounds (as N)
Sodium chloride	0.0005	0.0005
Magnesium sulphate	0.001	*0.002*

Multiple zeros after the decimal point are intended to impress, but the units of concentration are "percent", and these represent potential impurity levels that are far from satisfactory. Take for example, the case where both phosphate and nitrate are present in these two salts at only one tenth of their respective maximum concentration limits, and the N is exclusively nitrate. There is still a serious problem; the ASW specified in 5.1 will contain phosphate and nitrate at 0.27 and 2.5 μmol/l respectively. Both of these concentrations will exert appreciable bias on a system set up for typical seawater concentrations if the ASW baseline is simply *defined* as zero-concentration.

This does not preclude the use of ASW, but, if it is to be used as a concentration reference, its users not only need to find ways of measuring its nutrient concentrations before use, they need also to ensure that these concentrations remain constant during use.

8. Pre-treatment of Samples

It is self-evident that visible flora and fauna, and other suspended material, should be removed from the sample or at least be prevented from taking part in the analytical chemistry. Some samples may be considered free enough from suspended material to permit direct analysis but for coastal work this may not be the case and the relative merits of filtration and centrifugation need to be considered.

8.1. Filtration

Assuming the nutrients sub-sample will require filtration, an intermediate container will be required for the journey from the rosette to the filtration apparatus. 1-litre

polyethylene bottles with plastic insert seals and separate screw-caps are recommended. Both seal and bottle should be adequately rinsed with sample and filled directly from the tap of the rosette bottle without recourse to additional drain-tubing of any kind.

The filtration process is a potential source of serious contamination. Where sample size permits, a 1-litre Buchner apparatus is recommended, and ample rinsing of the filter with the sample, and of the receiving flask with the filtrate. Glass fibre (GF/C) discs of around 50 mm diameter having a nominal pore size of around 1 μm are widely used, but, because of the very nature of their composition, they *will* contribute silicate to any passing filtrate. However, the extent of this contamination will in most cases be negligible; for example, if the passage of 500 ml of a sample containing > 2 μmol/l takes no more than one minute, the difference will be undetectable. More thorough filtration can be achieved using polycarbonate membranes if preferred, but, whatever the chosen procedure, the onus is on the analyst to verify its suitability, experimentally.

8.2. Centrifugation

To avoid the potential contaminating influence of filtration, Kerouel and Aminot (1987) have described a system whereby the sub-sample from the rosette is obtained in a 125 ml polypropylene bottle, and, without further transference, this bottle is first centrifuged then presented directly to CFA using a modified commercial sampler. Where facilities are available for centrifugation immediately after sampling, this approach clearly has some advantages.

9. Stability of Samples

The analyst needs to be aware that nutrients concentrations are liable to change due to the activity of micro-organisms naturally present in seawater, therefore, as a general rule, samples should not be exposed unnecessarily to any source of light. Analysis within minutes of sampling will always be the preferred and definitive procedure, but where this is not possible, methods of preservation and storage must be considered.

There is ample literature on this subject and the one thing that authors are agreed on is that there is no single universally applicable preservation/storage regime that will satisfy all requirements. For example, storage in glass is unacceptable if silicate is to be determined, while some workers claim that phosphate is removed from the sample by adsorption on to container walls (particularly polyethylene).

Some literature claims appear to be openly contradictory but a large part of the apparent confusion is almost certain to be because different authors are never exactly comparing "like with like"; for example, seawater samples from the same location at different seasons may contain micro-organisms of very different species and/or concentraions and it is possible that a given preservative regime could be effective in April but not in the previous (or following) October.

Nevertheless, there are two popular approaches to preservation, both in their own ways aimed at arresting the biological processes that cause depletion of nutrients concentrations; these are *refrigeration* and *poisoning.*

9.1. Refrigeration

9.1.1. Freezing

Freezing is the method of choice of many workers, but several important details require attention. Bottles should be frozen, stored, and thawed, in an upright position, and they should not be completely filled. The reason for these recommendations is that during the freezing process the last few millilitres to freeze will have a very different composition from the bulk, and if the bottle is completely filled some of this liquid may be expressed past the seal of the closure during the freezing process. Subsequent thawing will then result in a sample of unrepresentative composition.

Dissolved silicate is reported to polymeriselcrystallise during the freezing process and several authors warn that when samples are thawed prior to analysis, sufficient time must be allowed for de-polymerisation/redissolution. According to Van Bennekom (1992)), 24 hours is insufficient time for this process, and a separate aliquot of sample, reserved for the silicate determination, stored in darkness at 4 °C is recommended.

9.1.2. Non-Frozen Refrigeration

Non-frozen refrigeration is recommended by some workers, particularly below 4 °C but the majority appear to find frozen storage more convincing, for example, Kremling and Wenck (1986) showed that for nitrate and phosphate, storage at 4 °C was totally inadequate for samples of (unfiltered) Atlantic water.

9.2. Poisoning

An alternative approach, particularly where refrigeration is not possible, is the addition of chemicals with the intention of poisoning the species responsible for consuming the nutrients to be determined.

Of the various chemicals that have been investigated, only three have ever achieved widespread popularity; these are, sulphuric acid, chloroform, and mercuric chloride.

9.2.1. Sulphuric Acid and Chloroform

Both of these preservatives have had their critics and adherents and were recommended in specific cases by Standard Methods for the Examination of Water and Wastewater, up to the 12th Edition. However, without explanation, the 13th Edition (1971) and subsequent including 17th Edition (1989) clearly states *"Preservation with acid or chloroform should be avoided."*

Probable reasons for this "about-turn", and chemical preservation in general are discussed in detail by Kirkwood (1992).

9.2.2. Mercuric Chloride

The use of mercuric chloride in this or a similar context was reported before 1920 but Ibáñez (193 1) appears to be the first to describe its mode of action in any detail - "The bactericidal action of mercuric chloride prevents the troublesome activity of micro-

organisms present in the sea water whilst its very small dissociation constant (a = 0.013) is without effect on the pH".

Ibáñez used four drops of a saturated solution per 100 ml of sample, which is approximately 80 μg mercuric chloride per ml of sample, depending on the effect of ambient temperature on saturation concentration. (Translating "four drops" into 0.12 ml and "saturated" into 6.9 g/100 ml (the solubility at 20 °C given by the CRC Handbook of Chemistry and Physics).)

Several authors have reported the use of mercuric chloride at a variety of (predominantly lower) concentrations but over the years it seems to have lost favour, no doubt due to the concurrent increase in interest in the determination of mercury in seawater at nanogram per litre levels, and perceived contamination problems in the use of any form of mercury on board ships used for that work.

In their investigation of the effectiveness of three preservative regimes for nutrients, Kremling and Wenck were somewhat critical of mercuric chloride. They used 10 μg per ml of sample and concluded that this concentration was "probably.. .inadequate preservation against the ongoing biological activities" at that temperature (4 "C). Their objection to its use at a higher (unspecified) concentration was apparently based on their experience of "erratic nitrate measurements if copperised cadmium reductors are used".

The author has demonstrated that 20 μg/ml causes no analytical problems in his particular method and that this concentration was an effective preservative at room temperature for filtered North Sea (winter) water containing nitrate ≈ 5.5. μmol/l and phosphate ≈ 0.5 μmol/l. (Six laboratories from UK, Norway and France analyzed sub-samples of this water over a period of several months and produced remarkably good agreement for both nitrate and phosphate.)

As that work (Kirkwood (1992)) and that of Kremling and Wenck are not necessarily contradictory, as they may first appear, the use of mercury in this context should not be totally discouraged, indeed, in the continued absence of a more acceptable alternative, further investigations with a view to establishing a well-founded recommendable dosage concentration are clearly required.

10. Calibration of the Analytical System

Because of the well known but inadequately understood problems of biological activity in seawater, and the uncertainties associated with preservation techniques, there are no Certified Reference Materials (CRMs) available at this time for nutrients in natural seawater, consequently analysts have had to rely on their own resources.

It is generally prudent to assume that seawater matrix salts may exert some influence on the kinetics of colour-forming reactions; for example, in the most widely used method for the determination of nitrate there is a well known "salt-effect" that can give rise to significantly different calibration slopes for fresh water and sea water matrices. Due attention should be given to such effects otherwise systematic errors will be introduced.

10.1. Working Calibration Solutions

Working calibration solutions (those actually presented to the analytical system) are normally produced by "spiking" low-nutrients seawater (LNSW) with standard solutions of nutrient salts.

By diluting for example, a 1 ml aliquot of a standard solution to 200 ml using LNSW as diluent, the salinity of the product is close enough to that of the diluent to ensure that their refraction effects will be indistinguishable, and the resultant chemical matrix will remain a close approximation to that of a typical seawater sample. (Modern liquid handling devices can dispense a 1 ml aliquot with highly acceptable accuracy and precision, but they should be checked gravimetrically from time to time to ensure they maintain performance specifications.)

When using LNSW as a base-water for the preparation of working calibration solutions, it is important to appreciate that with or without filtration LNSW must be assumed to contain micro-organisms, consequently when it has been spiked with a mixture of nutrients the depletion process is liable to resume. Working calibration solutions are therefore best prepared immediately before their intended use; they may be stored in darkness for short periods but should not be trusted for more than a few hours.

Certified standard solutions specifically for marine work have been commercially available for several years ("Sagami" Standards, from Wako Chemicals, GmbH, Neuss, Germany) but only a minority of laboratories use these routinely. These solutions consist of a sodium chloride matrix of stated nominal salinity and as their nutrients concentrations are appropriate, they are analyzed directly without dilution. Though it may be said that they are "not quite the real thing", use of these solutions should be encouraged because the credibility of results is considerably enhanced if the measurement technique has been validated by the simultaneous successful analysis of certified solutions.

10.2. Standard Solutions

These can be prepared in the laboratory by classical gravimetric and volumetric techniques. The chemicals and water used should be of the purest grades available to the analyst. Storage under refrigeration is widely practised but solutions must be returned to room temperature before use, otherwise systematic volumetric errors will be introduced,

Concentrated primary standard solutions (containing hundreds of milligrams of a single salt per litre) can be assumed to have an indefinite shelf-life if stored in darkness, well protected against evaporation. Silicate is an exception; solutions may be supersaturated and unstable at concentrations exceeding 100 mg per litre.

When using a multi-channel analyzer for the simultaneous determination of three or four nutrients, it is very convenient to combine the serial dilution of primary standard solutions into a single mixed solution of nutrients of intermediate concentration which can be used as a spiking solution as described in 10.1.

However, a mixture of this kind should be assumed to have a composition that will support the same kind of biological action as occurs in natural samples, therefore its

working life should be considered very short (hours) unless effective steps are taken to prevent such action. Kirkwood (1992) with the help of 18 collaborating laboratories has demonstrated excellent stability (> 12 months) for a combined solution of nitrate, phosphate and silicate stored in polycarbonate and protected from biological action by the addition of 20 μg $HgCl_2$ per ml.

It should be noted that as nitrate is the eventual end-product of the oxidation of such nitrogen-containing ions as nitrite and ammonium, solutions containing combinations of these need to be treated with some circumspection.

11. Analytical Methods (CFA)

The following sections 11.1 - 11.4 contain a selection of methods for the determination of nutrients at natural concentrations in North Sea waters.

In the author's laboratory each of these methods is part of a four-channel simultaneous system whose auto-sampler is effectively limited to cups containing around 7 ml. This imposes constraints on sample uptake rates and 0.8 ml/min has been adopted as maximum; the chemistry is tailored to suit, but as far as possible these methods are faithful to the manual methods on which they claim to be based.

11.1. Determination of phosphate

11.1.1. Chemistry (phosphate)

Every phosphate method that uses molybdate, sulphuric acid, potassium antimony tartrate, and ascorbic acid as reducing agent, whether manual or automated, is almost certainly descended from Murphy and Riley's (1962) manual technique.

A recent re-investigation of the original Murphy and Riley technique by Pai, Yang and Riley (1990), described a comprehensive study of the effects of variation of acidity and molybdate concentration. The acid/molybdenum ratio was shown to be crucial, influencing not only the form of the final reduced complex but also playing a vita! role in the control of the reaction kinetics. The message is clear; the underlying chemistry of the original manual method should not be tampered with. Nevertheless, the ICES Intercomparison Exercise, Kirkwood, et a!. (1991) (which included a survey of phosphate methodology) shows that workers and equipment suppliers alike, are capable of making apparently arbitrary changes to their own and to each others' methods, sufficient to cause substantial divergence from the parent methods on which they claim to be based. Conclusions from chemical interference studies on the original method may be invalidated by such changes.

Pai et al's re-investigation strengthens the case for considering only Murphy and Riley's method as "standard", and CFA users might be we!! advised to abandon their particular adaptations unless they adhere very closely to the concentrations and conditions specified in the manual method. These consisted of 40 ml of sample plus 8 ml of a "single mixed reagent", diluted to volume in a 50 ml flask, then stood at room temperature for a minimum of 10 minutes before Absorbance measurement at 880 nm.

Their mixed reagent was prepared by mixing the following ingredients.

sulphuric acid (5 N)	2550	mmol/l	125
ammonium molybdate (40 g/l)	32.37	"	37.5
ascorbic acid (0.1 M)	100	"	75
potassium antimony! tartrate (1 mg Sb per ml)	8.21	"	12.5

(consequent volume 250 ml)

The final concentrations of these constituents in the cuvette are as follows - (mmo!/!)

sulphuric acid H_2S0_4 (mol. wt 98.1)	200
ammonium molybdate $(NH_4)_6Mo_70_{24}.4H_20$ (mol. wt 1235.9)	0.777
ascorbic acid $C_6H_80_6$ (mo!.wt 176.1)	4.8
potassium antimony! tartrate $KSbC_4H_40_7$ (mo!.wt 324.9)	0.0657

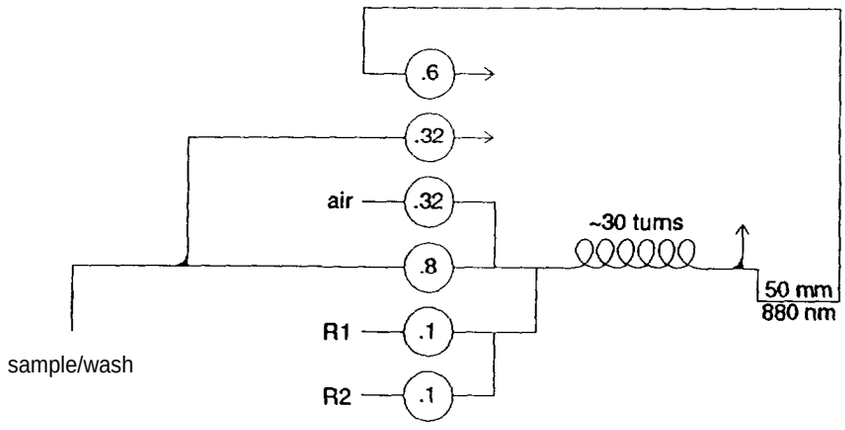
11.1.2. Sensitivity (phosphate)

From Murphy and Riley's (1962) experimental data, a molar absorptivity (ε) of around 23,000 at 882 nm can be derived for the phosphoantimonylmolybdate complex. A 50 mm path-length cell containing sample with a phosphate concentration of 1.0 μmol/l (taking account of the small but significant dilution by the added reagents) should produce an Absorbance of 0.091. Adopting 0.001 A as an empirical limit of detection for manual spectrophotometric techniques, it becomes evident that the measurement of sub-micromolar phosphate concentrations with a precision of $\pm 0.01\,\mu$mol/l is about the very best that can be expected, but the task is made even more difficult if a CFA system subjects the sample to dilution appreciably greater than the x 1.25 (40 ml $\rightarrow$ 50 ml) of Murphy and Riley.

11.1.3. Hydraulics (phosphate)

The flow-diagram (Figure 1) shows an example of CFA which adheres closely to the parent manual method; it also contains Grasshoff's popular "split-reagent" refinement which solves the instability problem associated with Murphy and Riley's single mixed reagent.

Figure 1. Determination of phosphate in seawater by continuous-flow analysis.



Reagent 1	**sulphuric** acid (5 **mol/l**)	5000	**mmol/l**	200 ml ⎫
	ammonium molybdate (40 **g/l**)	‘32.31	"	120 ml I → 500 **ml**
	potassium **antimonyl tartrate** (5.3 **g/100** ml)	163.1	"	2 **ml** ⎭
Reagent 2	ascorbic acid			**4.2 g** ⎫
	Levor IV (wetting agent)			0.5 **ml** ⎭ → 500 **ml**

Assuming the nominal flow-rates for pump-tubing are accurate, the final concentrations of these constituents in the flow-cell are within ± 1 % of those of Murphy and Riley’s manual method listed in 11.1.

Manufacturers generally include a 37 °C heating cartridge but this is not entirely necessary: however, if reaction time is to be maintained around or below the 10 minutes minimum of the manual method, it is essential that room temperature should be above 20 “C. (A convenient arrangements is a 40 or 60 W lamp directed at the reaction coils from a distance of a few centimetres.) Completeness of reaction should, of course, be checked by addition/subtraction of coils. An excessive number of coils may cause hydraulic problems as well as contributing to adsorption/desorption effects understood to be a consequence of the colloidal nature of the phosphoantimonyl-molybdate complex. When peak-tailing due to this effect causes sample separation to deteriorate to an unacceptable level, satisfactory conditions can generally be restored by pumping a solution of sodium hydroxide (1.0 mol/l) through the sample line for a few minutes.

Atlas et a!. (1971) described a method, known as the "OSU" method (Oregon State University), which uses hydrazine (at 70 “C) as the reductant and claims 15 % more sensitivity than Murphy and Riley. Their measured phosphomolybdate complex must be different from that of M&R, as the latter contains antimony in a 1: 1 P:Sb ratio, while OSU omits the antimony-containing reagent. This, according to Gordon (1989), eliminates flow-cell coating.

Using the manifold shown (Figure 1), the author has had highly satisfactory results from the following adaptation of the OSU method. (The reaction is heated at 65 - 70 °C and is thus compatible with the temperature requirements for the ammonia manifold described in 11.3).

Reagent 1	sulphuric acid (5 mol/l)	**200** ml ⎤	
		→	500 ml
	ammonium molybdate (40 g/l)	120ml ⎦	
Reagent 2	hydrazinehydrochloride	0.5 g ⎤	
		→	500 ml
	Levor IV (wetting agent)	**0.5** ml ⎦	

11.2. Determination of nitrate and nitrite

11.2.1. Chemistry (nitrate and nitrite)

In the absence of a suitable reaction for the direct colorimetric determination of nitrate, the most widely used method relies on reduction to nitrite which is then quantitatively incorporated into a diazo-couple compound. Any nitrite originally present in the sample requires separate determination and subtraction, but this is not a serious drawback of the method, as generally nitrate >> nitrite, and the proportion of the response attributable to nitrite is small relative to the precision attainable for the nitr(ate+ite) determination.

Bendschneider and Robinson (1952) were first to investigate the use of sulphanilamide as diazotising agent and N-(1-naphthy!)ethy!enediamine dihydrochloride as coupling agent in a seawater context. They stated that for maximum colour development the sample should be mixed with the sulphanilamide and hydrochloric acid before adding the coupling agent, and that the final solution should have a pH < 2.4.

Bendschneider and Robinson’s manual method consists of 50 ml sample plus 1 ml sulphanilamide reagent (containing hydrochloric acid), then after 2-6 minutes, 1 ml of coupling reagent followed by Absorbance measurement at 543 nm at least 10 minutes later.

Bendschneider and Robinson’s two reagents were prepared thus:

a. sulphanilamide (5 g) in 500 ml of 1.2 N hydrochloric acid

b. N-(1-naphthy!)ethy!enediamine dihydrochloride, "NEDD" (0.5 g) in 500 ml water

The final concentrations of these constituents in the cuvette are as follows - (mmo!/l)

sulphanilamide $C_6H_80_2N_2S$ (mol.wt 172.2)	1.12
hydrochloric acid HC! (mo!.wt 36.5)	23.1
NEDD $C_{12}H_{14}N_2.2HCl$ (mo!.wt 259.2)	**0.074**

Nitrate to nitrite reduction can be off-line but most workers now prefer on-line systems based on copperised cadmium in the form of granules, wire or tube.

The role of ammonium chloride in the reduction process ins unclear but it is necessary, widely used, generally referred to as a "buffer", and recommended for filling reductores when not in use.

Several authors stress the need to carry out the reduction at around pH8. Most waters with an appreciable salinity naturally have a pH in this region and have enough buffering capacity to be unaffected by the relatively low concentration of ammonium chloride used in the method described here. However, if for example, fresh water samples with a naturally low pH are to be analyzed directly, steps must be taken to adjust the ammonium chloride solution to pH8-8.5 so that it will act as buffer, otherwise a substantial loss of sensitivity and precision may result (see Collos *et al.* (1992).

Some workers claim that such additives as EDTA, sodium tetraborate and ammonia prolong reductor life by preventing the formation of cadmium hydoxide or carbonate, but whichever solution is selected, it must not prevent the colour reaction from reaching $pH < 2.4$.

Reductor efficiency should be checked by comparing steady-state signals from identical concentrations of nitrate and nitrite passed separately through the system in nitr(ate+ite) mode.

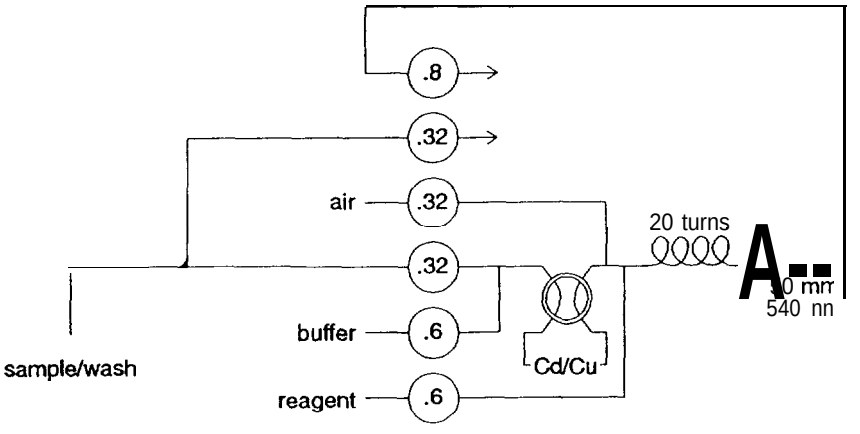
11.2.2. Sensitivity (nitrate and nitrite)

From Bendschneider and Robinson's experimental data, a molar absorptivity (E) of around 50,000 at 543 nm can be derived. This gives the method a potential sensitivity approximately double that of Murphy and Riley's phosphate method.

11.2.3. Hydraulics (nitrate and nitrite)

The flow-diagram (Figure 2) shows a dual-purpose system which can be readily switched between nitr(ate+ite) and nitrite and is suitable for typical North Sea concentrations.

Figure 2. Determination of nitrate and nitrite in seawater by continuous-flow analysis.



Buffer solution	ammonium chloride	3	g /l	
Combined Reagent	sulphanilamide	500	mg l	
	conc. hydrochloric acid (- 12 mol/l)	5	ml	→ 1 litre
	NEDD	50	mg I	
	10 % BRIJ-35 (wetting agent)	0.5	ml	

Assuming the nominal flow-rates for pump-tubing are accurate, the final concentrations of the constituents of the mixed reagent are within ± 3 % of those of Bendschneider and Robinson.

Most published methods use air-segmentation and mixing coils for the buffer addition step but this requires a debubbler before the reductor, all of which adds unwanted dead-volume to the system. The manifold shown, which includes a single combined colour reagent, works well, suggesting that adequate mixing of sample and buffer is achieved despite its simplicity.

A 4-way valve (Pharmacia LV-4) protects the reductor from unwanted incursions and is compatible with many CFA fittings; for example, if a PT11 junction is used at the confluence of the sample and buffer lines, it can be mounted directly in a valve port. Using this configuration, wetting agent need not be added until after the reductor, and this absence of wetting agent has been found to have a beneficial effect on reductor life and stability.

This dual-purpose system is necessarily a compromise, and if for example the determination of nitrite is required at higher sensitivity, then a greater proportion of sample in the circuit than that shown (21 %) should be used, and reagent concentrations altered accordingly.

11.3. Determination of ammonia

11.3.1. Chemistry (ammonia)

The most widely used colorimetric method for ammonia is based on a reaction attributed to Berthelot in 1859. Under alkaline conditions (pH 8-11.5) ammonia reacts with hypochlorite to form monochloramine, which, in the presence of phenol and excess hypochlorite forms indophenol-blue.

Various authors have adapted this reaction for the direct CFA determination of ammonia in seawater and the method described here is based on that of Treguer and Le Corre (1975). Their method produces a response which varies only slightly over the entire salinity range and this was a substantial improvement over others that were in use at that time. Following many years of experience with the method, Aminot and Kerouel(l992) have suggested some minor modifications and these have been taken into account. Although final reagent concentrations in the flow-cell are identical to those of Aminot and Kerouel, individual reagent concentrations have been reduced to minimise precipitation problems, and reagent flow-rates increased to compensate.

The main points of difference between Treguer and Le Corre's method and that described here is that reagent stability has been improved by altering the combinations used. There has also been a general increase in reagent concentrations accompanied by a lowering of the reaction temperature from 80 to 65 °C in deference to those authors who have expressed fears that extra ammonia may be brought into the determination from hydrolysis etc. of nitrogen containing molecules if high temperatures are used.

Final concentrations in the flow-cell are as follows (mmol/l)

	this method	Treguer & Le Corre
trisodium citrate $Na_3C_6H_5O_7.2H_2O$ (mol.wt 294.1)	61.8	24.2
sodium nitroprusside $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ (mol.wt 297.9)	0.488	0.274
phenol C_6H_6O (mol.wt 94.1)	17	9.44
sodium hydroxide NaOH (mol.wt 40)	25	14
chlorine Cl, (mol. wt 71)	1.3	0.43

11.3.2. Sensitivity (ammonia)

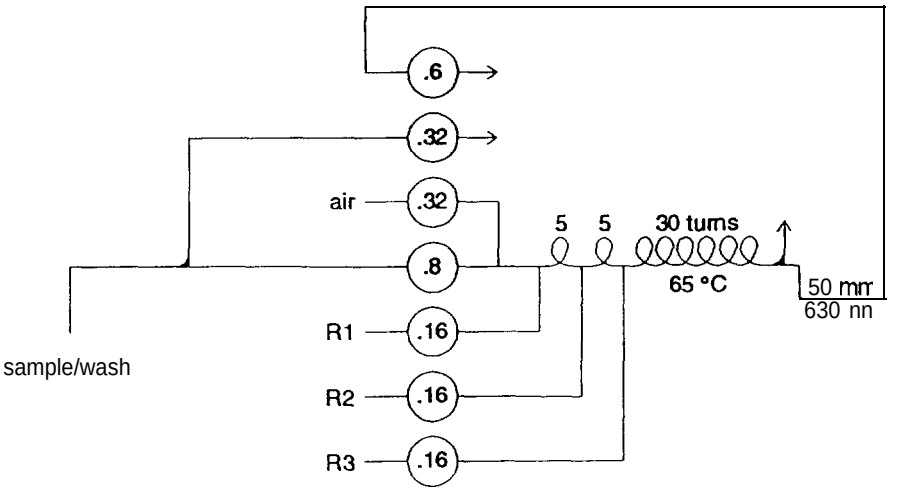
From Treguer and Le Corre's experimental data, a molar absorptivity (ε) of around 10,000 at 630 mm can be derived for indophenol-blue.

Richards and Kletsch (1964) described a method whereby ammonia is oxidised to nitrite which can then be determined as in 11.2. While their method is potentially around five times more sensitive than those based on indophenol-blue, the subtraction of nitrite from (ammonia + nitrite) in order to arrive at ammonia, presents problems when concentrations are near to detection limits and/or of the same order.

11.3.3. Hydraulics (ammonia)

The entire manifold (Figure 3) should be protected from sunlight. Failure to do so may result in serious baseline instability.

Figure 3. Determination of ammonia in seawater by continuous-flow analysis.



Reagent 1	trisodium citrate	62.5 g	⎫		
	sodium nitroprusside	0.5 g	⎬	→	500 ml
	10 % BRIJ-35 (wetting agent)	0.5 ml	⎭		
Reagent 2	phenol	5.0 g		→	500 ml
Reagent 3	sodium hydroxide	3.4 g	⎬	→	500 ml
	chlorine donor*	1.0 g	⎭		

* Solutions of hypochlorite have lost favour due to their instability and have generally been replaced by salts (Na or K) of dichloroisocyanuric acid, for example $C_3Cl_2N_3O_3Na$ (mol. wt 219.9) Eastman product no. 10511.

11.4. Determination of silicate

11.4.1. Chemistry (silicate)

Koroleff thoroughly investigated the determination of silicate in seawater and his 1971 manual method described in Grasshoff et al. (1983), appears to be the first to use ascorbic acid as reductant in this context.

Under acidic conditions, dissolved silicate reacts with ammonium molybdate to form silicomolybdic acid, of which there are α and ß isomers, and the kinetics of their formation are complex. Once formed, the a is the more stable but its formation is slow.

The ß is formed rapidly and preferentially at low pH but the presence of seawater salts favours its transformation into a faster than would be the case in fresh water. Reagent rations and pH are optimised to favour the formation of ß which is then reduced by ascorbic acid to a highly stable blue-coloured complex. Oxalic acid prevents reduction of the excess molybdate which would otherwise interfere in the colorimetry. Koroleff's manual method consisted of 25 ml sample plus 1.0 ml of an acid/molybdate reagent, then after a salinity dependent time-interval, 1.0 ml of oxalic acid solution followed immediately by 0.5 ml of ascorbic acid solution, then Absorbance measurement at 810 nm after 30-60 minutes.

Obviously a salinity dependent time-interval cannot be accommodated in CFA, but Grasshoff's concentrations (substantially different from Koroleff's) achieve a compromise whereby the response to silicate varies only slightly (3 %) over a wide salinity range; this variation can be neglected in all but the most demanding applications.

Final concentrations in the cuvette/flow-cell are as follows-(mmol/l)

	Koroleff	Grasshoff
sulphuric acid H_2SO_4 (mol. wt 98.1)	81.8	25.4
ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ (mol. wt 1235.9)	1.86	1.42
oxalic acid $(COOH)_2.2H_2O$ (mol. wt 126.1)	28.8	11.9
ascorbic acid $C_6H_8O_6$ (mol. wt 176.1)	2.89	22.7

11.4.2. Sensitivity (silicate)

Koroleff reports molar absorptivities (E) of 19,000 in seawater and 22,000 in distilled water and attributes this 15 % difference to the effect of salinity on the rate of transformation of the B-form into the a-form of silicomolybdic acid.

The absorption spectrum of reduced ß-silicomolybdate lends itself to the use of alternative wavelengths to extend the range of measurement; 810 nm is used for maximum sensitivity but an attenuation of around 2.5 is available at 660 nm.

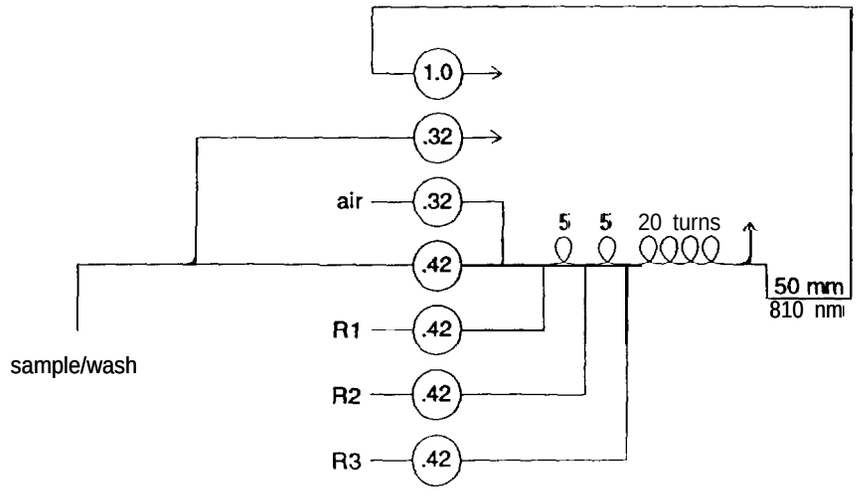
11.4.3. Hydraulics (silicate)

Grasshoff was evidently aware of a potentially serious temperature dependence which has been mentioned by other workers. He chose to counter this by constructing the entire manifold from polyethylene or Teflon tubing wound around a temperature-controlled cylindrical former.

Good results have been obtained in the author's laboratory by winding 0.5 mm i.d. polyethylene tubing externally on a SKALAR 30 mm o.d. heat exchanger, then insulating the entire assembly with "bubble-wrap" packaging material (Figure 4).

The choice of temperature is less important than its stability, but to ensure it will not be overtaken by room temperature, 30 °C is probably more suitable than Grashoff's suggested 25 "C.

Figure 4. Determination of silicate in seawater by continuous-flow analysis.



Reagent 1	sulphuric acid (5 mol/l)	5000 mmol/l 9.4 ml	
	ammonium molybdate (40 g/l)	32.37 " 88 ml	→ 500 ml
	Levor IV (wetting agent)	0.5 ml	
Reagent 2	oxalic acid	3.0 g	→ 500 ml
Reagent 3	ascorbic acid	8.0 g	→ 500 ml

The chemistry is identical to that of Grasshoff.

12. Miscellaneous Technical Considerations

12.1. Over-range Concentrations

The foregoing sections are concerned primarily with oceanic or open-sea concentration ranges which generally demand that the instrumentation be configured to produce high sensitivity towards the determinands. For exclusively estuarine or coastal work where typical samples may contain very much higher concentrations, there are several points the analyst must consider.

A linear relationship between Absorbance and concentration (i.e. compliance with the Beer-Lambert law) must not be assumed.

Non-linearity of system response may be due to colorimetric (instrumental) non-linearity, or to chemical non-linearity, or a combination of both. The use of short path-length flow-cells and/or alternative measurement wavelengths will have a beneficial effect on the former, but it is generally more satisfactory to manipulate the chemistry. For example, the efficiency of a cadmium/copper reduction system (and its life-expectancy) is much reduced by exposure to excessive nitrate concentrations, consequently these require dilution to acceptable levels.

Samples may be diluted externally, or within the CFA system either by a dilution loop, or in extreme cases, dialysis can be considered. Whichever method is chosen, due attention should be given to the salt effect described in 10.0. If using an internal dilution loop in CFA, it is not sufficient to calculate the extent of the dilution from the nominal flow-rates of pump-tubes. High concentration working calibration solutions, appropriate to the samples, must be put through the complete system, and at suitable time intervals to compensate for flow-rate drift during the working life of the pump-tubes.

Where only a small proportion of samples are over-range and a high sensitivity configuration is retained, external dilution using LNSW as diluent ensures that normal calibration procedures retain their validity and no appreciable salt-effect errors are introduced.

12.2. Hydraulics

Unsegmented flows, such as between the auto-sampler probe and the point of segmentation, should be kept as short as possible to minimise mixing across sample/wash interfaces.

Reagent liquid levels, manifold components and waste outlets should be approximately in the same horizontal plane, as unnecessary pressure heads generally contribute unwanted signal-noise.

Ionic and non-ionic wetting agents have very different modes of action and they are definitely not interchangeable. They may even be unnecessary; if a system will run smoothly without them, there is no reason to include them. In any case, recommended concentrations should be treated as maxima; exceeding these may cause precipitation in manifolds of high salinity and the resultant turbidity will produce false positive Absorbance signals that will bias results, (see example in Grasshoff et al. (1983) p 369).

12.3. Ship-board Operation

The ship's motion induces vertical accelerative/decelerative influences in the hydraulics of CFA systems: finely balanced systems that give trouble-free operation on terra firma may give problems on board ship and must not be assumed to be satisfactory without testing. Debubblers are generally a weak point and their flows may have to be reconfigured to allow for a greater proportion of sample loss to the debubbler waste. This may degrade the system's wash-out characteristics to some extent, but not seriously.

12.4. Photometer Developments

Recent developments in instrumentation employ a dual-wavelength compensation system which is analogous to "background correction" in atomic absorption spectrophotometry. This effectively removes the refraction component which contributes equally to the analytical wavelength channel and to the (substracted) non-analytical wavelength channel. It seems likely that this approach will become increasingly popular as it also implies automatic correction for turbidity in samples.

13. ICES Intercalibration/Intercomparison Exercises

The earliest attempts to study the intercomparability of nutrient determinations were multi-ship events in which freshly obtained samples were distributed and analyzed by participants within hours; see Koroleff (1965) and Grasshoff (1966) for details of what were, in effect, "NUTS I/C 1" and "NUTS I/C 2", respectively.

In 1969/70 in an ICES/SCOR exercise organised by the ICES Working Group on Chemical Analysis of Seawater, 45 laboratories in 20 countries analyzed solutions prepared and distributed by the Sagami Chemical Research Center, Japan. The collection and compilation of results for this exercise "NUTS I/C 2" took some time and the final report, Koroleff, *et al.* was not available until 1977. This was the first I/C to report on nitrate.

The most recent ICES Exercise, "NUTS I/C 4", Kirkwood, et *al.* (1991) in an attempt to achieve more realism, made a point of using materials that had at least begun their life as natural seawater.

Sample 1.

One sample was of oceanic water from a sampling depth of 1000 m in a water depth of 2700 m near Greenland. Sub-samples from a similar depth at a nearby location one year earlier had shown a remarkable and potentially useful stability after several months of storage in glass bottles, at room temperature and without treatment of any kind. The apparent stability of this water was a surprise to many of the participants but the measure of agreement achieved for its nutrient concentrations appears to confirm that it was at least "stable enough" for its use in this context.

Sample 2.

The second was a shelf-seas sample which was filtered, bottled, capped, then sterilised by heat treatment in an autoclave. This appears to be a promising approach to the eventual production of a Standard Reference Material for nutrients. (See Aminot and Kerouel (1991)).

Sample 3.

The third was a sample that had been allowed to become depleted and therefore should have produced nutrient concentrations below the detection limits of participants' techniques. Two bottles of this sample were supplied, one glass the other polypropylene, but participants were unaware that these bottles contained the same sample. Their inclusion proved highly effective in the identification of those laboratories whose techniques suffered from bias from various sources.

Intercomparison and intercalibration (I/C) exercises, both formal and informal have a vital role to play in the development of techniques and in the improvement of analysts' performance in the application of these techniques. For example, of the 68 laboratories which participated in NUTS I/C 4 (the great majority of them well-accustomed to nutrient determinations at marine concentrations) it was perhaps something of a surprise to discover that 16 were "unacceptable" for nitrate, and 10 "unacceptable" for phosphate. Surprise or no, it was something well worth finding out since unacceptable performance can be rapidly upgraded once recognised. In many cases it would appear that analytical sensitivity and precision were adequate but the major problem is a lack of quality control in calibration procedures. This is the same conclusion as was reached

by Jones and Folkard in their contribution to the NUTS I/C3 (1977) report; twenty years later not much has changed.

For this reason, a well designed I/C exercise should not simply come to an end with the listing of results, but should find ways of helping the poorer performers to improve. To aid in this process, it is immensely valuable to the organizers to have detailed information form the participants on how they obtained their results, as this can often be much more revealing than the results themselves. Furthermore, I/C exercises offer opportunities for workers to communicate with each other and this helps to improve understanding of and control over the various processes that complicate the task of measuring nutrient concentrations.

It goes without saying that since techniques, equipment, and analysts, all change with time, the skills of a given laboratory will also be subject to change and should be tested by formal intercomparison at regular intervals, not just once. ICES is committed to further similar exercises in 1993 and 1997 (NUTS I/C 5 and 6).

14. Summary

To recommend that there should be a Standard Method for each determinand, in a nutrients context, is unrealistic, impractical and unnecessary. Technical and instrumental constraints can make it impossible to produce a faithful copy of a method using equipment B if the method was originally designed around the characteristics of equipment A. However, what can be and should be standardised is the underlying chemistry on which the methods are based.

Because the requirements and equipment of individual workers are so varied, it is difficult to formulate consistently positive advice that will apply in all situations, nevertheless, a comprehensive list of "don'ts" particularly if accompanied by detailed "why nots", as this work has attempted, should help improve the novice's feel for the subject.

15. Acknowledgements

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Standards, Reference Materials and Matrix Problems in Marine Nutrient Analysis

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Problems encountered in the preparation of standards and reference materials for nutrient measurements in the marine environment are not simply the preparation of defined concentrations. An accurate amount of standard material in a defined volume is easily prepared using calibrated glassware and standard analytical balances. In most cases analytical grade reagents are sufficiently pure. However, regular tests and checks against reference material are required. Stock standards of concentrations $> 1\ \mu mol/L$ sealed in ampoules are stable up to decades. Sterile filtration or addition of a trace of chloroform may be used to inhibit bacterial effects. Aliquots of stock standards should be "archived" and new stock standards should be checked against references and previous batches.

The exact definition of standards and reference materials is, however, only one requirement. A standard and a natural sample of equal concentration must result in identical analytical signals, i.e. displayed or calculated concentration value. Natural seawaters, however, are not neutral matrices as e.g. deionized water and they may affect the chemical reaction used to identify the parameter in question (nutrients).

There are at least two ways by which the analytical results of nutrient samples can be falsified by the seawater matrix as compared to standards in pure water.

Most modern methods of nutrient determination are colorimetric or photometric measurements of coloured dyes produced from the respective nutrient and special reagents. Both, intensity and spectral absorbance of some of these dyes can be affected by the electrical properties of the matrix, i.e. the ionic strength. The photometric signal of e.g. the silicomolybdate formed in the determination of silicate is reduced by up to 0.5 % per salinity unit.

Some of the analytical reactions are also very sensitive to changes of the pH. The formation of the indophenol blue in the analysis of ammonia is reduced by a shift of the reaction pH from 11.4 (pure water) to 9.8 (36 PSU natural seawater) as an effect of the buffer capacity of seawater. The resulting analytical error may be up to 9 %.

Unfortunately a wide variety of natural seawaters have to be considered and different measures are required to match standards and samples. In principle two procedures are applicable to overcome the problem.

1. Standards and reference materials are prepared in a matrix as close as possible to that of the samples. This can be achieved by a matrix of artificial seawater (recipes see e.g. Kester, D.R., Duedall, U.W. and Pytkowicz, R.M., Limnol. Oceanogr. 1967: 12,176-178) or a low or zero nutrient natural seawater (e.g. filtered and aged Atlantic or Mediterranean surface water). For sample

salinities other than 35-36 PSU the matrix may be adjusted by corresponding dilution. The results thus obtained are correct within the accuracy of the method. Reference materials are commercially available in either pure water or a sodium chloride matrix (e.g. SAGAMI).

2. In estuaries and seas with strong salinity gradients (Baltic Sea, Black Sea etc.) the matrices of the samples are variable, and this means that no standard matrix can be used to match all samples. In these cases the matrix effects ("salt effects") have to be determined experimentally and the analytical results obtained using standards in a common matrix must be corrected correspondingly.

   As most nutrient analysis are currently done by continuous-flow "Auto-Analyzers', a matrix of sodium chloride or zero nutrient artificial or natural sea water is recommended because most instruments require compensation of the densities of sample and standard (disturbed photometer signal due to inhomogeneous optical densities).

   The sodium chloride (analytical grade) solution or sea water matrix is adjusted to the mean sample salinity. Correction equations have to be established for ammonia and silicate, however, it is recommended to check possible salinity influence for other parameters too.

Figs. 1, a and b illustrate the establishing of correction equations for salinity effects. In this example silicate is analyzed in several dilutions of filtered Mediterranean surface water, which is low in nutrients. Samples of each salinity value were spiked with added silicate using a calibration standard of 20 μmol/L silicate in deionized water to provide a range of concentration up to 20 μmol / L. For each salinity a regression line is drawn through the analytical values (Fig. 1 a).

If the blank concentration is significant, the regression lines are corrected by parallel shift to the origin. The resulting recovery concentrations for 20 μmol/L (Fig. 1 b) are plotted against relevant salinities. The linear regression function can be converted into a correction function of the form

$$Si_{corrected} = Si_{uncorrected} * (1 + F_s * S)$$

where S is the sample salinity minus standard salinity.

The correction factor F_s (0.025 in the above example) is an experimental factor depending also on water types and analytical parameters and may vary from about 0.02 to 0.05.

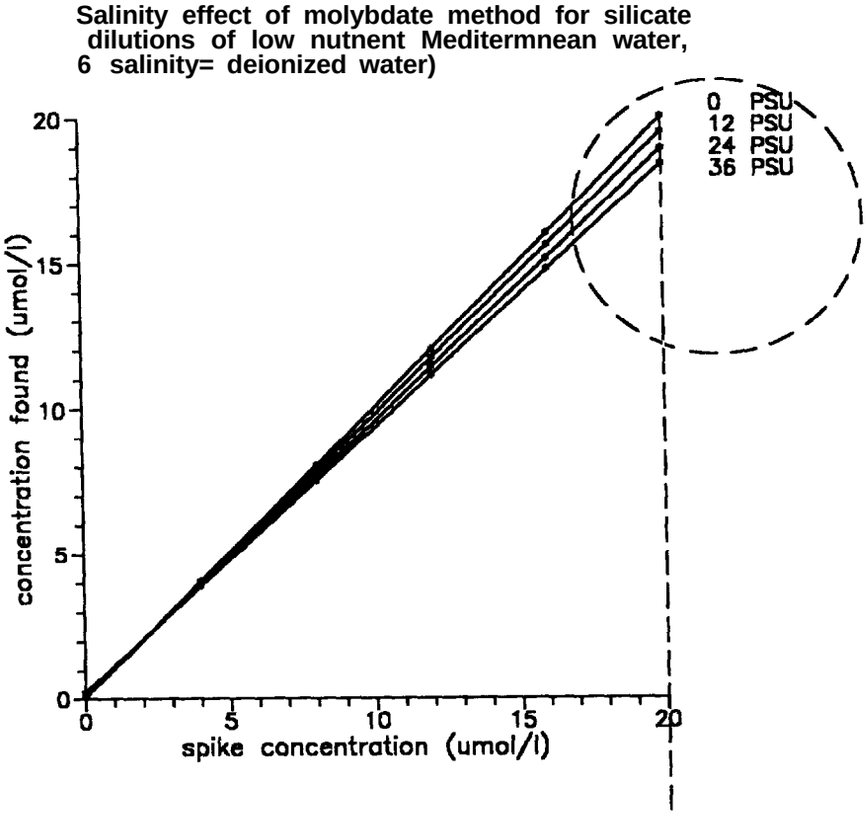
Due to the wide variety of matrices and the different chemical mechanisms involved no general standard or reference matrix can be recommended. For open ocean applications, however, where the salinity variability is only about 1 PSU, a standard matrix based on artificial seawater or pretreated natural Atlantic or Mediterranean water (see above) may simplify and improve the analysis.

Figure 1. Salinity effect on the analysis of silicate using the molybdate method,

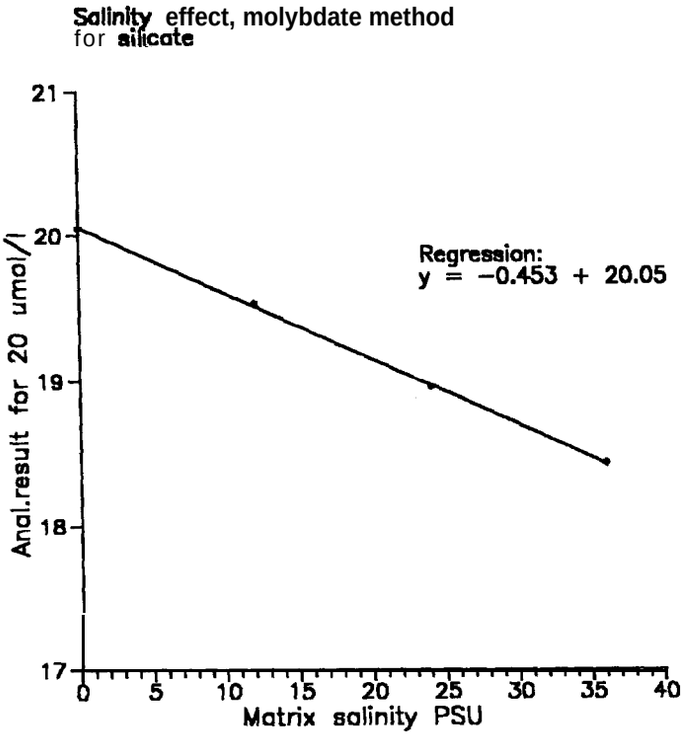
a) spiked waters of different salinities versus standards in deionized water, and

b) regression of the 20 μmol recovery versus salinity.

a



b



For the silicate determination a NaCl-matrix would compensate salinity effects, but this approach does not apply for ammonia determinations. Using manual (individual parameter) analyses, individual standard matrices could be applied. However, in the application of automated multiparameter methods one has to decide upon one common standard. So it will always be the task of the analyst to check the analytical procedures for salinity effects and decide upon the optimal compromise between matrix adjustment or application of correction calculations. Whatever method is chosen, the salinity effects have to be reduced to a maximum error of about 1/10 of the methodical accuracy. As the accuracies of most methods are about 1 %, salinity (matrix) errors have to be reduced to about 0.1 % (absolute accuracies depend on the calibration range).

On the Quality of "Old" Oceanographic Phosphate Data

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Abstract

Temporal trends of substances in the environment can only be recognized if the old and the new data are reliable. For the quantitative chemical analysis of inorganic phosphate in sea water the historical development during the past hundred years is reviewed briefly. A critical examination of the different methods shows that phosphate data can be considered reliable from 1923 onwards, if a number of analytical precautions were observed.

The discussion on temporal trends of plant nutrients in the sea has become of general interest, because scientists have recognized negative effects of hypertrophication in certain coastal areas, e.g. unusual plankton blooms and oxygen deficiencies. As the nutrient inputs from land into the sea have increased considerably during the past decades, it seems natural that the nutrient concentrations in estuaries and coastal areas have increased accordingly, at least in the biologically inactive period of the year, i.e. during winter. In the North Sea, such an increase has been proved for some coastal regions. Nevertheless, there are still some scientists who call these results into question. Their arguments are e.g. the natural variability and the unreliability of old nutrient data. In order to find out which of the old data are reliable and useful for trend analyses and which are not, it is necessary to throw some light upon the history of nutrient analysis. I have chosen the analysis of phosphate (= dissolved inorganic phosphate), because phosphate is one of the most important nutrients and because many oceanographers, even marine chemists, seem to know very little about the history of phosphate analysis.

The first attempt to measure the phosphate concentration of sea water was made more than 100 years ago, e.g. by G. Forchhammer and C. Schmidt. The values reported by Forchhammer lay between 1 and 40 g/m^3, i.e. by a factor of 1000 too high. A little better were the values of Schmidt: 0,5 to 4 g/m^3.

However, considerable progress was made by E. Raben (1916). The various steps of his method were: concentration of sea water (10 l samples) by evaporation, isolation of the phosphate by precipitation as ferric phosphate, dissolution of the precipitate, removal of silicate, precipitation as phosphomolybdate and finally weighing the precipitate. Raben analyzed ca. 120 samples from the North Sea, Baltic Sea, N-Atlantic and the Barents Sea during the years 1904 to 1914. His values were all ca. 25 mg/m^3 P too high. The reason for this systematic and rather constant error was probably the presence of additional MoO_3 in the precipitate and the contamination of the samples by the reagents.

A further improvement was made by D. J. Matthews (1917). He used the nephelometric strychnine-nitro-molybdate method (Poujet and Chouchak, 1911). In this method, the

phosphate in 0,5 1 sea water samples was co-precipitated with ferric hydroxide and the precipitate dissolved in HNO_3. After the addition of strychnine molybdate, the nephelometric analysis was made. In the English Channel off Plymouth, Matthews found 20 to 25 mg/m^3 P in winter (which is very close to reality) and 3 mg/m^3 P during spring.

In the years 1920 and 1921, S. Dénigès published a new method for the determination of phosphate using the development of phosphomolybdenum blue. This method was adapted to the special requirements of sea water analysis by W. R. G. Atkins (1923). Around this time a similar phosphomolybdenum blue method was being used by E. G. Moberg at the Scripps Institution of Oceanography in La Jolla (USA). Although Moberg did not publish his method, details of it were given to H. Wattenberg (Kiel) who subsequently used a modification of this method during the German "Meteor" expedition to the Atlantic Ocean 1925 to 1927 (H. Wattenberg, 1933, 1957).

All the methods which are in current use for the determination of phosphate in sea water are variations of the Dénigès method. They are based on the reaction of the sample with an acidified molybdate reagent, to yield a phosphomolybdate complex, which is then reduced to phosphomolybdenum blue, the light extinction of which is measured.

During the past decades, a number of modifications of the phosphomolybdenum blue method were published. Considerable progress was made by Murphy and Riley (1962) who used ascorbic acid instead of stannous chloride as a reducing agent, and a small amount of trivalent antimony. This method is now in common use in chemical oceanography, because it is superior to all the previous methods, mainly in terms of the ease of analysis.

The step from the manual analysis of single sea water samples to continuous automatic analysis was made by G. Weichart (1963). He obtained the first horizontal profiles of the phosphate concentration in the sea (see also J. P. Riley, 1965, p. 364; H. P. Hansen and K. Graßhoff, 1983, p. 348; G. Weichart, 1970 and 1990). The results of the continuous analysis, which was also based on the phosphomolybdenum blue method, were in full agreement with the data of the single phosphate analyses (G. Weichart, 1963).

Conclusions

Oceanographic phosphate data can be considered reliable from 1923 onward, if

the phosphomolybdenum blue method was used;

the analyses were made immediately after sampling. If the samples were preserved, the effectiveness of the preservation must be known;

the calibration was made in sea water, or the results were (or can be) corrected for the salt error. If stannous chloride was used as a reductant, the salt factor can be up to 1,25 at S = 35, depending on reagent concentrations (K. Buch, 1929; K. Kalle,1934;, H. Wattenberg, 1937; L. H. N. Cooper, 1938). For the reduction with ascorbic acid the salt error is much smaller (ca. 1 % at S = 35, Murphy and Riley, 1962);

the general guidelines of a good analytical laboratory practice (normally taught at universities and at schools for chemical technicians) were followed;

the analyses were performed carefully by a trained analytical technician or scientist.

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Phosphate Analysis in Ship- and Land-based Laboratories, a Case Study

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Certain types of errors may be introduced by automatic determinations. Consequently one must have a reference method validated by intercomparison exercises.

This case study deals with phosphate measurements, all performed with the phosphomolybdenum blue method but with different procedure or apparatus.

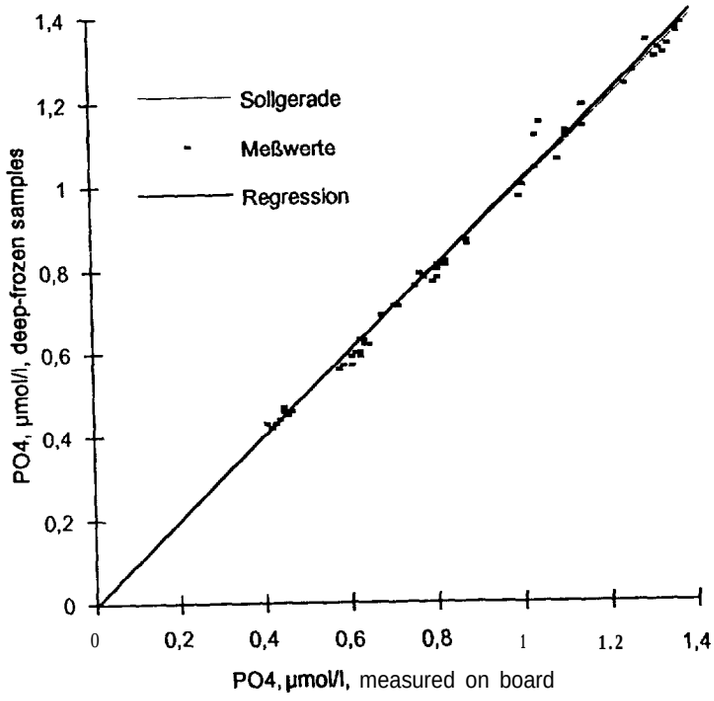
For two projects monthly nutrient measurements in the German Bight were planned. Aims of the projects were to get better knowledge of transport, turnover and variability of pollutants and nutrients and to optimize the North Sea monitoring (BSH, 1993). For several reasons we decided to carry out only sampling and preservation of samples on board, and to analyze them here in our laboratory immediately after the relatively short cruises.

There seemed to be no problem for the storage by deep-freezing of samples collected for silicate and nitrate analysis, also not for samples collected for the analysis of total nitrogen and total phosphorus. But we only had very few experience with the storage of samples collected for the analysis of dissolved inorganic phosphate.

1. Influence of storage

In order to find out if the storage of samples would affect the results we made comparison measurements: From each sample analyzed on board, a subsample was taken immediately after filtration, quickly deep-frozen, and stored at - 20 “C. An example for the results gained one or two weeks later in comparison with the measurements on board is shown in Figure 1: Obviously no problem to store deep-frozen phosphate samples for a short time.

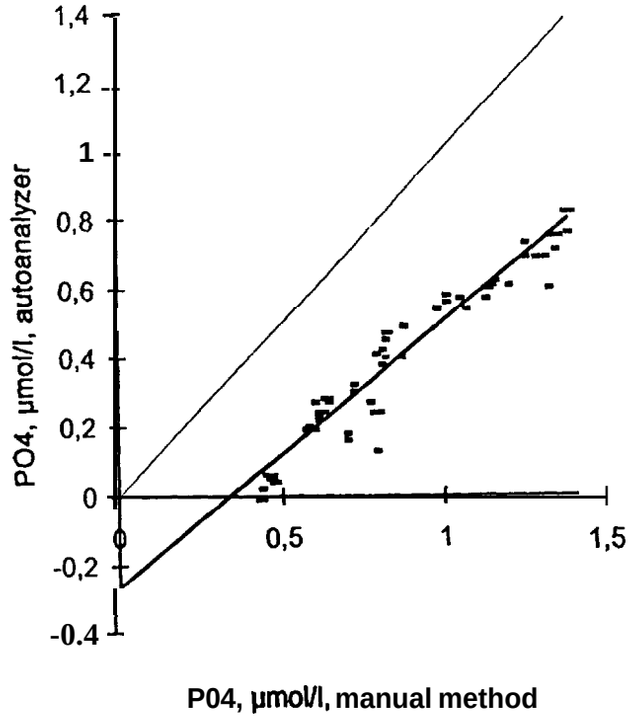
Figure 1. Manual measurements of deep-frozen samples compared to manual measurements on board (January 1992).



2. Influence of procedure

Problems did arise when we started to analyze the samples with an autoanalyser: The concentration values were astonishing low and checks by the manual method according to Murphy and Riley (1962) showed considerable differences (Figure 2).

Figure 2. Autoanalyser results (channel A-B) compared to manual measurements of the same samples (January 1992). Autoanalyser standards prepared in artificial sea water.

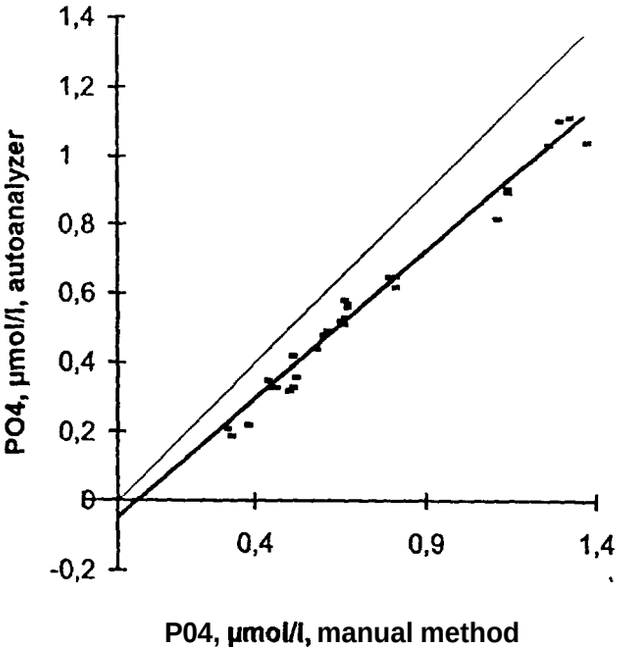


So we continued to make reliable measurements with the manual method directly on board - especially for our trend monitoring during the winter months (Weichart, 1986; Körner and Weichart, 1992). As often as possible subsamples were deep-frozen and analyzed later, in order to find out the reason(s) for the difference between results obtained by the manual and autoanalyser method.

The autoanalyser was operated using artificial seawater (prepared according to Strickland and Parsons, 1968), also the standards were prepared with artificial seawater. Of course we first checked the phosphate content of the artificial seawater by measurements with the manual method. Result: a contamination of 0,05 to maximal 0,08 µmol/l in different charges - too less to explain the discrepancy.

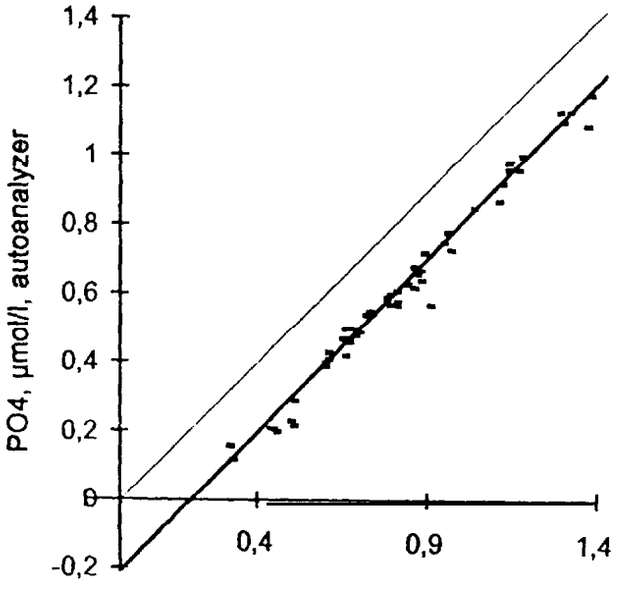
Nevertheless, preparing the standards with natural seawater the differences to the manual method were smaller (Figure 3).

Figure 3. Autoanalyser results (channel A-B) compared to manual measurements of the same samples (March 1992). Autoanalyser standards prepared in natural sea water.



In order to make a correction for turbidity and for refractive effects, in our autoanalyser the absorbance is measured at two wavelengths, at 880 nm (channel A), and at 1010 nm (channel B). The difference of both readings gives the net absorbance, which is converted into concentration by means of a calibration factor. Taking the reading of channel A only, the different slope disappears (Figure 4).

Figure 4. Autoanalyser results (channel A) compared to manual measurements of the same samples (March 1992). Autoanalyser standards prepared in artificial sea water.



A similar result - only parallel deviation of the regression line - was also obtained by using our older one-channel autoanalyser system.

Further experiments are necessary to solve the problems. But whatever the reasons for the different results will be - the more important point is that the autoanalyser gives the illusion of a perfect performance, the calibration line is excellent, and it is difficult to realize that the results contain a systematic error, if there are not done comparison measurements.

Therefore it is essential to have an intralaboratory reference method to rely on. This is not a new finding, but it is good to remember and to realize the necessity. Of course for a short time we also were doubtful about our manual method. But fortunately, this method had been verified by the fourth ICES intercomparison exercise (ICES, 1991) not too long ago, and also recently (in December 1992) by the fifth ICES intercomparison (Figure 5).

Another device for phosphate measurements, the automatic pump photometer (APP) is designed for in-situ recordings over longer periods. The results obtained with this system until now were sometimes good-looking but were far from being realistic (Figure 6: APP recordings compared to mean values of manual photometer measurements of the same samples).

Figure 5. Phosphate results of NUTS I/C5: Frequency of the concentration values of 3 samples, submitted by different laboratories. Our results obtained with the manual method, are marked by arrows.

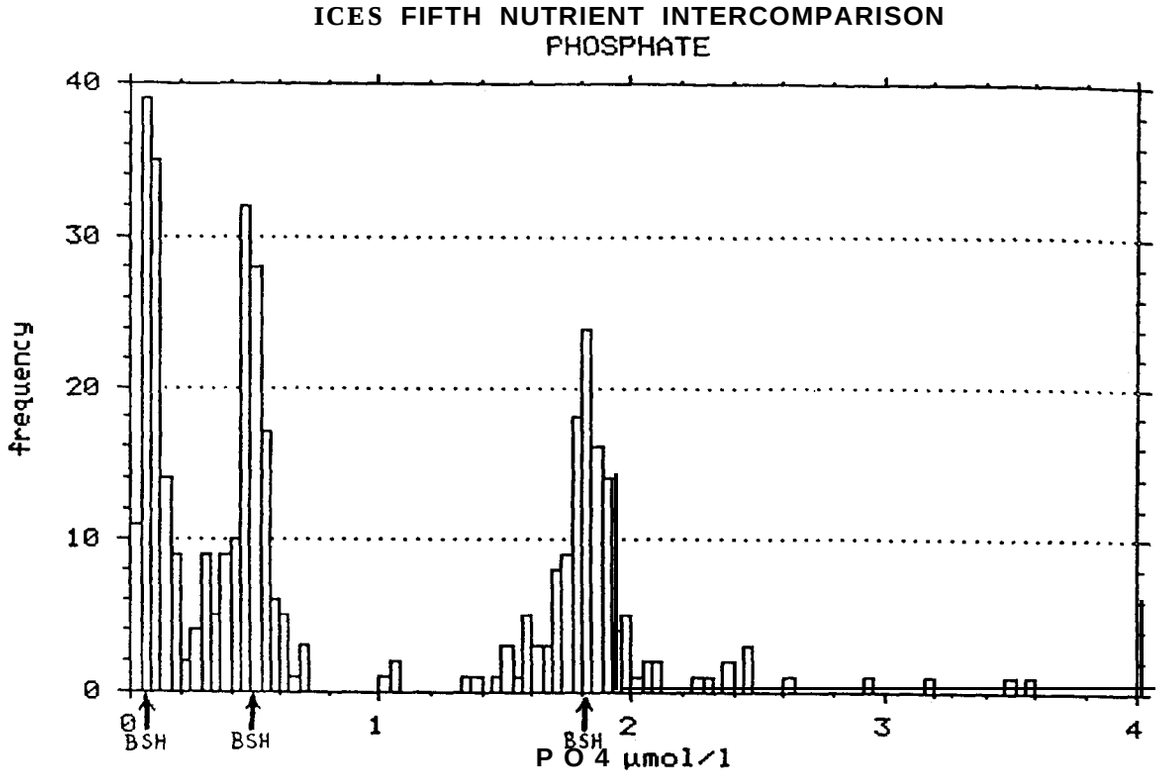
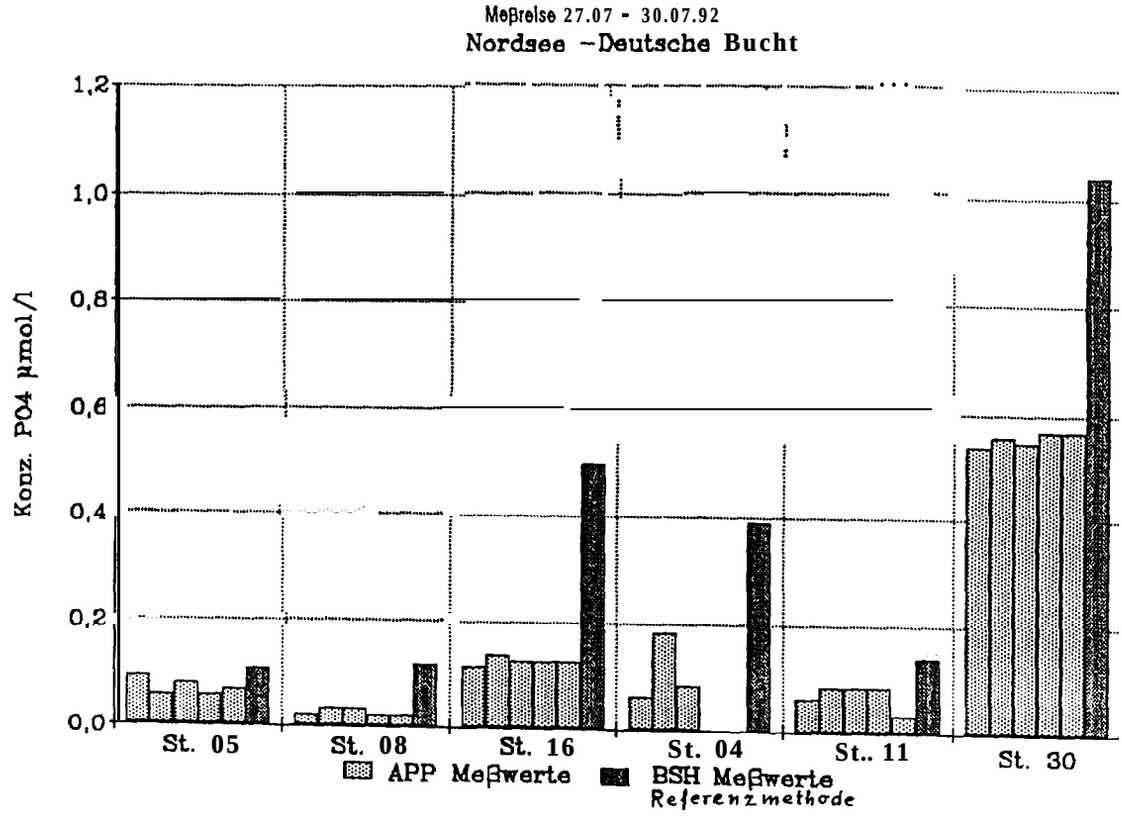


Figure 6. Phosphate results obtained with an Automatic Pump Photometer (APP) compared to mean values of manual photometer measurements (BSH) (July 1992).



Therefore the conclusion of this case study:
It is of vital importance to have an intercalibrated reference method - and each change of procedure or apparatus has to be verified by this method.

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Quality Assurance of Chemical Analytical Procedures for the Determination of Trace Metals in Biota

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The Role of Analytical Chemistry in Research or Monitoring Programmes

Analytical Chemistry has developed a tendency to a scientific discipline with a multidisciplinary character. Remarkable instrumental evolutions have occurred during the last decades with the consequence that far reaching information can be gained in many fields such as material chemistry, environmental research, clinical chemistry, toxicology, biotechnology and microelectronics.

The objective of Analytical Chemistry is to obtain chemical information about materials or systems concerning their specific qualitative and quantitative composition and structure (Danzer, 1992). Analytical methods are the fundamental tools of the analyst. They are based on chemical reactions and electrochemical processes as well as on interactions with all forms of energy, particularly radiation. Most of the techniques of measurement are based on physical principles. However, the connection to chemistry is given through the material aspects of the sample.

Verification of results in quantitative elemental analysis is only possible, if the objectives of the investigations are clearly formulated and the analytical task is well defined.

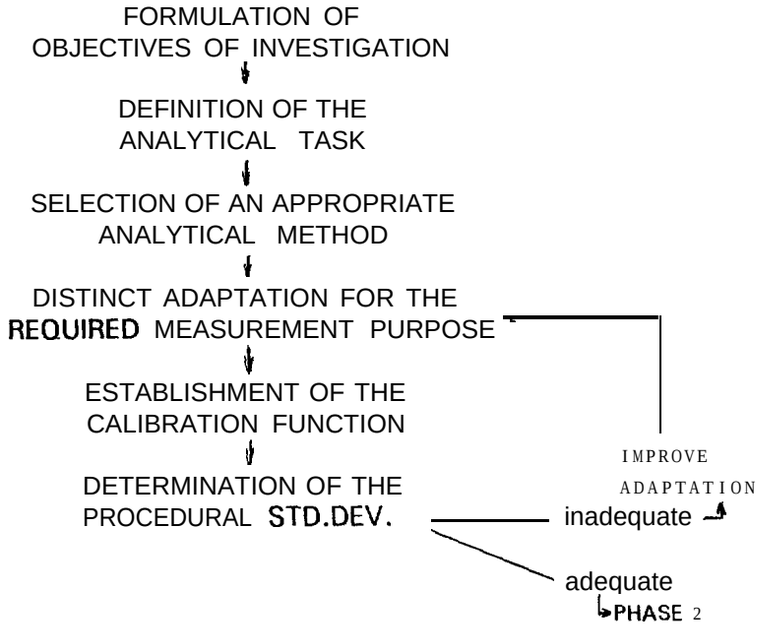
The intended use of the data should be stated explicitly at the beginning of the analytical process. Good data are those that answer a question or provide a basis on which a decision can be made. Kaiser (1970) postulated: The precise and complete formulation of an analytical problem is the first step to its solution. Following these considerations, we can conclude:
The distinct adaptation of an *analytical method* for the selected *analytical tusk* is a prerequisite if the *objectives of the investigations* shall be attained. In each case it is important that these key elements of analytical investigations comply with each other.

Validation of Analytical Methods

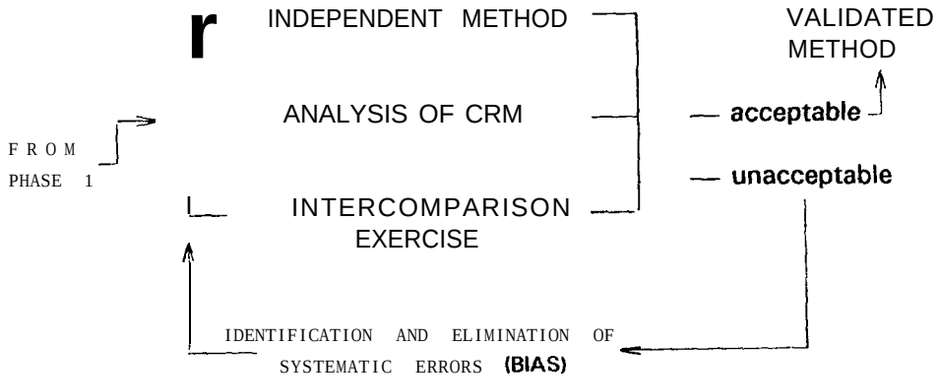
Before an analyst uses an analytical method on a routine basis he must demonstrate that it will actually produce data of the required accuracy to meet the needs of the investigations. The general process of validation of a method is outlined in the following scheme.

PRINCIPLE PROCEDURE FOR VALIDATION OF ANALYTICAL METHODS

PHASE 1 OF THE VALIDATION PROCESS

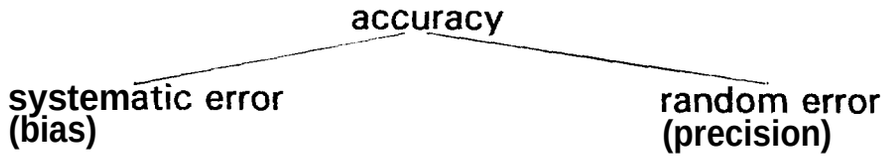


PHASE 2 OF THE VALIDATION PROCESS



Types of Error in Analytical Chemistry

The term “accuracy” is used to describe the difference between the expected or true value and the actual value obtained. Accuracy can be divided into two components:



Random errors make an analytical result uncertain; systematic errors make it wrong.

Because of the random error, results obtained from replicate analyses scatter around the sample's true value. The mostly unknown content of a sample lies within the variation of the results.

A systematic error, on the other hand, shifts the measured values in the one or other direction.

Random errors and systematic errors therefore have a totally different influence on an analytical result. Nevertheless, there are certain *interdependencies between both types* of error: Random errors must be well established, before systematic errors can be identified and adequately eliminated (Doerffel, 1984).

The Need for Calibration in Quantitative Elemental Analysis

In most cases an analytical procedure does not directly provide data but measured values of "indicating quantities" (weight, volume, meter-reading, absorbance etc). These "measures" must be *evaluated* and ***interpreted*** to get the final analytical data. The relation of the measure to the concentration being sought is called the ***calibration function***. Such a function always stands between the measure and the concentration; it *is an important part of the analytical procedure* (Kaiser, 1970).

Random Errors and Linear Calibration Function

Random errors of the analytical results in calibration dependent procedures originate from uncertainties in the whole analytical process. Such uncertainties are also reflected by the random variability of the calibration function. Therefore, intralaboratory quality assurance must start with quantifying, examining and if necessary optimizing the *precision of the calibration function.* A straight line or linear function is generally preferred. This is justified, because then the work required for calculation is rather easy. The calibration procedure must meet some basic criteria or assumptions in order to give a best estimate of the true (but unknown) element content of the sample analyzed:

1) The masses or concentrations of standards for the establishment of the calibration line must be prepared without bias.
2) The chemical and physical properties of the calibration standards must closely resemble those of the sample under investigation.
3) Sample and calibration standards must be subject to the same operational steps of the analytical procedure.
4) Signals of repeatedly analyzed calibration standards must scatter only with homogeneous variance in the linear part of the calibration line.

Precision of the calibration line, in terms of reproducibility, should be estimated from a series of replicate standard analyses. Information obtained is the *standard error of estimation* s,. The value of s_y can be useful in identifying the sources of errors if the random variability of the total analytical procedure does not meet the analyst's requirements.

The quotient of standard error of estimation and slope of the calibration function gives the *procedural standard deviation* which may serve as performance characteristic by which the quality of the analytical method can be controlled and, if necessary, improved according to the objectives of the analysis.

Identification of Systematic Errors

According to Taylor (1981), systematic errors or biases are of two kinds: concentration-level independent (constant systematic or additive systematic errors), and concentration-level related (proportional or multiplicative systematic errors).

When assessing an analytical procedure, it is important to recognize the type and extent of the systematic errors as this knowledge allows the analyst to draw conclusions about their sources and correction for them to the extent possible.

Independent Analytical Procedure

The analyst can test for systematic errors in the analytical procedure under investigation by using a second, independent analytical procedure (Stoeppler et al., 1979). A t-test can be done to check for differences on condition that the reproducibility of both methods being used are comparable.
A significant difference between the results obtained by both procedures indicates that one of them contains a systematic error. Without further information, however, it is not possible to say which one.

Use of Certified Reference Material

In order to detect simultaneously constant and proportional systematic errors, samples of known composition should be analyzed. Several "certified reference materials" are currently available. An analytical procedure is sufficiently accurate if it produces results for a certified reference material that do not differ from the certified value more than can be accounted for by within-laboratory statistical fluctuations.

In practice, when performing, such tests, one should ensure that the material to be analyzed and the certified reference material have a similar macrocomposition (a similar matrix). However, in reality it is difficult to meet this requirement as it is only fulfilled in the strictest sense in a few cases.

Intercomparison Exercise

In an intercomparison exercise the bias of the participating laboratory's analytical procedure is estimated with respect to the assigned value for the concentration of the element in the sample which is distributed to participants. The assigned value is an estimate of the true value and is predetermined by some "expert" laboratories. In some

instances the assigned value is a consensus value established by the coordinator after critical evaluation of results returned by the participants. The bias is equal to the difference between the determinand concentration reported by the participant and the determinand concentration assigned by the coordinator.

If a target standard deviations representing the maximum allowed variation consistent with valid data can be estimated, the quotient $z = (x-X)/s$ is a valuable tool for appropriate data interpretation. If z exceeds the value of 2 there is only a 5 percent probability that the participating laboratory can produce accurate data (Berman, 1992).

Elimination of Systematic Errors

The aim of sample pretreatment in an analytical procedure is to obtain reaction products whose type and amount are appropriate for subsequent measurement of their element contents by instrumental techniques. Usually, sample pretreatment requires as a first step a *decomposition* and *dissolution* of the material under study.

Because chemical reactions are balanced reactions, the analytical chemist tries to ensure that the equilibrium is shifted as much as possible in favour of the desired reaction products.

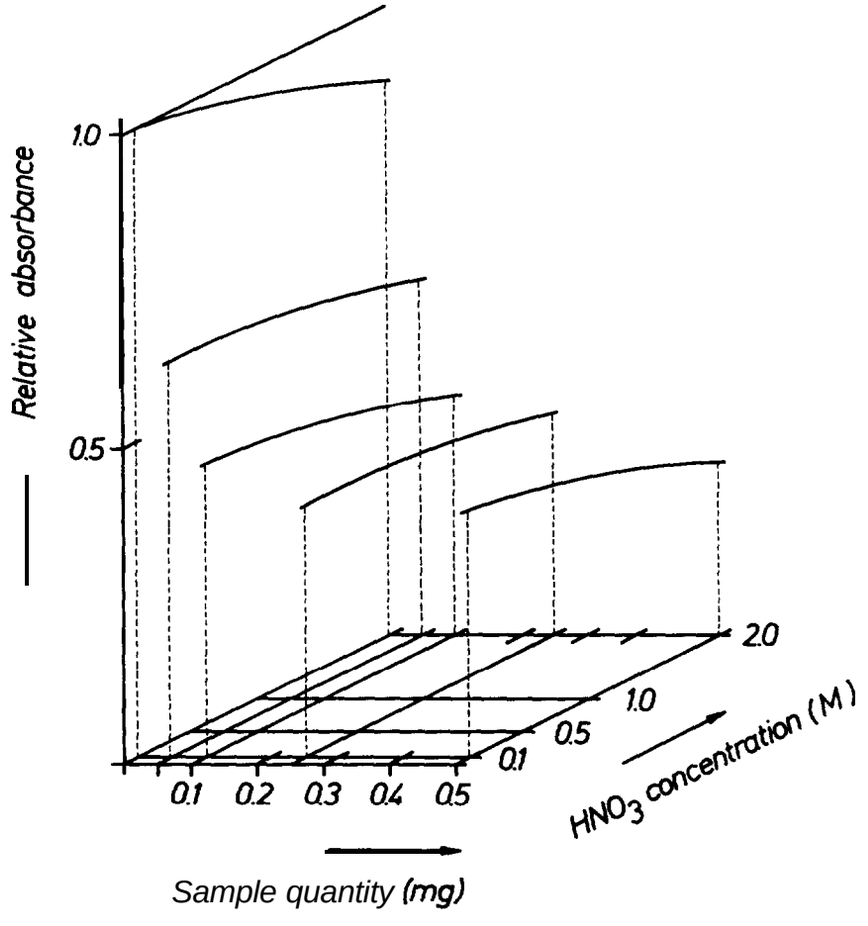
During chemical reactions, however, unwanted secondary reactions, so-called "matrix effects" can cause uncontrollable multiple systematic errors. They are mostly due to complicated reactions of the determinand with sample components and reveal themselves in the form of suppression or distortions of the determinand signal. In the case of atomic absorption spectrometry with a graphite furnace, for instance, matrix effects take the form of gas-phase interferences during the atomization process (non-spectral interferences). This is illustrated in Figure I where the influence of a biological matrix on the atomization process of lead is clearly seen.

Multiple influences of the above types can be compensated by using the *method of standard addition.* The results from the standard addition method however will only be correct, if the linearity of the standard addition calibration function is sufficiently proven and if some assumptions are met (Rechenberg 1991). These include:

the masses of the added standards must be without bias,
measurements in the linear part of the standard addition calibration line must scatter only with homogeneous variance,
the added standard and the analyte in the sample must behave identically during the whole analytical process.

It is often difficult to fulfil the last-mentioned condition which generally requires that the standard and the determinand have the same chemical structure.

Figure 1. Matrix effects on lead response, observed in graphite furnace atomic absorption spectrometry (GFAAS).
Signal suppression in dependence of HNO, concentration of sample solution and absolute sample quantity injected.

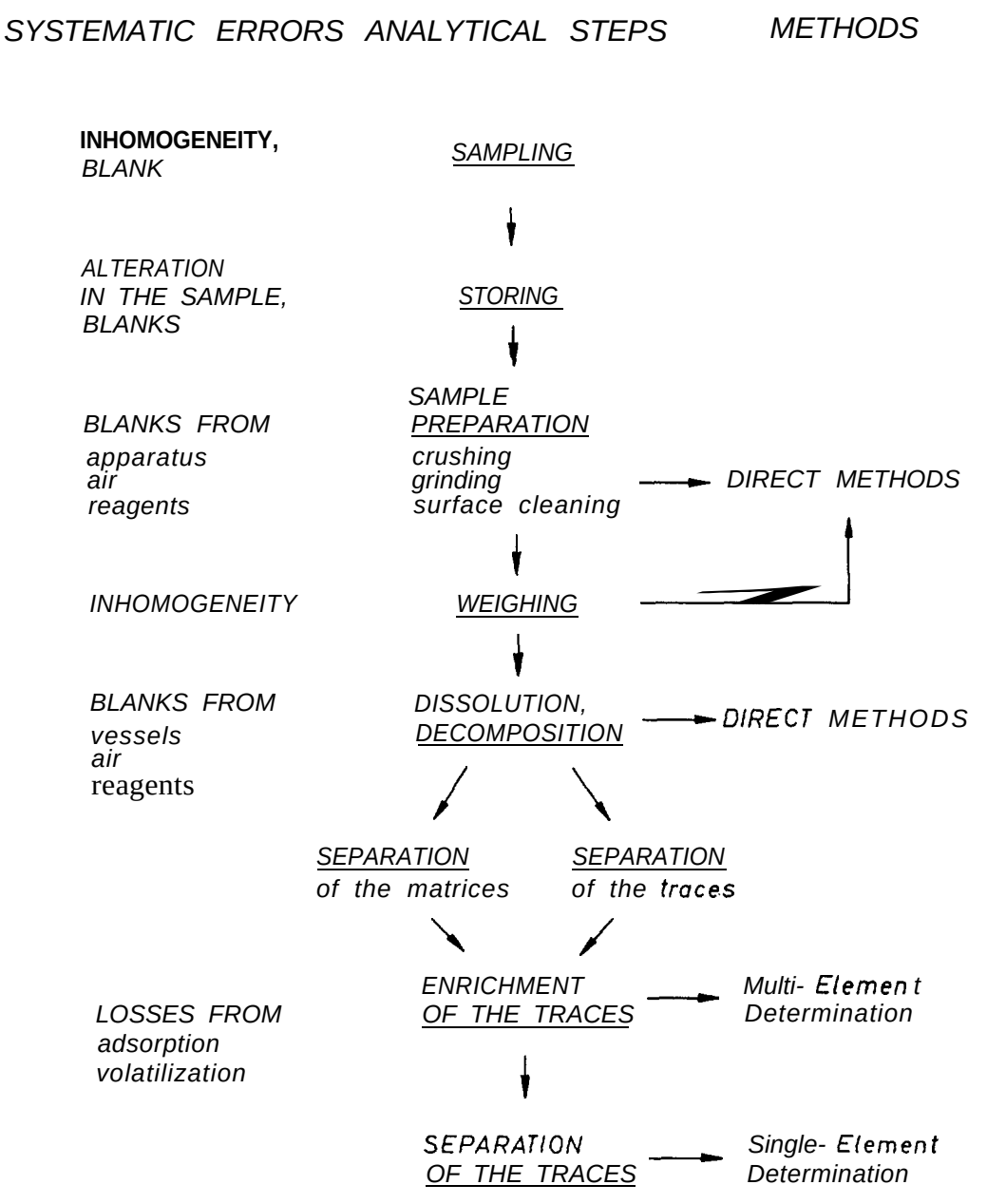


In contrast to the methods of standard addition *methods of determination which incorporate chemical separation techniques* are much easier to calibrate. With this approach, simple standards with a minimum of matrix matching are required to interpolate the analysis of unknown samples from conventional analytical calibration functions.

The disadvantage of chemical separation procedures that consist of several stages, however, is that they can produce systematic errors. Disturbances, which result in constant systematic errors, are a) analytical contamination; b) boundary-surface interactions (ie adsorption effects) of the very small absolute quantities to be determined which can be important particularly when very diluted analytical solutions are being handled, and which result in uncontrollable losses (Tölg 1972, 1974, 1977, 1979; Zief and Mitchell, 1976; Stoeppler et al., 1979; Tschöpel et al., 1980; Harms et al., 1982; Moody, 1982; Veillon, 1986).

Tölg's scheme for trace analytical procedure as reproduced here shows that in a multi-stage analytical procedure the advantage of easier calibration is reduced by an *increase* in possible systematic errors.

SCHEME FOR TRACE ANALYTICAL PROCEDURE"



"From Tölg (1974)

In the 1970s, Tölg, aware of these fundamental difficulties, made proposals on how to optimize multi-stage analytical procedures.
In his "Guidelines" Tölg (1979) showed that systematic errors of multi-stage analytical procedures are minimized, if

1) all analytical steps are carried out in dust-controlled rooms (clean benches), to eliminate contamination through air,

2) all vessels used consist of materials such as PTFE or quartz to exclude both adsorption losses and/or contamination by desorption of impurities,

3) a favourable ratio of sample amount to surface area, and a small excess of thoroughly purified reagents, are used.

For these reasons, precautions have to be maintained through the analysis which include the thorough cleaning of labware and purification of reagents prior to use. Sample container and reaction vessels for sample decomposition represent potentially one of the largest sources of systematic errors, since impurities leached from the materials for construction may lead to uncontrollable sample contamination. Therefore, much of the analytical reliability will depend upon the choice of appropriate container materials and reaction vessels and the method of cleaning their surfaces (Boutron, 1990, Kosta, 1982, Mitchell, 1982, Moody et al., 1982).

Conclusion

Within the framework of decision processes considerable importance is attached to analytical information. This is also the case for decisions related to environmental protection, where growing demand for data has been realized. However, increasingly, analysts find themselves in areas of conflict with the customers of their data, which result from the presentation of unreliable data. To improve this situation, the implementation and mandatory application of a sound **Quality Assurance** system are considered indispensable.

When providing analytical data, we must recognize that reliability of measurements and the cost of producing data are decisive factors which are closely linked. Savings made at the expense of the reliability of results can often lead to wasted time and effort if they produce unacceptable results. This is why an optimization of both of these criteria is necessary if analytical work is to be efficient. The costs of routine **Quality Assurance** (including the costs of certified reference materials) can be as much as 15 % of the costs of total analytical performance in a laboratory. However, the benefit of implementing a sound **Quality Assurance** system are substantial:

Participating laboratories will be able to provide reliable data and thus contribute successfully to an international monitoring programme, such as the BMP.

Traceability of the measurement process is achieved, i.e. the analyst is in the position to trace objectively an analytical problem back to its source.

Within the framework of such a system, laboratories can improve and demonstrate their technical qualification and competence for conducting specific analyses or types of analyses in specified fields of investigations.

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Biota Sampling with Respect to the Baltic Monitoring Programme

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Introduction

Quality of monitoring data does not depend solely on the quality of the final analytical measurement. All parts of the complete procedure influence the quality of the final result, for instance the sampling strategy, sampling locations and frequencies, season, investigated organisms, normalisation and evaluation procedures. To assess differences between natural variability and anthropogenic influences, variances in the measurement have to be reduced on every stage of the overall operation, particularly considering the trace concentration levels existing in the marine environment. This demands great efforts to avoid incorrect (analytical or unsuitable) data and thus misinterpretations. To fulfil the defined objectives of a monitoring programme, the achieved data should be checked regularly and programmes should be revised if required.

Since it is not possible to review all monitoring activities and existing programmes, this paper will be restricted to an examination of the Guidelines of the Baltic Monitoring Programme (BMP, 1988), paying particular attention to sampling of biota, and some aspects of data evaluation and statistical treatment.

Aim and definitions

The aim of the Baltic Monitoring Programme, as it is defined in the BMP Guidelines, is to follow the *long-term* (annual and long period) change (trends) of selected determinands in the Baltic ecosystem. The resulting Monitoring data form part of the background information for an appropriate *assessment of the state* of the marine environment and the *forecast* of possible man-induced changes. However, in order to assess such man-induced changes, monitoring of the natural changes of different elements of the ecosystem must also be carried out. In its most restricted sense, the term *Monitoring* is also applied to the regular measurement of contaminant levels in relation to set standards, or in order to judge the effectiveness of a system of regulation and control. The latter point is of particular interest to politicians and managers. Monitoring, by the BMP definition, does not encompass experimental laboratory studies and scientific investigations. However, if monitoring is to be effective, additional research is required to develop methods and to investigate the underlying processes of the influences of the co-variables. However, it is very difficult to define an exact boundary between monitoring and research. Since it is frequently influenced by non-scientific reasons, it should be stressed that accompanying research is essential and should be an established part of any monitoring programme.

Conditions of the monitoring programme

The monitoring programme is based on measurements of *obligatory* (mandatory) and *tentative* determinands. The latter include substances for which suitable intercalibration among laboratories must be carried out successfully before they can be included as obligatory, and others which still require considerable effort with regard to both development of methods and intercalibration.

Species and sampling procedure

The species chosen as test organisms and the sampling procedures recommended for monitoring harmful substances in biota are intended to provide a picture of the levels of harmful substances in the studied organisms, and to determine trends in their levels over time.

Sampling strategy

The BMP is based on a relatively small number of strategic stations in different parts of the Baltic Sea with sufficient determinands to provide a reasonable overall picture of the ecological changes in the sea. The actual number of HELCOM BMP positions is 45, the number of areas is 14.

Sampling frequency and de terminands

The sampling frequencies are listed in Table 1, the investigated species and harmful substances to be determined in biota are listed in Table 2:

Table 1. Sampling frequencies

Subject	Area	Frequency	Remarks
Harmful substances in biota		1 /year	
Pelagic biology	Baltic Proper	12/year	summer
	other areas	6/year	
	representative stations	minimum 6/year	

Table 2. Harmful substances investigated in biota

Species (obligatory)	Contaminants	
	obligatory	**tentative**
Herring (Clupea harengus)	PCBs (IUPAC Nos. 28, 52, 101, 118, 138, 153, 180)	Chlordane
Cod (Gadus morhua)	HCB, alpha-HCH, gamma-HCH	Dieldrin
	pp'- DDT, pp'- DDE, pp'- DDD	
	Hg, Cd, Pb	Zn, Cu

Sampling for the BMP

Trends of environmental contaminants have to be studied in *defined areas* (same place every year). However, sampling in several areas of a marine region at the same time will provide a general assessment of contamination in any one year.

The *species* and the part of its population selected as study material for trend monitoring programme have to be representative for the defined study area. HELCOM has selected herring and cod as study organisms in the BMP, because: (a) they can be caught in all parts of the Baltic (important for comparison), (b) they are easy to collect, (c) they are suitable size for pre-analytical sample treatment, (d) their biology is fairly known, and (e) they are an important commercial species.

However, they are migratory so that sampling must pay due attention to periods of life, at which the species are representing the area of investigation.

To reduce the variability and the uncertainties when interpreting the measured values, the most available and stable conditions should be chosen. The part of the species to be analyzed, the sampling period, age, size and sex of species, and the sampling size are summarized in Table 3. Additionally, total body weight and degree of maturity has to be recorded.

The period in late summer selected for herring and cod is relatively stable for the organisms in terms of migration and physiology of these species.

The prespawning age classes of herring seems to be a local or regional species at young age. In all probability, samples of younger fish originate from the same stock and are thus representative of the local environment.

Table 3. Specification of sampling conditions

Species/ analysed part*	Time	Age	Size	Sex	Sample size
Herring liver / muscle tissue*	Aug.- Sept.	1 + ,2 + ,3 +	not specified	female	20 fish
Cod liver / muscle tissue*	Aug.- Sept.	Length	stratified	female	2.5 fish

*depending on the contaminand to be determined

For length stratification of cod samples, the range is divided into five classes with at least 2-3 cm length interval. The lower boundary is given by the minimum weight needed for analytical purposes, the upper boundary by the minimum number of individuals, that can be readily obtained. It is recommended that the samples should not be restricted strictly to these five classes. The reason is, that in case of a significant shifting of the length distribution, the year to year comparison of fixed length intervals would cover different parts of the population. Thus in order to obtain a correlation between length and contaminant levels, the length ranges have to be selected each year.

So, for the data the correlation between contaminant level and the co-variable “length” has to be controlled from year to year.

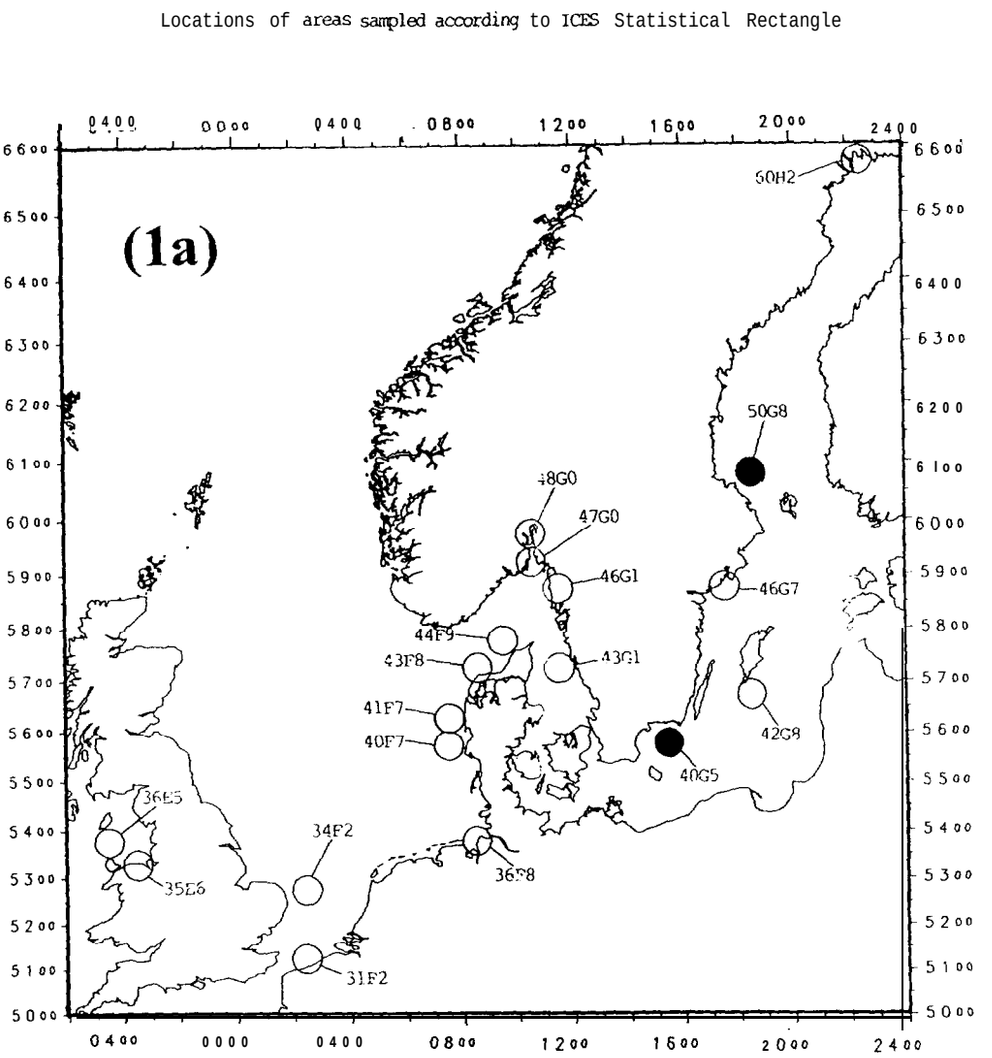
Evaluation of data

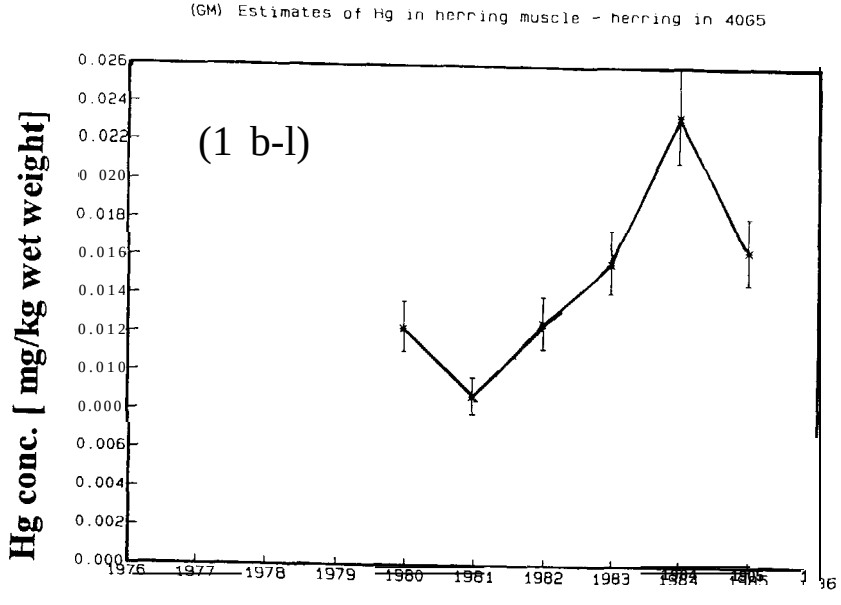
Evaluation of monitoring data needs statistical tools, for example to distinguish between time trends or randomly varying situations. Statistical analysis of contaminant levels must be viewed in the general context of environmental and biological information. Thus, simple correlation calculations are unsuitable. Some of the parameters which the result of the statistical analysis of a certain data set depends on, are variability, number and form of distribution of data , or the observation time for trend analysis. In the following, some short examples of the evaluation of field data are presented linked to statistical procedures and parameters.

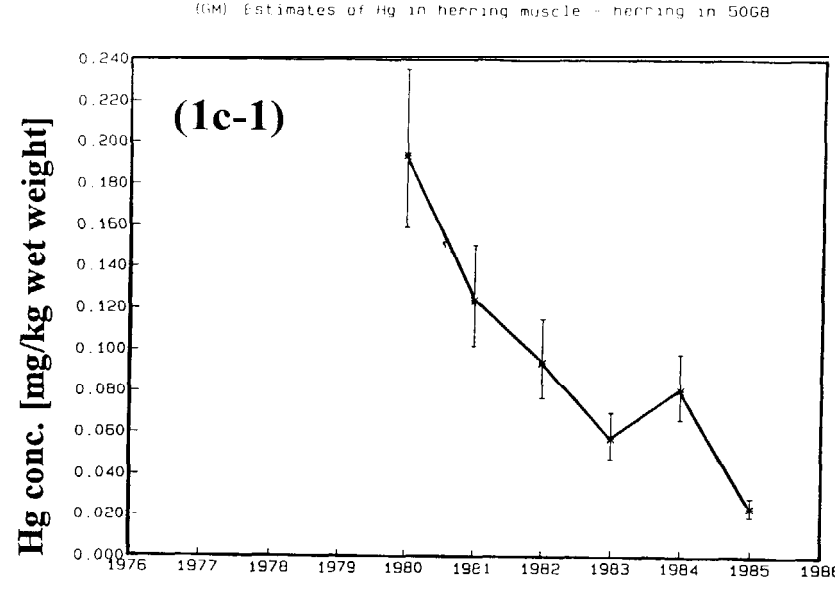
The intention is to demonstrate the need of adequate statistical procedures for evaluation and interpretation of environmental data. Some information to existing approaches are given in the text.

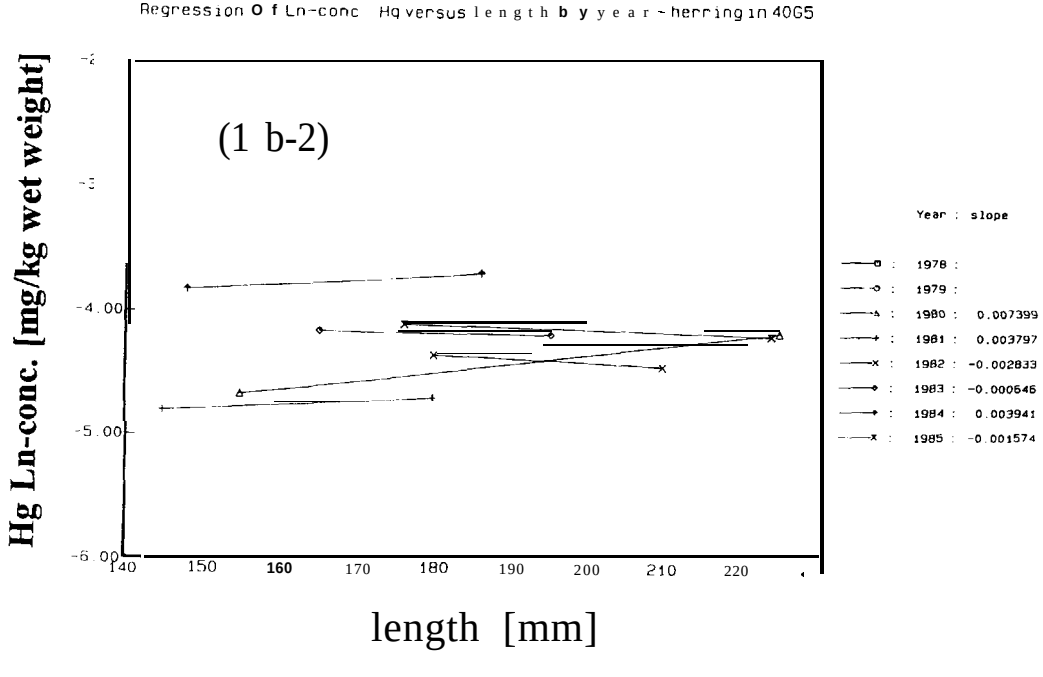
In the first example from the ICES Cooperative Monitoring Programme (ICES, 1989), the development of mercury concentrations in herring tissue for two separate areas in the Baltic Sea (Fig. la), obviously shows temporal upward and downward trends (Fig. lb-l and lc-1). The significance of these trends has to be checked by special statistical tests due to the small number of observations, in this case using the number of mean values.

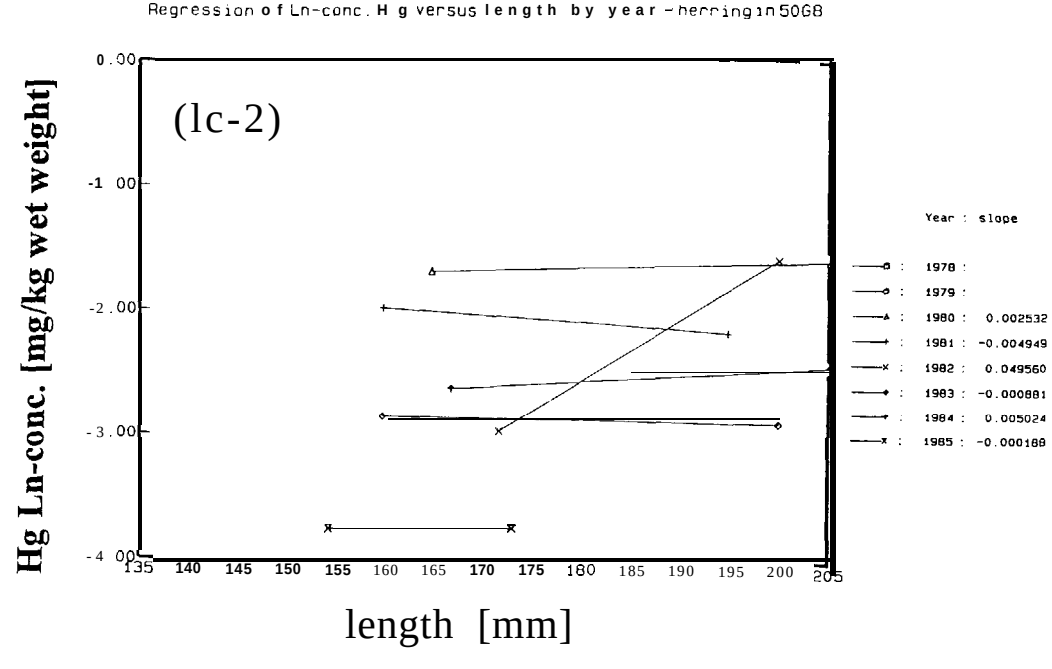
Figure 1. Example from ICES Cooperative Monitoring Programme 1978-85 (ICES, 1989): Location of areas sampled (la); sample location 40G5: Estimates of Hg in herring muscle (lb-l), regression of Hg Ln-concentrations versus length by year (lb-2); sample location 50G8: Estimates of Hg in herring muscle (lc-1), regression of Hg Ln-concentrations versus length by year (lc-2).




(GM) Estimates of Hg in herring muscle - herring in 4065
(1 b-l)
Hg conc. [mg/kg wet weight]
sampling year


(GM) Estimates of Hg in herring muscle - herring in 5068
(1c-1)
Hg conc. [mg/kg wet weight]
sampling year


Regression Of Ln-conc Hg versus length by year - herring in 4065
(1 b-2)
Hg Ln-conc. [mg/kg wet weight]
length [mm]
Year : slope
1978 :
1979 :
1980 : 0.007399
1981 : 0.003797
1982 : -0.002833
1983 : -0.000646
1984 : 0.003941
1985 : -0.001574


Regression of Ln-conc. Hg versus length by year - herring in 5068
(lc-2)
Hg Ln-conc. [mg/kg wet weight]
length [mm]
Year : slope
1978 :
1979 :
1980 : 0.002532
1981 : -0.004949
1982 : 0.049560
1983 : -0.000881
1984 : 0.005024
1985 : -0.000188

The principles for the statistical six-stage procedure applied by the ICES cooperative monitoring programme, in which sampling conditions are identical with those of the BMP, presuppose that concentrations are correlated to one or more variables, (every serious correlation requires a traceable explanation of the possible relation between the variables), and that concentrations are adjusted for the effect of co-variables, e.g. length stratification. The data are tested against six models, which are compared using the principle of reduction and the residual sum of squares. The data are tested for different variables (length, sex etc.) and the procedure will supply information about linearity, error structure, irregularities of data and outlying values.

Another aspect which has to be emphasised is the form of distribution of data from one area and one year. This is illustrated by an example of Cd and Hg data for cod and herring, the results of which are given in Figures 2 to 7.

The data from the bight of Kiel from 1989 are evaluated by the aid of a non-parametric procedure. The notched box- and whisker-plots of the achieved median value indicate that the data are not normally distributed. The medians for cadmium in the liver of herring and cod are significantly different, as the confidence intervals do not overlap (Fig.2.). When adjusted to the length of the individuals, an effect for cod but not for herring is recognised (Fig.3). So the covariable "length" influences the variability of cadmium concentrations only in cod liver. This supports the recommendation of the BMP of length stratification only for cod. For mercury in tissue, the variability is higher. Without length adjustment, the medians are identical for both species (Fig. 4), but after length adjustment, the medians become significantly different (Fig.5). This should be supported by a good correlation between length and concentration value. In this case, the correlation is not existent for cadmium (Fig.6) and rather poor for mercury in cod (Fig.7). Thus, the indicated improvement for the cadmium values by length adjustment seems to be only randomly.

Generally, the number of observations influences the band width of confidence limits or standard deviations in non parametric as well as parametric procedures. Therefore, it is necessary to differentiate between the number of individual data, from which a mean or median value with its standard deviation or confidence interval, respectively, is calculated and which is representative for a special area and a certain year, and the number of means for the annual comparison.

For comparison of different areas, it is not always necessary to have identical numbers of observations, if one is using non-parametric tests. One example is the calculation of the required numbers of samples for a significant distinction of the medians of mercury concentrations in seawater in the German Bight and the central North Sea. On the basis of the variability measured in summer 1986 (summer cruise of the ZISCH project), twenty samples from the central North Sea, but eighty samples from the German Bight are required for a confidence level of 95 %, due to the high variability of data from the German Bight.

Figure 2. Cadmium concentrations (referred to wet weight) in liver of cod (code "c") and herring (code "h") of fish from the Kiel Bight in autumn 1989. Data from Uwe Harms, BFA für Fischerei Hamburg, Germany.

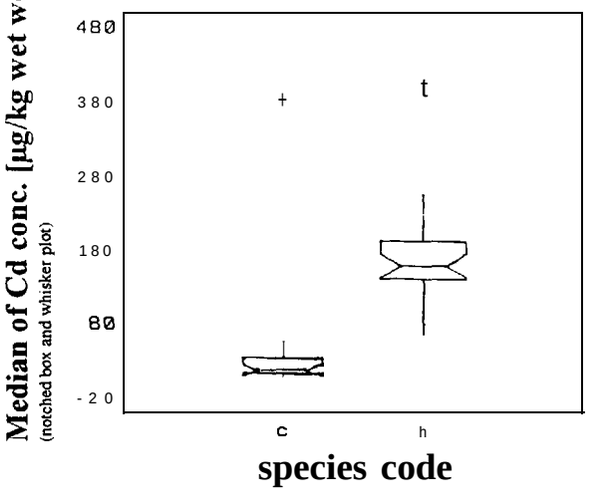


Figure 3. Cadmium concentrations (referred to wet weight) in liver of cod (code "c") and herring (code "h") of fish from the Kiel Bight in autumn 1989, adjusted to length of individuals. Data from Uwe Harms, BFA fir Fischerei Hamburg, Germany.

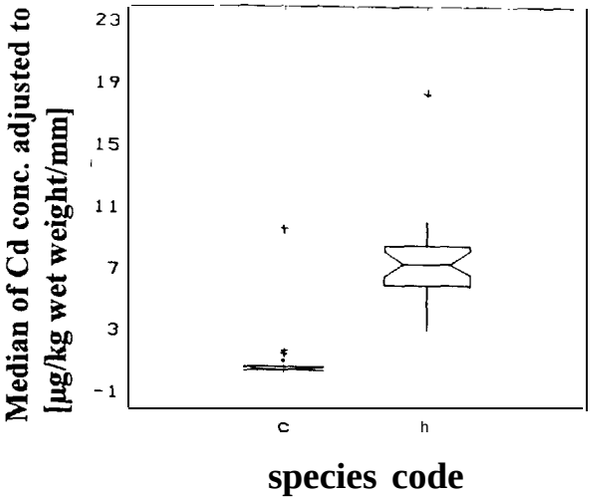


Figure 4. Mercury concentrations (referred to wet weight) in liver of cod (code "c") and herring (code "h") of fish from the Kiel Bight in

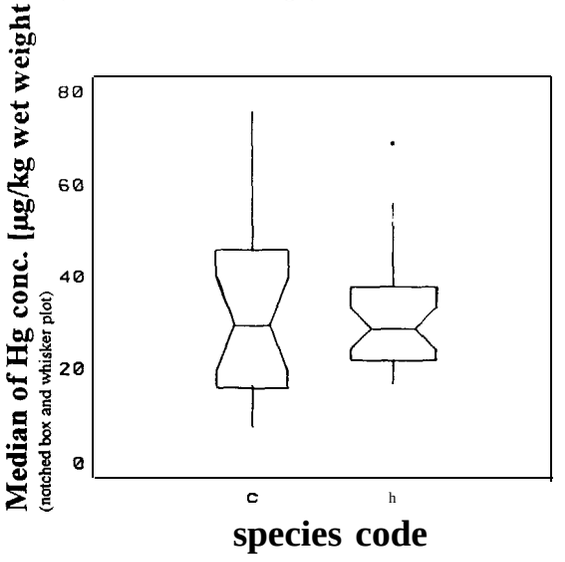


Figure 5. Mercury concentrations (referred to wet weight) in liver of cod (code "c") and herring (code "h") of fish from the Kiel

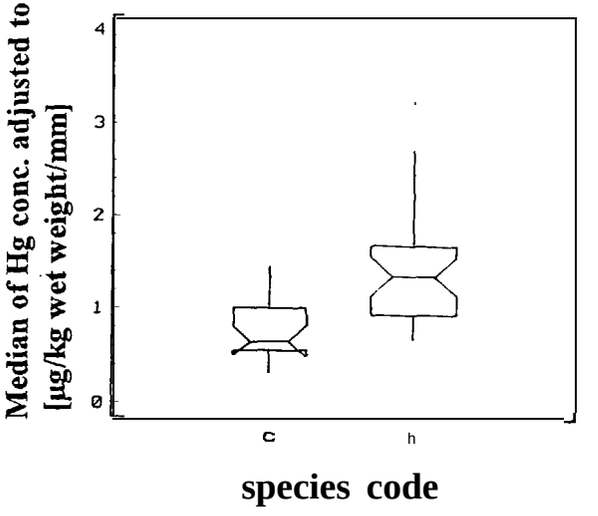


Figure 6. Regression of cadmium concentrations in cod liver versus length of individuals. Samples from the Kiel Bight in autumn 1989. Data from Uwe Harms, BFA fir Fischerei Hamburg, Germany.

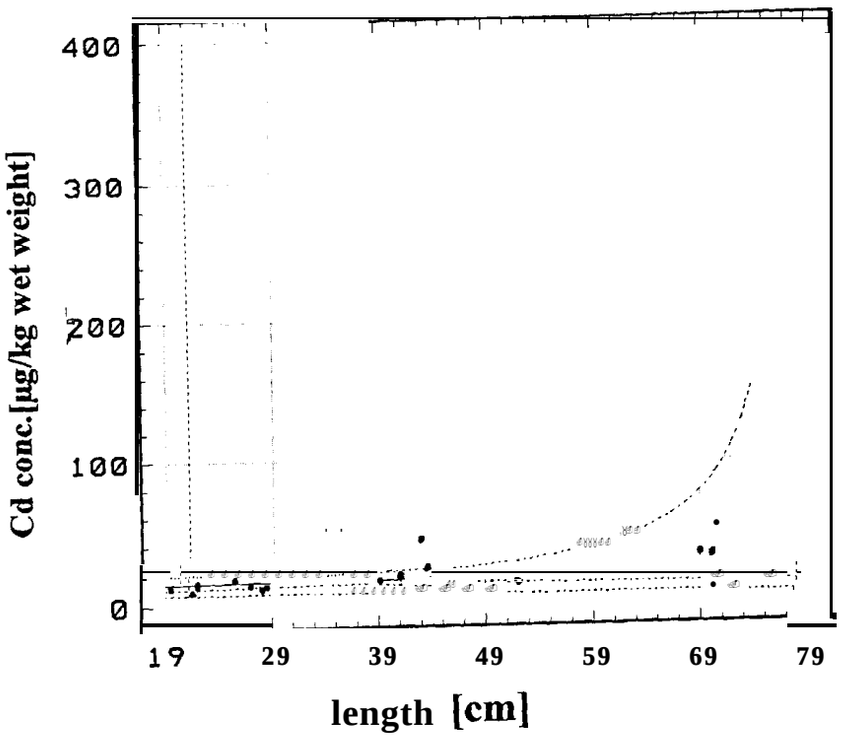
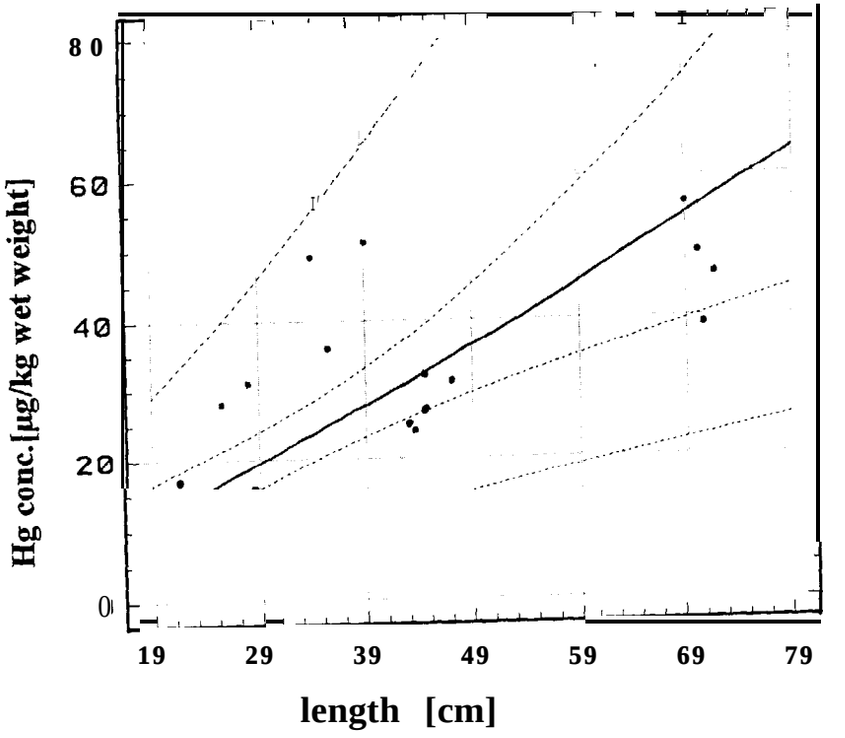


Figure 7. Regression of mercury concentrations in cod liver versus length of individuals. Samples from the Kiel Bight in autumn 1989. Data from Uwe Harms, BFA fiir Fischerei Hamburg, Germany.



There exists some non-parametric tests that are suitable for trend analysis, for example the trend test by *Wilcoxon* or the iteration test by *Wallis and Moore*. (in: Bohle-Carbonell and Huber, 1992). These tests provide information about significant differences between the first and the second part of a data records of time series for a chosen confidence level. However, the precondition is that the number of compared values are twelve or more. Additional tests are recommended, if the data record is small, i.e. less than 25 pairs of values.

Statistical procedures applicable to trend analysis have been the subject of investigations published in the recent years. One question is how to optimise the monitoring under the given restrictions, particularly with the budget allocated to the work. "How many samples are necessary to detect important trends", is the subject of the contribution of R. Fryer presented at the fourth meeting of the Environmental Committee of the Baltic Marine Environmental Protection Commission (Fryer, 1993). In this paper, Fryer emphasises the need to establish different programmes for specific objectives, i.e to discover time trends or to assess the local distribution. The number of samples mainly depends on the measured or estimated variability of contaminant data and the required statistical power. A description of appropriate statistical tests for detecting linear trends and incidents is published by Fryer and Nicholson (1993). The authors describe tests which were used to examine time series of contaminant levels in fish muscle collected in the ICES Cooperative Monitoring Programme (ICES, 1989). One conclusion from this paper is that for most of the heavy metals in cod the time series are too short to detect trends of less than 10% per year. This does not only apply to the Baltic and the North Sea, but to contaminants in tissues of Atlantic cod also (Uthe et al., 1991 b).

One problem of statistical approaches to evaluate trends in the marine environment is to consider the complex interdependencies of contaminant levels, for example the co-variables. The step from procedures using conventional univariate to multivariate analysis of covariance (ANCOVA and MANCOVA) has been recently described for Canadian cod contaminant data (Misra et al., 1993).

Finally, two papers have to be mentioned which stress the importance of biological variation and an adequate biological sampling in ecotoxicological investigations (Bignert et al., 1993a, 1993b). The problems dealt with are once again the necessary sampling periods and frequencies to detect temporal trends or disclose spatial variation, and the dependency of organic contaminants in herring from the co-variables age, season and lipid content. The question whether to use pooled samples or individual samples, has not been a problem to the BMP: the instruction to analyze individual samples has been part of the guidelines since many years.

Conclusions

Monitoring for the assessment of the state of the marine environment and particularly for the determination of long-term changes requires data of high quality. Variability in measurements induced by the sampling strategy, the sampling procedure, the analytical procedure and the evaluation of data should be minimised in order to identify and separate man-induced changes from natural variability. This depends on the right choice of the test organisms, the test variables, the sampling area, sampling period, technique and preparation of samples, the treatment on board and in the home laboratory, e.g. with regard to trace analytical conditions.

For comparison of different parts of the Baltic Sea, the comparability of the involved laboratories has to be secured by intercalibrations and quality assurance procedures.

As it is not realistic to fully meet the requirements of statisticians, particularly to satisfactorily supply ecological simulation models with data, one must decide between sampling in selected areas with a higher frequency and preferably many co-variables, and the entire area but with a lower frequency. For the demands of the Baltic Monitoring Programme with regard to the aim of trend monitoring, the first mentioned strategy seems to be the more efficient version. However, in practise, a monitoring programme is restricted by the resources of the laboratory and the allocated budget.

Therefore, in practise, the sampling for analysis of harmful substances in biota, according to the demands of trend testing procedures, the frequency has possibly been too low to get reliable information in acceptable time with regard to the demands of politicians and managers. Considering the high inter-annual variability of some environmental data, the statistical procedure (Fryer and Nicholson, 1993) will give the answer, that a minimum period of years is needed to detect temporal trends, predominantly independent of the intra-annual frequency or number of duplicates of samples. The relatively poor correlation between contaminant concentrations and the primary covariable “length” requires more co-variables to be considered in such cases. It is questionable, whether it is sufficient to use log-transformed values with the aim to get a linearity in correlation, in order to use simple linear statistics, and to use tests designed for normally distributed data. This is not adequate to the complexity of the subject and not necessary considering the power of computers nowadays. Generally, non-parametric tests should be preferred for evaluation of environmental data.

Many of the past monitoring activities in the North Sea and elsewhere have not delivered data suitable for indicating both temporal trends and incidents, or in some cases unfortunately not satisfied any of the main objectives of monitoring. It has been observed that natural conditions are varying too much to allow only one approach to be successfully applied to a number of different purposes. For marine biota, a recommendable introduction to the study of contaminant levels is given by Uthe et al. (1991a).

Statistics is only one tool to interpret the obtained data. Equally important is the experience and expertise of the scientists and the knowledge of environmental and biological correlations, and procedures which ensure quality assurance from the very beginning of designing a monitoring programme to the final assessment.

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Quality Assurance During Sampling Onboard

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Introduction

Quality assurance is not only essential in the different steps of sample pretreatment, analysis and evaluation of results, it is especially necessary in the first step of the whole measurement procedure i.e. in the moment when the catch comes on board and the samples are selected. Sampling means collecting specimen from the catch according to criteria defined by the scientist. During sampling a number of severe faults and defects can be made which can have detrimental influence on the results even if all other steps in the analytical procedure are perfectly performed. Therefore before entering a vessel a clear and concise sampling plan has to be set up including:

- species to be sampled
- number of individuals per species
- sex, length ranges, weight, age of individual
- treatment of samples (alive or dead, how to sacrifice, store, label)

Personnel and Crew

Onboard it is of major importance that besides scientists and technical personnel the captain and the whole crew are motivated and do their duties skilfully. This can be achieved by explaining why the samples have to be taken in a particular way, what will be done with the samples afterwards at land and what is the reason and background of this research. The scientist should make clear what he (she) expects as results and how the data will be used (modelling, restrictions, legislation etc), Everybody on board should feel involved and be aware of his (her) importance in the whole process. It is advisable to train any inexperienced scientific personnel and crew, unfamiliar with the procedure for handling biological material to demonstrate how the catch and the samples should be treated. It is advisable that the whole sampling procedure with all details and possible faults is well documented and available in written form. Clear advice should be given to the crew what is detrimental for your samples (also *simple* and obvious facts should be mentioned, not everything which is clear to a scientist is clear to a sailor) and questions should be answered.

Some defects in sampling procedures, which may influence the quality of the sample, will only be evident after the analysis is completed, these are:

casting offal and litter overboard during trawling

discharging waste water or sanitary tanks during trawling

spitting on the catch (e.g. chewing tobacco)

stepping on samples

using hooks for sorting (if tissue to be analyzed is affected)

smoking, discharging chewing gum and stubs into the haul

Contamination During Trawling

The investigator must be aware of the fact that the biological material in the catch coming on board may be contaminated even when the fish in the open sea are defined as uncontaminated. This is due to the fact that the biological material in the net has been in contact with a number of different materials and organisms:

trawl material: (organic polymers or natural net material), metals, rubber, plastics

during trawling: sediment, mud, plants, corals, algae, stones, offal, benthic organisms (sponges, sea-urchin, sea- stars), other fish species

on board: oil, paintings, anti-fouling, anthropogenic contamination by rubber boots, hooks, cigarettes etc.

mechanical force: during trawling (function of trawling time, speed of vessel and catch size), onboard by crew or scientific personnel

The trawling time and the trawl used has therefore considerable influence on the composition of the catch and the condition of the biological samples.

Selection of Samples

When the catch is onboard the samples, for later analysis, should be carefully selected. Fish showing wounds, parasitic infestation, bruises, misshaped fish and somewhat damaged fish should be avoided. Fish covered with foreign material like mud, slime, etc. should not be taken.

The selection of fish for later analysis is also dependent on the aims of investigation:

If the average content of a trace element in the fillet of a fish species from a certain catching ground in a certain catching season is being examined, the fillets of the fish selected can be pooled and thus only a limited number of analyses is necessary.

If detailed knowledge of the trace element concentration with variations within a species is wanted or if a correlation between trace element content and fish specific parameters like length, age, sex, state of maturity etc. is aimed at, single specimen must be taken according to the specific requirements. Consequently the number of analyses will be rather high which may limit the number of samples.

Sampling and subsequent sample treatment (chopping, wrapping etc.) can vary, depending on the type of contaminant to be analyzed (inorganic versus organic analysis).

Sampling is different for different size classes of fish. Regardless of the fish size it is important to know whether or not the trace element concentration is homogeneously distributed over the whole fish muscle. The posterior part of the fillet (tail muscle) shows higher concentrations of copper and zinc than the anterior part (dorsal muscle). In molluscs and crustaceans the same situation is found, especially in the abdominal muscle of crustaceans. Therefore it is necessary to take the whole muscle or to take a sub-sample which is representative for the whole muscle or to limit the investigation on a well defined part of the fish body.

small fish (< 200 g) are collected as whole fishes according to length or weight

medium sized fish ($>$ 200 g up to 5000 g) can be filleted and fillets stored until further treated. Fillets from both sides of the fish must be taken because trace element content can vary between sides.

from big fish ($>$ 5 kg) sub-samples can be taken, but it is important to define the sampling part before taking the sub-sample, e.g. *Musculus adductor mandibulae* (chewing muscle black side) in White Halibut (*Hippoglossus hippoglossus)* .

If possible live fish should be taken. Live fish is a better defined material because the time of death is determined by the investigator. In dead fish the history from death on is generally unknown and the fish is not bled to death. In scientific hauls which normally take 30 or 60 min the majority of fish coming on board are alive. The samples of fish should be transferred to the hold, where they are killed and allowed to bleed before sub-samples are taken.

Summary

Quality assurance is an integral part of sampling. The quality of results can be improved by good sampling practice and can be badly influenced by poor sampling leading to irregular results.
Sampling can be influenced by various factors. Among these are of major importance:

- Skilfullness, motivation and training of personnel and crew
- Contamination by the catching technique itself
- Well defined description of posing the problem and formulation of the question
- Selection of samples and correct sampling plan

Critical Review on the Suitability of Different Decomposition Procedures for the Analysis of Trace Metals in Biota

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Introduction

Trace and ultratrace element analysis of samples from marine biota or biological material is generally influenced by an excess of organic compounds (proteins, lipids, carbohydrates), which represent the matrix. Complete elimination of all organic matter is normally unavoidable before the subsequent steps of dissolution, preconcentration, separation and finally measurement.

The organic matrix can be eliminated by either extracting the trace metals from the matrix or volatilising the matrix by ashing (digestion, decomposition). Extraction, however, is limited to only a few samples where the trace metals can be extracted quantitatively and where the matrix is completely insoluble. The most important step in trace element analysis is decomposition.

The decomposition of biological material is the most time consuming and work intensive step in trace metal analysis. If we assume that the sum of sampling, storage, sample preparation, decomposition and analysis is 100% the decomposition amounts often to 80% or more. Furthermore decomposition is the step with the highest risk of contamination because the samples come into contact with a number of chemicals, decomposition aids and storage materials.

Decomposition may be defined as the removal of the organic matrix by converting it into suitable gaseous components which are then volatilised, leaving behind an inorganic residue for element analysis.

Criteria to be Considered for Decomposition of Biological Material

A decomposition procedure for trace element analysis should ideally involve:

- complete destruction of all organic components and mineralisation of the sample
- retention of all elements to be determined quantitatively within the inorganic residue
- avoiding all possible contamination

Furthermore the decomposition method of choice should preferably:

- need only small amounts of ultrapure reagents and chemicals

- be not dangerous in handling
- be simple to clean
- not use $HCLO_4$ (explosion)
- not use H_2SO_4 (coprecipitation with insoluble salts)
- be a closed system
- work at a low temperature
- use only silica as material for containers
- have low costs for equipment and consumables
- be simple in handling

Some analytical methods like neutron activation analysis (NAA), X-ray fluorescence (XRF) and others allow the direct analysis of liquid or even solid samples without destroying or modification of the matrix.

The different decomposition procedures can be classified in different ways. A possible way is the classification according to “open” or “closed” systems. An approximate price for a complete system is given in TDM (Thousand Deutsche Mark).

Classification and Relative Costs of Procedures used for the Digestion (Decomposition) of Organic Matter for Trace Metal Analysis

- “open" wet digestion systems with convective heat transfer (pressureless digestion) TDM 5-20
- “open” microwave digestion systems (pressureless) TDM 2-4
- “open” UV digestion system (pressureless) TDM 13
- Digestion system under pressure with convective heat transfer TDM 10-70
- microwave digestion system under pressure TDM 2-30
- “open” Combustion system (pressureless) TDM 20
- Combustion system under pressure (in bombs) TDM 2-5
- Combustion system (cold plasma digestion) TDM 25-70 (100)
- “open” digestion by dry ashing TDM 20
- Digestion by fusion TDM 9-25

Advantages and Disadvantages of Different Digestion Methods

Of major importance for trace element analysis in organic matrices are dry ashing with oxygen piasma at temperatures below 150” C, combustion in a stream of oxygen or oxygen/hydrogen at very high temperatures and wet ashing by HNO, or $HNO_3/HClO_4$ with microwave heating.

The main advantage of wet ashing methods over dry ashing methods are the lower temperatures resulting in lower losses of trace elements, although losses of trace elements are reported in wet decomposition methods due to reaction with container walls.

The choice of the decomposition method is strongly dependent on the analytical procedure used for the determination of elements in the decomposed sample. Some analytical methods do not need decomposition steps. Others like Zeeman-AAS are less sensitive to incomplete mineralisation, whereas some methods like DPSV (differential pulse scanning voltammetry) give reliable results only in a solution which is free of any organic matter or residues.

Some decomposition methods for which sophisticated instruments were developed in the past like UV-digestion are not suitable for use in marine biota. Normally the complex organic matrix cannot be destroyed by the use of UV only. Digestion aids like acids or H_2O_2 must be added and the method can only be used for almost fat free samples. In our laboratory it was possible to decompose a diluted emulsion of a lean fish (haddock) muscle in a UV digester for later analysis for cadmium and lead by AAS.

The two most promising methodologies are wet ashing procedures and dry ashing by oxygen plasma. In Table 1 a general validation of the most commonly used methods is shown.

From Table 1 it can be concluded that the risk of contamination is highest in dry ashing at high temperatures in open systems and in wet ashing methods if an open system is used. The lowest risk of contamination is found in low temperature oxygen plasma ashing, in combustion in oxygen stream and in closed wet ashing systems.

A satisfying or complete decomposition of organic matter is achieved by all methods.

With regard to costs of analysis it is evident that systems with a low risk of contamination and a complete decomposition of organic matter are generally most expensive (see above).

Samples of marine biota are characterised by a high water content ($> 60\ \%$), an organic matrix with a high content of protein and fat and - mostly - with a very low content of toxic heavy metals.

Table 1. Most frequently applied decomposition methods in trace and ultratrace analysis of biological matrices (after M. Stoeppler, 1991).

Method	Risk of contamination	Complete Decomposition	costs	Sample Throughput	Remarks
Dry ashing high temperatures	***	***	*	***	Usually less applicable for low contents and high precision analysis
Low temperature ashing, oxygen plasma	*	**	***	**	Often duration of decomposition long due to formation of residues if no special systems are used
Burning in oxygen stream	*	**/**a	***	**	Recent improvements, sample throughput and performance to note
Wet ashing open systems, glass or silica	**	**/***	**	**/***	Automated systems with considerable costs allow high sample throughput. Also reflux systems. Various acids and acid mixtures. Complete decomposition only possible at higher temp. and by addition of, e.g. $HClO_4$, and/or H_2SO_4
Wet ashing in closed systems under pressure ≤180 °C with PTFE vessels	*	**	**	***	Difficult for mercury analysis. Mainly only HNO_3 and mixtures with HNO_3, also with HF. Subsequent voltammetric analysis requires posttreatment with $HClO_4$
Wet ashing in closed systems under pressure with microwave energy PTFE etc.	*	**	**	***	Rapid and effective method, however, in principle at present similar effects like HNO, decomposition. Precautions should be taken in routine analysis to avoid accidents
Wet ashing in closed systems at higher temperatures silica vessels temp. up to 300 °C	*	***	**	**	Particularly useful for subsequent voltammetric analysis and mercury systems. Still very expensive

Scores: * low, ** medium, *** high

This means that samples from marine biota have to be dried as a first step if a dry ashing method is used. Because of the low amount of trace metals present in marine biota independent of the analytical method it is necessary to reduce the amount or - if possible - avoid the use of excessive amounts of chemicals in the digestion procedure. The use of redistilled or even sub-boiling point distilled acids is recommended as this can reduce the contamination. However in later steps of the procedure, even if they are skilfully performed, some contamination may occur during dilution steps, adjusting pH or during transfer between containers. As mentioned before the choice of the decomposition method is closely correlated with the analytical method to be used for the determination of the trace metals. Since more than 90% of all analyses are performed with AAS-methods, ICP-methods or voltammetric methods decomposition methods are only reviewed in this respect.

Because of the advantage of closed decomposition systems there is a tendency towards the use of high pressure or high temperature microwave digestion systems. Some new developments are already on the market. For safety reasons (explosion risks) the size of the decomposition vessel is limited to 25-70 mL and the device has to be constructed to tolerate pressure peaks of approx. 100 bar. The sample size should be ca. 100 mg calculated as carbon, and the resulting temperature should be close to 300" C if working with nitric acid. Some microwave systems have a pressure regulated device to avoid overheating. The decomposition achieved by these systems is of a quality which allows application of voltammetry for the determination of heavy metals. The degree of mineralisation reported was 93 % for oyster tissue and 95 % for mussel tissue (measured as DOC, dissolved organic carbon). A disadvantage of the device is that only two samples can be decomposed simultaneously.

Since 1981 we have used and recommend microwave induced oxygen plasma under vacuum for the decomposition of marine biota. The method has the following **benefits:**

+ no contamination, no chemicals used, sample has contact only with oxygen,

+ energy and thus temperature can be adjusted according to the matrix to be decomposed (50-250 W)

+ approx. 20 samples can be decomposed simultaneously

+ complete destruction of organic matrix

+ best decomposition method for voltammetric determinations

+ reasonable costs during use (electricity and oxygen)

+ a safe process for the analyst (no toxic or hazardous chemicals involved)

the **disadvantages** are:

high costs of the equipment

because only small amounts of samples can be used in the instrument (approx. 10 g i.e. 20 samples of 500 mg each), the homogeneity of the sample must be good (drying and milling steps are necessary)

Loss of some elements like selenium or arsenic if energy (temperature) is too high

time consuming (the greater the fat content of the sample, the longer the digestion time). A complete digestion of 10 samples of fatty fish muscle can last up to 120 hours.

Figure 1. Schematic view of a low temperature oxygen plasma processor.

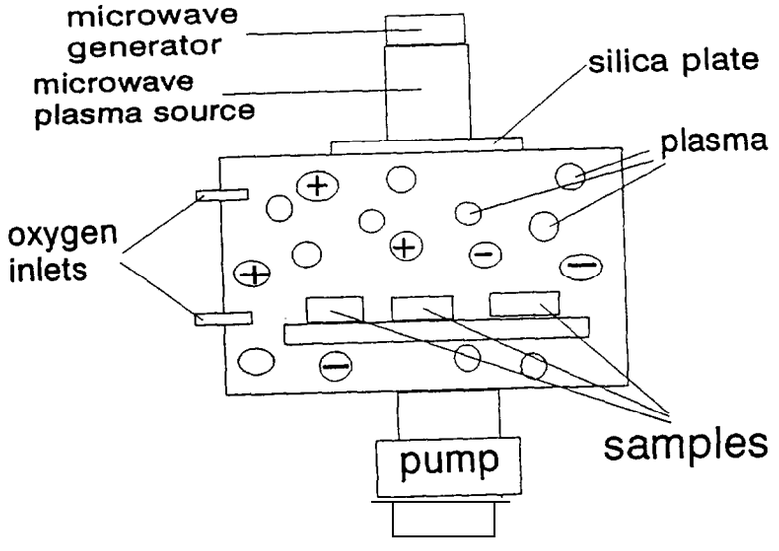
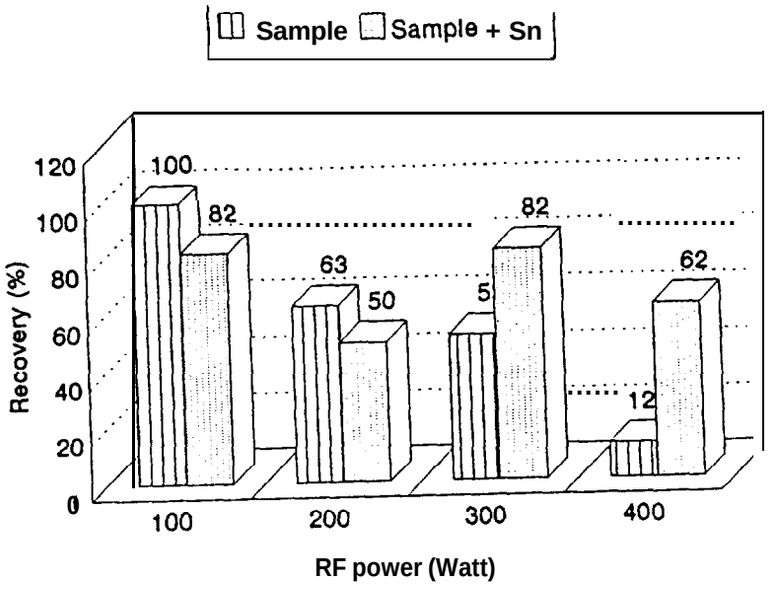


Figure 2. Change of recoveries of arsenic with RF (Radio frequency) power in low temperature ashing with oxygen plasma.



A schematic view of the principal of a low temperature oxygen plasma processor is shown in Figure 1. An example of the loss of elements is given in Figure 2, which refers to the change of recoveries of arsenic with RF (radio frequency) power. At RF power of 100 W the recovery is almost completely (100%). With increase of energy the recovery becomes lower. At 100 W the resulting temperature in the sample is approx. 70 °C and at 250 W approx. 140 °C. Selenium and mercury gave comparable results. For later analysis for selenium and arsenic samples should be ashed in a plasma at 150 W or less, mercury should be determined after a wet digestion procedure.

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Experience with Differential Pulse Scanning Anodic Voltammetry (DPSAV) in Trace Metal Analysis in Biological Matrices

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Summary

In this case study a brief introduction into the methodology is presented. Then the different steps of sample pretreatment which are a prerequisite for DPSAV will be mentioned and finally the measurement itself is demonstrated and typical results are shown.

Introduction

The "classical" method for the analysis of trace metals in biological matrices was the AAS (Atomic Absorption Spectrophotometry). In the 60s and even more in the 70s and 80s other methods became more popular for the analysis of trace metals e.g. electrochemical methods like polarography and voltammetry. Under an international convention, the name **polarography** is defined as the electrochemical method in which a dynamic (dropping) mercury electrode is used (Hevrovsky). If the electrode is a static one like the hanging mercury drop (HMDE) and current/voltage curves are recorded, the term **voltammetry** is used.

In our laboratory this method has been used since 1981. We now have the fourth generation of instruments in use, the last was bought in December 1992. The development of the instruments has gone very rapidly, While we started with an instrument in which all functions had to be controlled and adjusted manually including measuring time by a stop watch, we now have a fully automatised instrument.

Principle of voltammetric measurements

It is inappropriate to give a detailed lecture about the physical principle and the sophisticated instrumentations developed for differential pulse scanning anodic (or cathodic) voltammetry . It has therefore been decided to present an account of experiences with the application of this method in biological matrices.

The simplified principle of the voltammetric procedure is: At a microelectrode (e.g. a mercury drop placed in a solution containing trace metals) an electrode process formulated for a heavy metal as

$$\text{oxidised metal} + n \cdot e^- \longleftrightarrow \text{reduced metal}$$

occurs (where $n*e^-$ is the number of electrons transferred). In the solution of the analyte only a single form of ionic species is found, the oxidised or the reduced type, while the product of the electrode process (reduced metal in reduction process and oxidised metal in oxidation process) is formed only during the electrode process itself at the electrochemical double layer between electrode and solution in situ. The electrode potential E for a given redox reaction is defined and the corresponding current i, which is a function of the concentration of the metal in the solution, is measured. If the current/voltage correlation i = f(E)is recorded, a polarogramm or voltammogramm is obtained. Practically the DPSAV, Differential Pulse Anodic Stripping Voltammetry, which is a combination of different pulse polarography and anodic stripping voltammetry, is used for the determination of heavy metals. In this "inverse" technique the elements in a first step are deposited on the electrode and in a second step stripped off according to their electrochemical potential.

The consumption of substance during recording a voltammogramm is minimal because of the law of Faraday (faradaic current). Thus the concentration in the analyte remains almost constant even after a number of analyses.

This simple principle has been optimised by manufacturers of instruments and the state of the art is that a graph of the correlation between current and voltage with well defined peaks for trace metals is produced by the instrument.

Sample pretreatment

All samples for later investigation on trace metal content were collected during research cruises with the fishery research vessels of the Federal Research Centre for Fisheries. As an example of a biological matrix we look at a whole fish muscle. After hauling the fish is brought into the ships chemical laboratory. Here the fish is carefully sacrificed, gutted, filleted and skinned. The whole muscle is given to an second technician bearing all necessary protection aids to avoid contamination of the sample (white coat, gloves, mouth and hair protection etc.). This person prepares the core of the fillet. That means that the outer layer of the muscle (3-4mm) is removed with silica knives, PTFE forceps and a pair of ceramic scissors. The remaining core of the fillet is cut into small cubes of approx. 1 cm^3, which are then mixed. From this mixture two samples of approx. 15 g each are randomly taken and immediately frozen in silica or glass petri dishes covered with glass lid in PE-pouches.

At the land-based laboratory, the petri dishes containing the deep frozen samples are transferred directly into a freeze dryer for lyophilization. After drying, the samples are finely milled in a ball mill made from agate. The extremely fine powder is transferred to small silica dishes, which are placed on a rack made out of silica rods and put in a low temperature oxygen plasma asher.

After evacuation the asher is adjusted to approx 0.8 mbar oxygen and the plasma is initiated by the microwave generator. The MW force is adjusted according to the elements to be measured. For heavy metals we use generally 350 Watts. The samples remain in the asher for 24-120h depending on the type of matrix. As a general rule it can be stated that the fattier the samples the longer the decomposition time. If elements which tend to volatilize at higher temperatures (Se, As) should be determined later it is necessary to adjust the MW force to 150 Watts or less to avoid element losses.

When decomposition is complete, this can be controlled visually by a change in the sample colour from grey/brown/yellow to a bright white and a change in the colour of the oxygen plasma from cyan/red to a light blue, the ashed samples are quantitatively transferred into 100 ml volumetric flasks and dissolved in *suprapure* hydrochloric acid at pH 2. This solution is ready for measurement in the instrument.

Determination

50 ml of the solution are given in the electrolytic cell, 70 µl of perchloric acid are added as an help-electrolyte and the analysis can be started.

The procedure described below illustrates the steps used in the measurement of four heavy metals, lead, cadmium, zinc and copper. In the first step, a run is made covering the whole range of voltage. This allows the operator to see the differences in concentrations of elements in the solution and a improper decomposition of sample can be detected very easily. Any organic compound which is left leads to a misshapen peak and no baseline separation of peaks. In fish muscle there is normally a thousand fold excess of copper and zinc over lead and cadmium so that the trace elements lead and cadmium are usually not seen. The first step is followed by the preprogramme for the single segments of each element. Each element requires an individual programme (segment) of voltage range, enrichment voltage, drop size, enrichment time, sensitivities (µA or nA), volumes for standard addition etc. After "start" all four determination are run automatically in a sequence including standard additions, All segments are measured in duplicate. After a total time of ca. 40 min the concentrations of the four elements are printed out in mg/kg dry weight and the corresponding curves are shown on the display or on a hardcopy.

If the concentration of trace elements is almost the same two or more elements can be run in a single segment. The programming of different segments is only necessary if there are big differences in concentration.

Quality, precision and accuracy of the analysis are controlled regularly, at intervals, by analysing standard reference materials and by spiking the aqueous samples with known amounts of elements (the recovery rates should be better than 95 %) and by comparison of the results obtained from DPSAV with those from AAS.

In our laboratory all samples are measured in duplicate as individual subsamples. As a result two separate concentrations are obtained for the two subsamples. We accept the resulting data when the difference between the results for the subsamples is $< 20\%$. As an example: 1.8 and 2.1ng/g will be accepted and later reported as 2.0ng/g while 1.8 and 2.4ng/g would be rejected and the measurement would have to be repeated, starting from the original sample.

In Fig. 1 and 2 typical results of a voltammetric determination are demonstrated. This can give an impression of the simplicity, versatility and effectiveness of the method and the instrument.

Figure 1. Original voltammogramm of a fish muscle sample over the whole range between - 1.2 V and + 0.2 V showing the 1000 fold excess of copper and zinc over lead and cadmium.

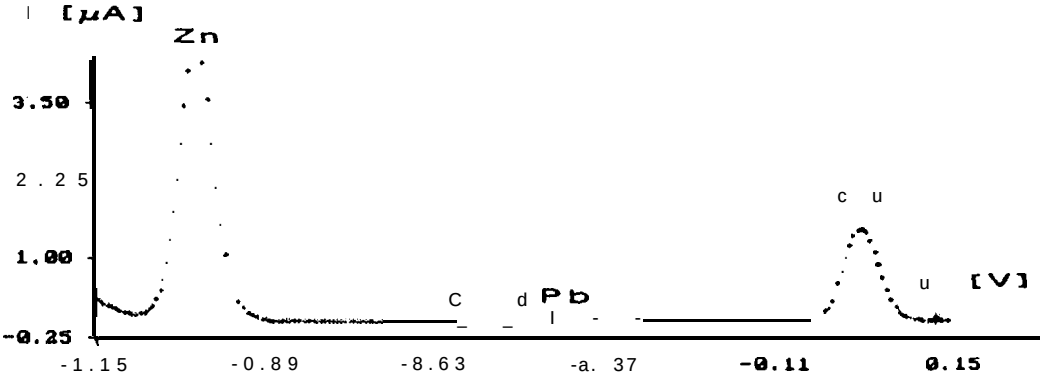
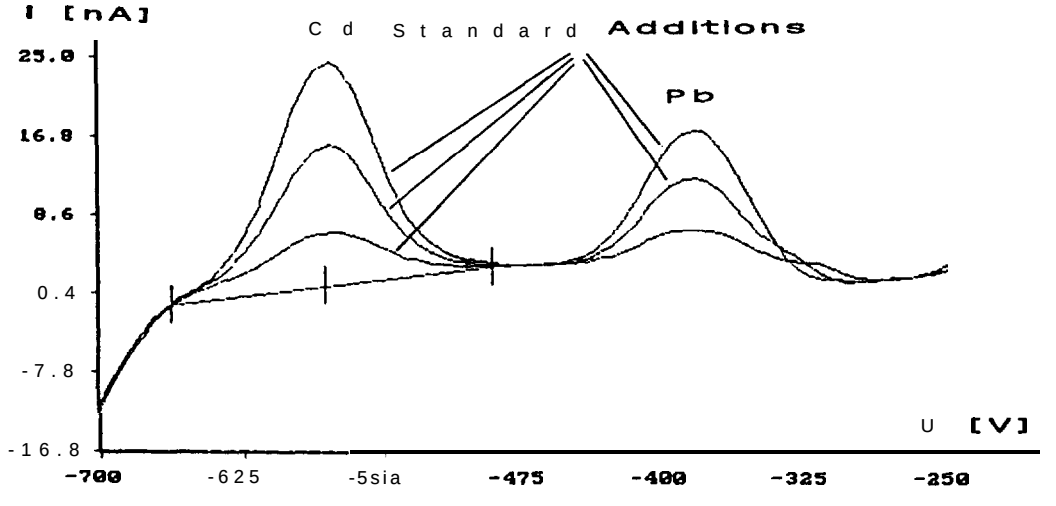


Figure 2. Voltammogramm of a segment showing the original curve for cadmium and lead and the resulting curves for the three standard additions.



The DPSAV with a partly or completely automatised instrument is a powerful tool for the analysis of trace elements in biological matrices. The only **disadvantage** is that all samples must be completely decomposed. Each organic compound present in the measuring solution disturbs the determination and may lead to incorrect results. Furthermore, the solution must be completely free from oxygen (nitrogen-stream).

The **advantages** of DPSAV are:

- Up to 4 elements can be determined simultaneously
- The equipment is available at a reasonable price compared with other instrumentation
- No clean room or clean bench are necessary (since operations are carried out in a closed system), and there is no risk to the operator. A single electrode filling is sufficient for > 5000 determinations, size of a single drop $\approx 5\text{-}10*10^{-5}$ cm^3
- The consumption of consumables is negligible (no graphite tubes etc.)
- Low risk of contamination. The sample comes into contact only with oxygen and hydrochloric acid
- The method is of high precision. The current (I) measured is direct proportional to the concentration (C) of the analyte in the solution (I = k * C). All measurement are made using the standard addition method, no calibration curves are used.

Summary

The DPSAV method has had a vigorous testing through the analyses of a 1500 samples of marine biological material for the presence and content of a no of heavy metals (lead, cadmium, zinc, copper) with DPSAV. Among the samples examined were whole fish muscle, muscle parts, liver, crustacean muscle, hepatopancreas, gills and eyes, cephalopode muscle, hepatopancreas, ink, gonads and mussel tissue. The concentration of trace metals in these samples varied between l-2 ng/g fresh weight for cadmium in fish muscle to > 30 μg/g fresh weight for cadmium in hepatopancreas of cephalopodes. All matrices and trace metal concentrations can be successfully measured by DPSAV. The most difficult matrices found were liver of lean fish species and the exosceleton of crustaceans.

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Certification for Low Level Trace Metals in Marine Biological Reference Materials

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The lecture given was a plea for a reference material containing extremely low concentrations of Pb, Cd and other heavy metals. A reasoning is given by a historical overview on the main topics of pollution research in relation to those metals.

Emphasis has changed from toxicological tests (with high concentrations) over "hot spots" in the environment (certain organisms and organs, such as kidney and liver, showing significantly elevated concentrations) to mechnisms of transport and modelling. That development has been accompanied and promoted by methodological improvement. Those concentrations, which have to be determined with a sufficiently high precision are now in the lower background range,

A proper standard material for such purpose could be fish muscle which, over and above, could be spiked, if necessary, for measurements in a higher concentration range. Now, the Bureau de Reference of the Commission of the European Communities has prepared and certified a cod fish muscle reference material. Some experiences gained in the course of it's certification have been given.

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Bureau de Reference of the Commission of the European Communities: Codfish muscle reference material CRM 422.

New Inorganic Contaminants

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Thallium (Tl) is a rare, scarcely used, and generally not well known heavy metal. The author lined out why for that metal, in particular, an occurrence study in coastal waters of the German Bight was being made. He argues, that mentioning of Cd and Hg in the black lists of a vast number of national and international regulations has created a research boom on the environmental behaviour of these elements, in particular, whereas other possible candidates for inclusion into the black lists are comparably discriminated. With reference to it's position in the periodic system of elements and viewing several similarities in it's environmental behaviour compared to that of Cd Tl is elected a possible candidate. For the selection of such candidates some criteria will be discussed.

A study on the occurrence of Tl in coastal waters of the German Bight demonstrates that this element is a contaminant. The expected background of about 10 ng Tl L^{-1} is, by far, exceeded in the Weser estuary (90 ng Tl L^{-1} at Imsum), and, to a lower degree, in adjacent regions. That result gives reason for further investigations on occurrence and effect in the biosphere.

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Quality Assurance of Trace Metal Analysis in Sea Water

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Introduction

The quality of analytical results is directly influenced by the methods applied, including sampling, the analytical procedures, and data evaluation, and by special techniques to secure and document the quality itself, like statistical methods, use of standards and reference materials and participation in intercalibrations.

It has been the principle within this group for more than two decades to keep the entire process from sampling at sea through analysis and evaluation of data to final publication of results in the hands of the members of the same small team, thus greatly enhancing possibilities to improve the quality of the data.

The intention of this paper is to present different aspects of quality assurance through the course of procedures outlined above as a "case study", using the example of the methods and experiences as tested and applied within the author's group at BSH rather than presenting an overview of different approaches as proposed, discussed and presented in the literature.

An outline of the major methodical improvements in the BSH (Bundesamt für Seeschiffahrt und Hydrographie) group "Trace Metals in the Sea" that directly lead to better quality results is given in Table 1:

Table 1. Overview of the major methodical improvements in the heavy metals group.

1972	Graphite furnace atomic absorption spectroscopy (Cd, Cu, Fe, Mn, Ni)
1977	Establishment of clean benches
1979	Cold vapour atomic absorption spectrophotometry (Hg)
1980	Development, construction and routine use of the PTFE water sampler "Mercos"
1985	Anodic stripping voltammetry (Cd, PB)
1985	Clean room container for sample pretreatment on board a research vessel
1986	Total reflection X-ray fluorescence spectrometry
1989	Move into ultra clean laboratories

Sampling

Sampling of sea water down to a depth of 100 m is achieved using the sampler system MERCOS (Freimann et al., 1983) which was developed and tested in this group. The sampler, which is commercially available, consists mainly of PTFE material and is lowered down through the contaminated water around the ship in a closed configuration; it is opened by a messenger (made from PTFE) in the conventional way and is lifted up open (the very small openings do not allow exchange of water). Sampling at depths below 100 m is mainly done using General Oceanics all plastic GoFlo samplers (Schmidt and Gerwinski, 1992).

The hydrographic wire consists of 4 or 6 mm diameter steel galvanized with zinc that is covered by 1 mm of polyethylene. Wire lengths of up to 5 000 m (8 mm outer diameter) have been used. Other equipment coming into contact with the sea water is made of plastic material (like the turning wheel) or covered with plastic material (like the ground weight).

Sample treatment

All handling of samplers, equipment, and treatment of sea water samples is performed in clean laboratory containers that can be placed aboard modern research vessels. Further details of these clean laboratories are given in Schmidt and Gerwinski, 1994, in this compilation of papers.

Filtration: All plastic (polycarbonate) apparatus made by Sartorius.
Nuclepore membrane filters of 0,4 μm diameter, rinsed with dilute HCl and H_2O, dried and weighed (Schirmacher and Schmidt, 1992).

Conservation of samples: Acidification of unfiltered and filtered water samples by nitric acid (for Hg measurements) or hydrochloric acid (for measurements of the other metals).
Freezing of sea water samples, previously employed by the author, has been discontinued recently.

Storage: Samples for mercury analysis are stored in special 500 ml teflon bottles as parts of the MERCOS system.
Other water samples are now mainly stored in polypropylene bottles.

Cleaning of equipment: Multiple rinsing with ultrapure dilute acids and water.
Storage in plastic bags and boxes prior to use.

Purification of liquids: HCl and HNO, of commercial suprapure quality or similar, purified in our laboratory by sub-boiling distillation. Organic solvents also undergo sub-boiling distillation. Ultrapure water is generated by commercial systems such as Millipore units,

Analysis

All ultra-trace analytical procedures are performed in the land-based laboratory ("Laboratorium Sülldorf").

Four different analytical methods have been applied in this laboratory and are summarized below. All make use of chemical separation procedures to achieve very low limits of detection by pre-enrichment of trace metals and separation of the salt matrix. The chemical separations are the limiting factor in terms of analytical effort and the available time.

1. Graphite furnace atomic absorption spectrometry (GFAAS).
   Separation procedure: Liquid/liquid extraction by MIBK and APDC complexing compound. For details see (Schmidt and Zehle, 1979; Schmidt, 1980; Schmidt et al., 1986; Schönfeld et al., 1992).
   Used here extensively for Cd, Cu, Fe, Mn, Ni.
   At present routinely used for Cd.

2. Cold vapour atomic absorption spectrometry (CVAAS) for Hg.
   Enrichment/purification by amalgamation on finely dispersed gold. (Freimann and Schmidt, 1982; Schmidt and Wendlandt, 1987; Schmidt, 1992)

3. Anodic stripping voltammetry (ASV).
   Enrichment and separation by the electrochemical method itself (electrodeposition from the solution into the mercury film electrode). (Dicke et al., 1987)
   Presently used mainly for Pb and Cd. Also applicable and used for Zn and Cu.

4. Total reflection X-ray fluorescence analysis (TXRF).
   Separation procedure by reverse phase column chromatography. Details are given in the following scheme:

Analytical scheme for the determination of heavy metals in sea water by TXRFA

200 g sea water sample (pH = 1.6)

+ 2.5 µg/kg Se (internal standard)

+ 1 ml Na-acetate / Acetic Acid buffer

+ ca. 0.5 ml NaOH (30 %) = > pH = 4.8

+ 2 ml NaDBDTC (2 %, aq.) Complexation

Reverse Phase Chromatography

(solid phase: Chromosorb W)

Elution with 3 ml Chloroform/Methanol

Evaporation of 100-200 µl eluate on a quartz sample carrier

TXRF-Measurement (3000 sec) Mo-tube

Elements: V, Mn, Fe, Co, Ni, Cu, Zn, Pb, U

The TXRF method has been proven to have a wide range of applicability and has turned out to be an extremely powerful analytical technique. In our group, it has gradually replaced most of the tasks of the GFAAS method which was used earlier. Since the TXRF method has, until now, not been used in trace metal sea water analysis to any significant extent by other groups involved in monitoring, a more detailed description is given here. The major components of the spectrometer unit are shown in Fig. 1; the principle of the analytical method is given in Fig. 2. A flow diagram of the chemical separation is presented in Fig. 3; the column chromatography equipment is outlined in Fig. 4. For more details reference should be made to previous publications (Freimann and Schmidt, 1989; Freimann et al., 1992; Schmidt et al., 1993), and to the paper by Schmidt and Freimann, presented at this workshop.

A typical example of a TXRF spectrum of a North Sea water sample is presented in Fig. 5.

Figure 1. Schematic view of the instrumental set-up for Total Reflection X-ray Fluorescence Spectrometry (TXRF).

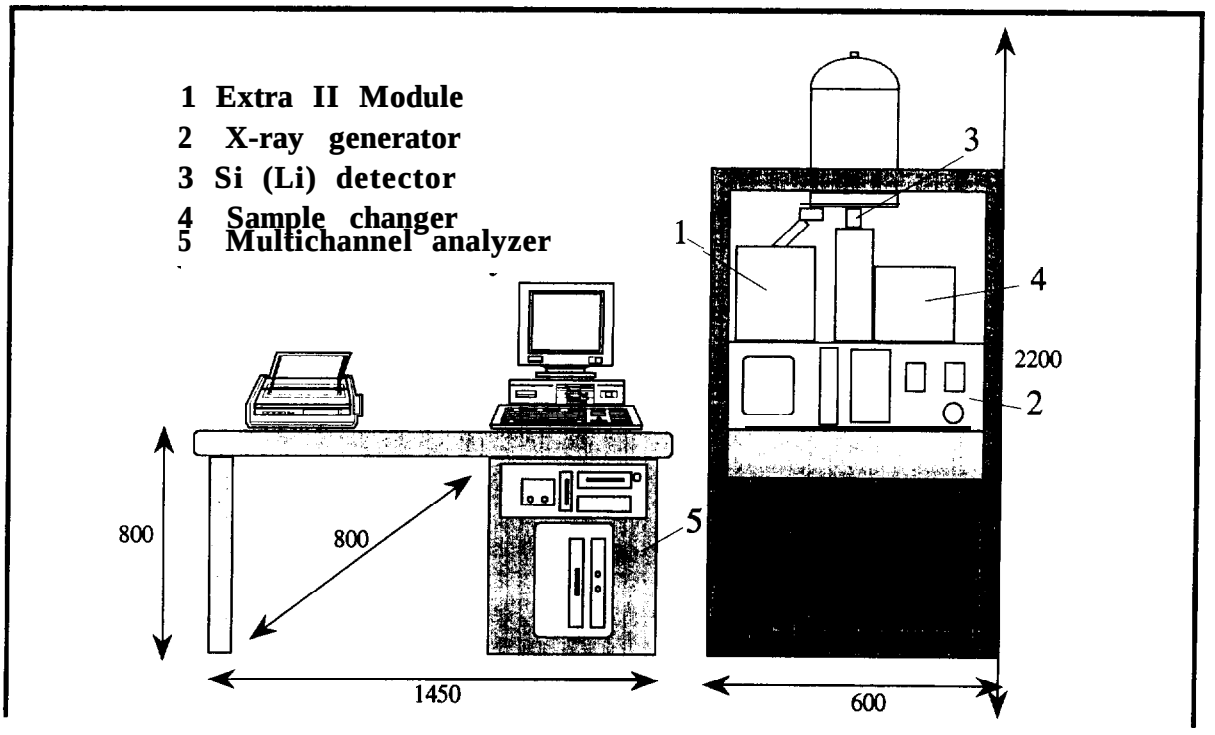


Figure 2. The principle excitation/detection geometry of TXRF.

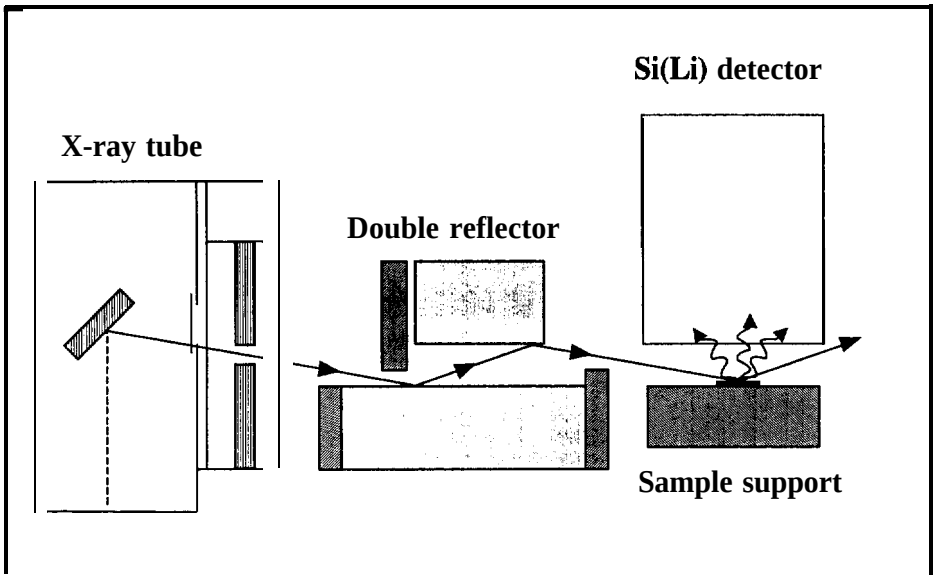


Figure 3. Flow diagram for the chemical separation of trace metals from the seawater matrix.

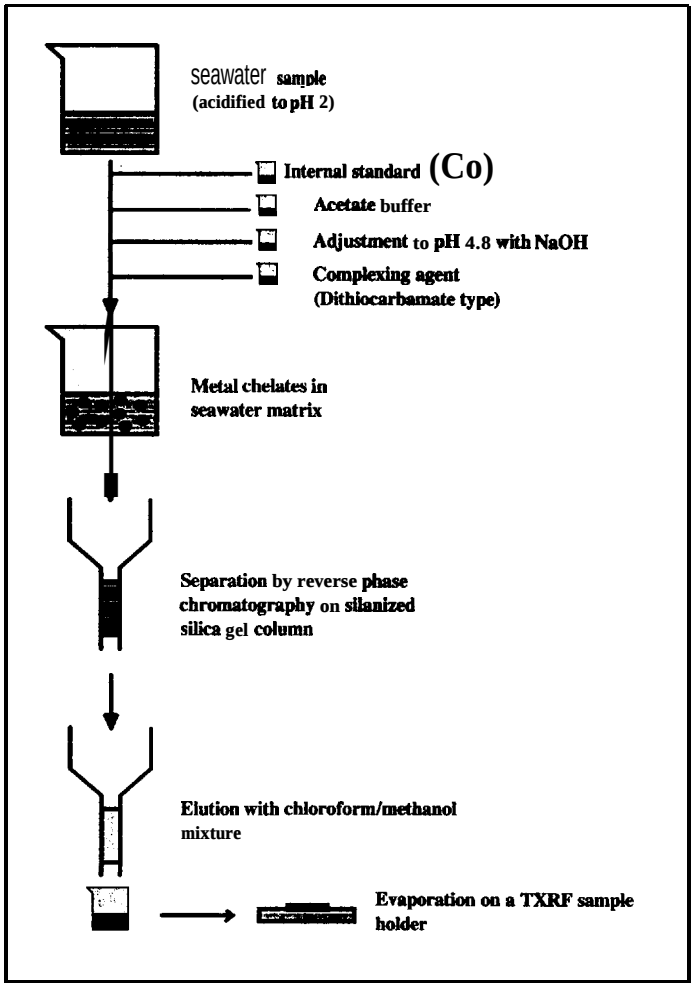


Figure 4. Column chromatography equipment used for the separation procedure shown in Figure 3.

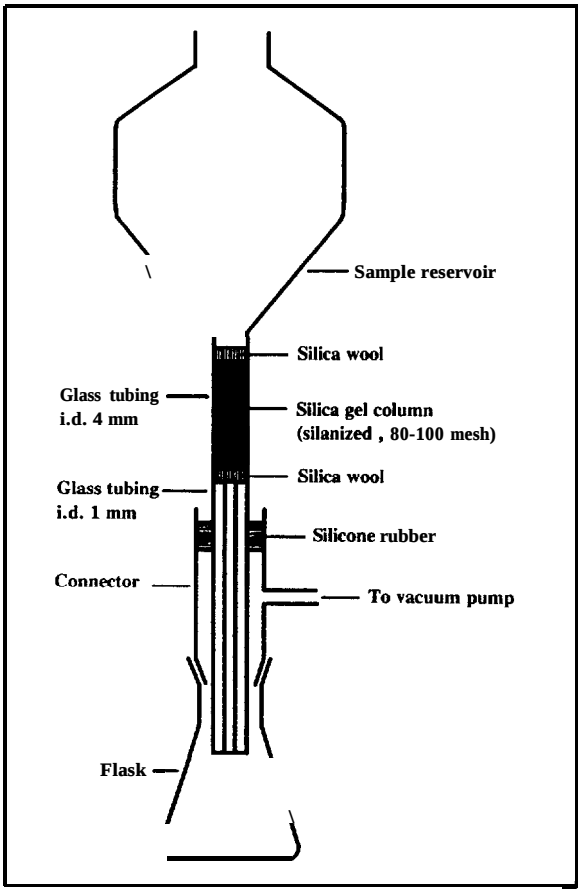
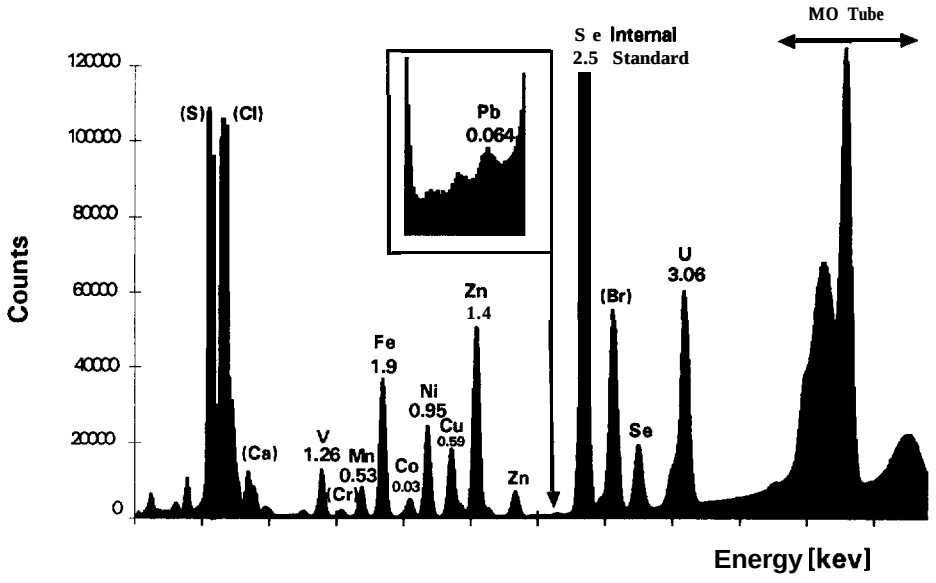


Figure 5. TXRF spectrum of a North Sea water sample; values are given in μg/kg.



Data evaluation

Calculation of concentrations, treatment of raw data, statistical evaluations and storage of data in a data bank are performed using Personal Computers and commercially available software.

Validation of results and quality assurance:

Precision checks are made by replicate analysis.
Accuracy determinations are made using certified reference materials (mainly Canadian NASS and CASS series sea water). A typical example from previous accuracy checks is given in Table 2.
Regular control of accuracy and precision is done by routinely drafting control charts from the data using the PC, see Fig. 6 and 7.

Table 2. Investigation of NASS-2 Open Ocean Sea Water Reference Material.

Element	Certified Value µg/l	TXRF (BSH) µg/l
V		1.53
Mn	0.022+/-0.007	0.024
Fe	0.224+/-0.034	0.306
c o	0.004+/-0.001	0.022
Ni	0.257+/-0.027	0.281
c u	0.109+/-0.011	0.104
Zn	0.178+/-0.025	0.175
Mo	11.5+/ -1.9	10.5
Cd	0.029+/-0.004	0.028
Pb	0.039+/-0.006	0.040
U	3.000+/-0. 150	3.17

Data from 1987

Figure 6. Quality control chart for copper in the certified seawater "CASS-2" of the national Research Council Canada.

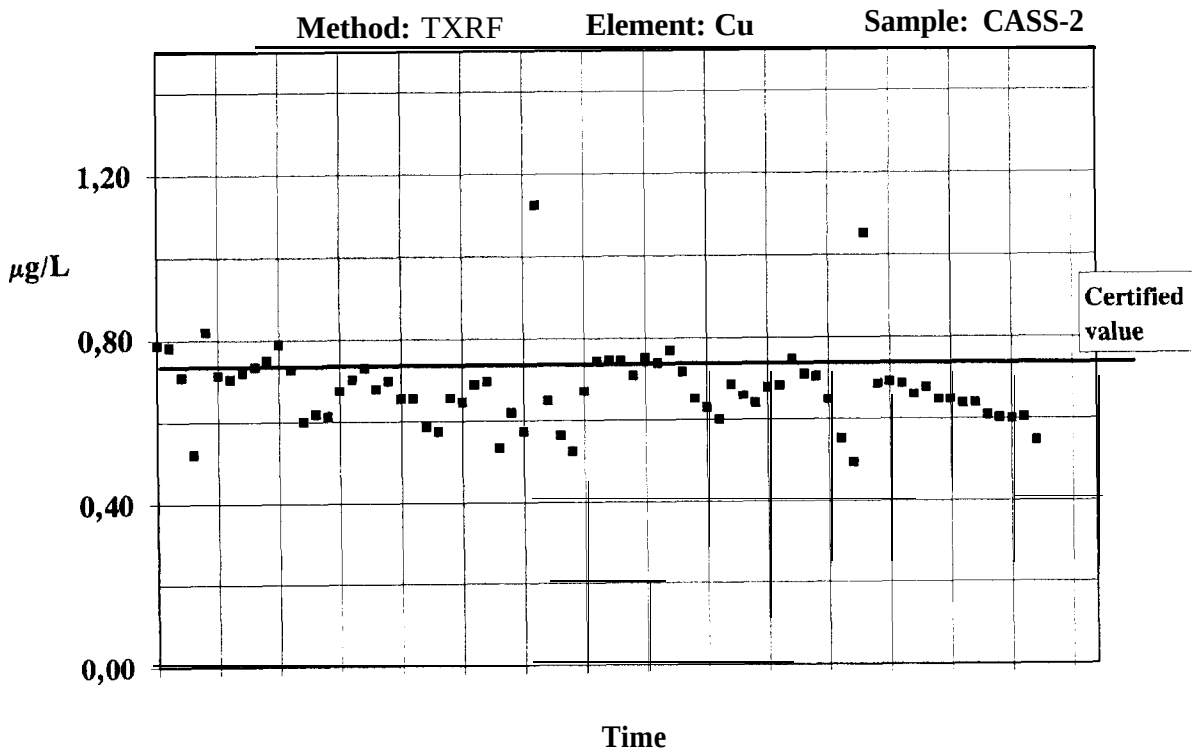
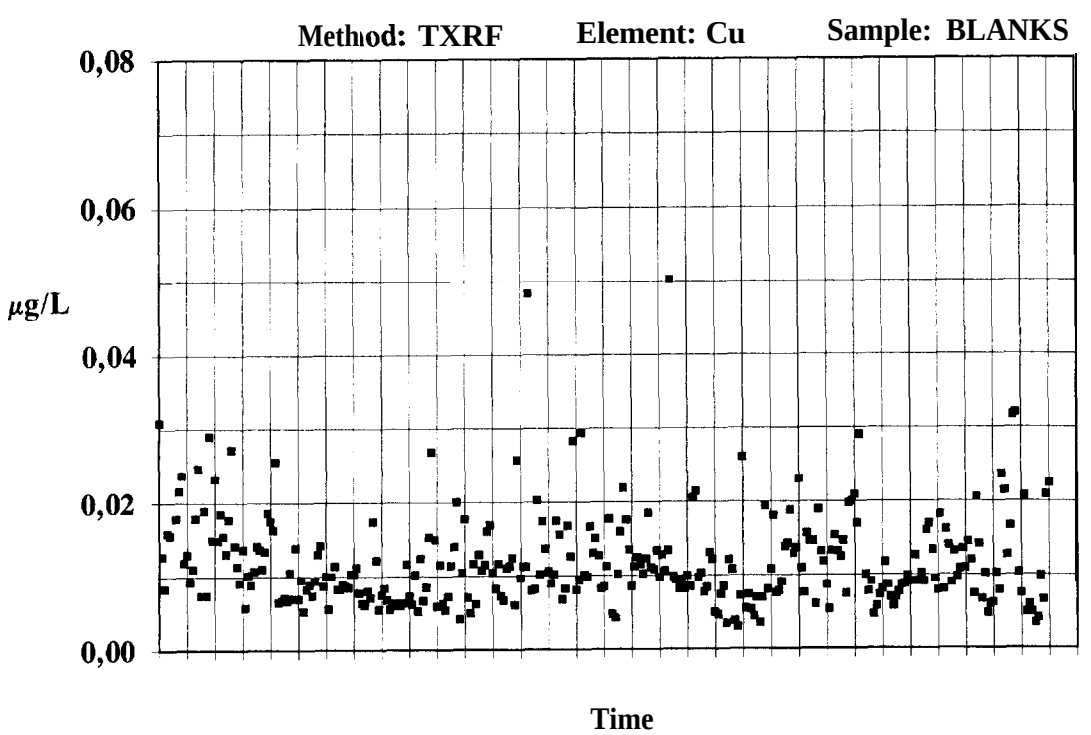


Figure 7. Quality control chart for the total analytical blank of copper.



Participation in external quality assessment

The BSH participated in numerous national and international intercalibration experiments during more than 20 years, generally with satisfactory to very good results. Details are given in (Schmidt, 1983; Bewers et al., 1985; Bewers et al., 1986; Freimann et al., 1993). Intercalibrations were organized by ICES, OSPARCOM/JMG, IOC, HELCOM/BMP, the German BLMP programme, and more recently by BCR of EEC.

An example of our participation with the TXRF method in the certification of a BCR reference sea water material is demonstrated in Fig. 8.

In this connection also a special paper on this subject is referred to (Schmidt and Freimann, this workshop).

Conclusion

It has been shown that the different steps of sampling, sample treatment, handling of equipment and chemicals, very sensitive and powerful analytical methods, data evaluation and banking, quality assurance procedures for validation including intercalibrations and use of certified reference materials are all necessary items that lead to a marked improvement in detection limits and reliability of data. A historical example of the improvement of measurements with time by BSH is shown in Fig. 9: This time series of medians of cadmium concentrations measured by essentially the same analytical procedure of GFAAS in unfiltered sea water samples taken from 1972 to 1986 in the German Bight show a substantial decrease of apparent cadmium levels. The same phenomenon has been found by many other researchers in the early years of trace metal sea water analysis. Generally it has been shown by other workers and ourselves that this apparent decrease is mainly caused by the improvement of analytical techniques.

Figure 8. Certification of "Seawater CRM 403" of the Community Bureau of Reference (BCR) of the Commission of the European Communities.

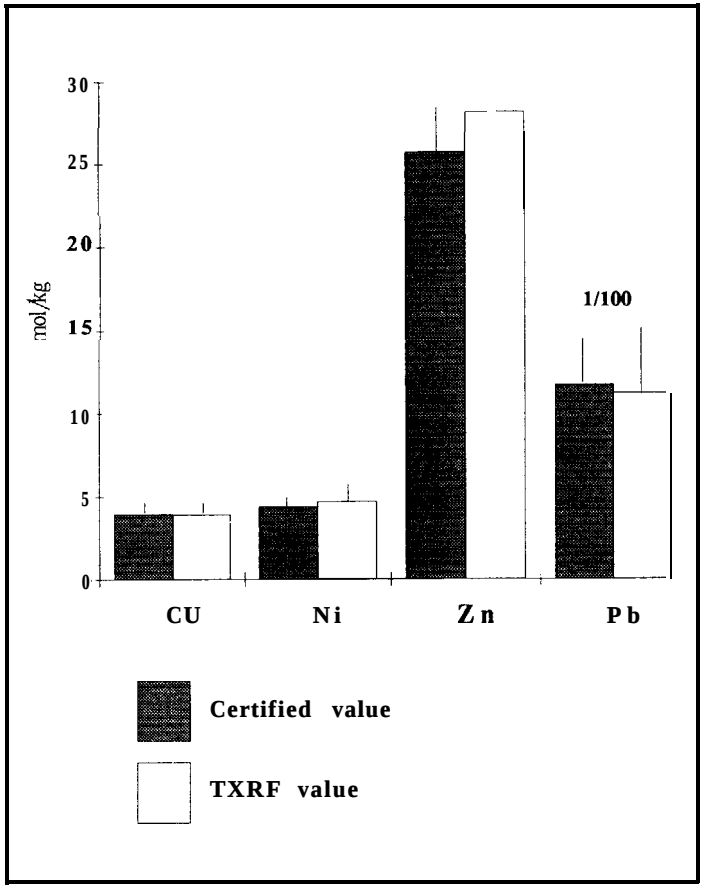
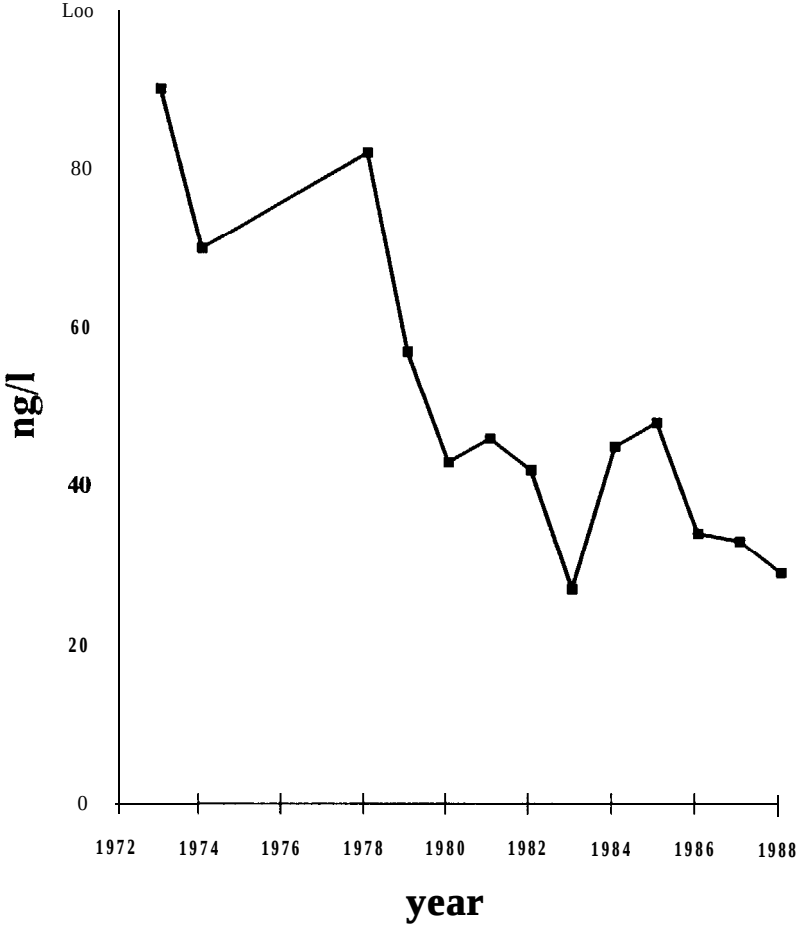


Figure 9. Time series of the medians of cadmium concentrations in the German Bight (measurement of unfiltered water samples by AAS).



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Design Principles of Clean Laboratories for Trace Metal Analysis

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Introduction

With modern techniques for sampling and analysis, extremely low levels of many trace metals have been found recently in open ocean water. Investigations of the open Baltic Sea also show very low concentrations. To achieve results with a high analytical quality it is increasingly necessary to avoid all possible sources of contamination. Contamination by heavy metals may occur through all stages of the sampling and analysis processes, e. g. by handling, clothes, equipment, samplers, storage bottles, contact with metals aboard a ship, contamination by the "cloud" of dissolved and particulate matters surrounding a research vessel at station for some time, reagents, solvents, airborne and waterborne metals etc. Most hazardous are those steps where handling of open samples is necessary. In these cases, contamination of the samples will most probably be possible by dust particles, clothes and shoes as well as parts of the body of the analyst. To avoid these contamination sources, clean laboratories have been proposed and used in many marine institutions.

Handling of open samples and contamination risks are mainly occurring at two stages of the entire process:

1. Sample treatment aboard the research ship: partition of samples, filtration, acidification, freezing, pre-separation procedures like column chromatography etc.
2. Chemical separation procedures in the land-based analytical laboratory and preparation of samples prior to measurement.

For both stages, clean laboratories have been designed and used by BSH for a number of years and this experience will be discussed here. However, for details of sampling and analysis methods, reference should be made to Schmidt, 1994, in this volume.

To illustrate the importance of avoiding contamination by dust a TXRF spectrum of a dust particle is presented in Fig. 1, showing numerous peaks for heavy metals, and this is compared with a similar TXRF spectrum of a cleaned silica sample carrier (Fig.2) showing only a minute peak for iron.

Figure 1. TXRF Spectrum of a dust particle.

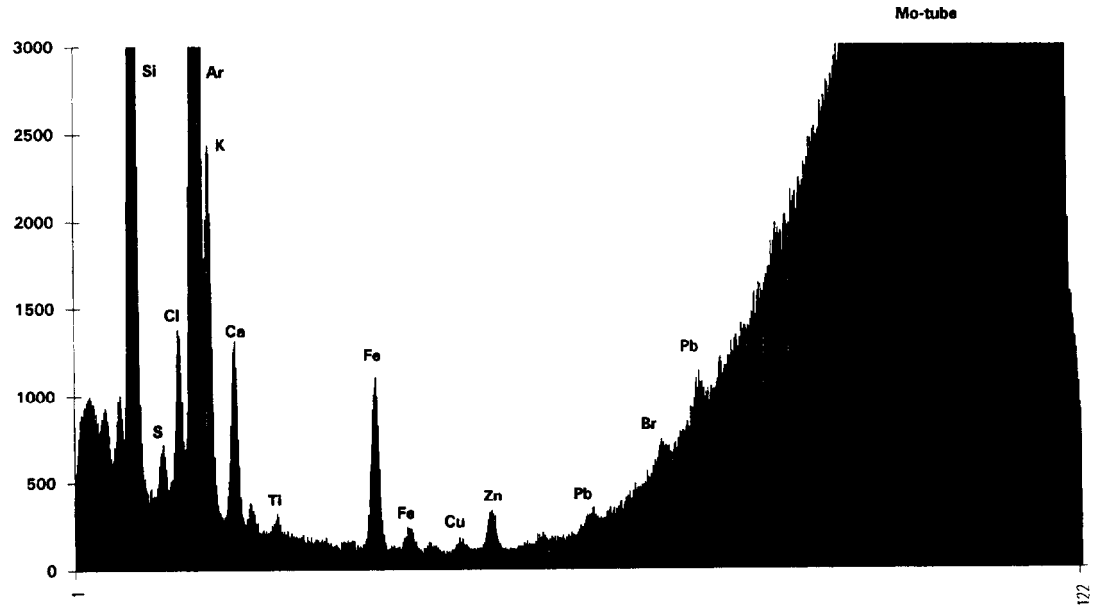
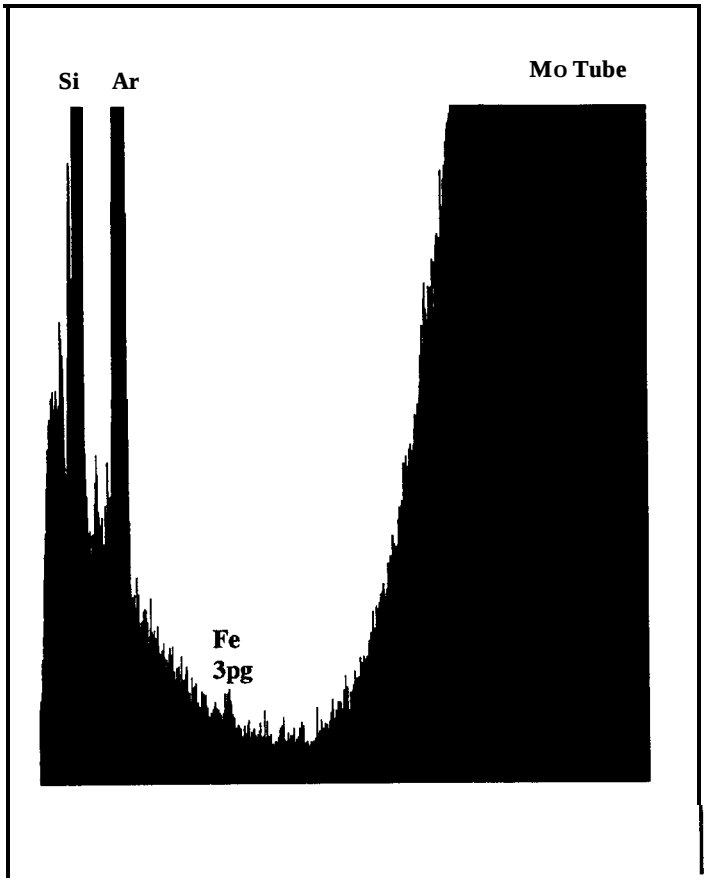


Figure 2. TXRF spectrum of a cleaned silica glass sample carrier.



Clean laboratories

In the past, it has been demonstrated that it is extremely difficult to convert existing laboratories in BSH to the necessary standard of clean rooms. With the construction of a new annex building of "Laboratorium Sülldorf" it was possible to plan in advance a number of clean room facilities for the trace metal laboratories. These were put into service in 1989.

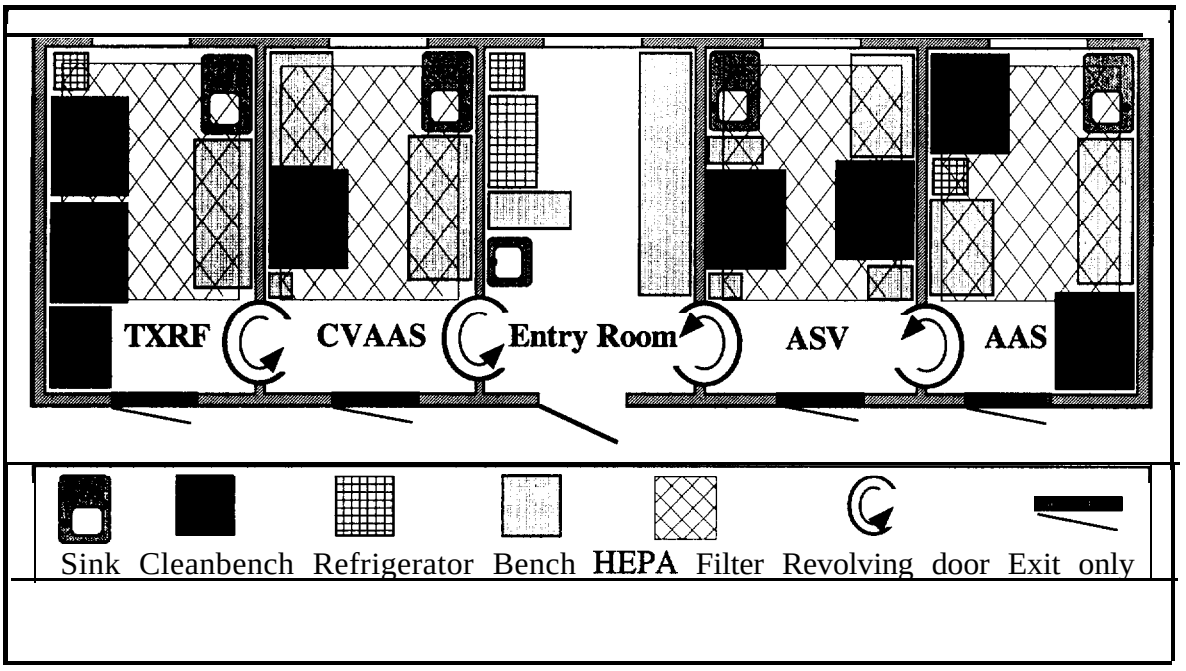
Details of the features of the BSH laboratories that have proven to be very advantageous, or essential, for accurate and precise trace metal work are presented below and illustrated in Fig. 3:

A suite of 5 laboratories, 4 clean rooms and 1 central service room.
Labs are adjacent to each other to provide better service, maintenance and procurement of purity standards.
Each of the 4 clean labs is equipped with an independent high efficiency (HEPA) filter unit suspended from the ceiling.
Access only through the central entry and service laboratory functioning as an air lock.
Doors of the 4 clean labs can only be opened from inside, and there is no access from the hall; they serve only as emergency exits.
Access to each clean lab is through a separate revolving door made from plastics (PVC and clear polycarbonate), according to our specifications: they serve as small air locks and save space.
Windows are sealed and cannot be opened under normal circumstances.
The entire laboratory system is kept under a slight overpressure to prohibit air and dust leaking in.

- Blue adhesion mats placed in the entrance area of each lab: dust particles from shoes stick on these; mats can be washed and re-used very often.

Each of the 4 clean labs is exclusively used for the chemical separation procedures of one single analytical method.
Cross-over contamination by mercury vapour through the air ventilation system has never been found, although macro amounts of liquid mercury are necessarily used in the ASV laboratory, and ultratrace concentrations of mercury are determined in the CVAAS laboratory.
For the highly instrumentalized methods of TXRF and GFAAS, only the chemical separation procedures are performed in the clean labs; the enclosed samples for measurement are then transported to an "instrumental laboratory" across the hall, where the complex and expensive instruments are operated together under acid-free atmospheric conditions.
For the methods CVAAS and ASV, the measurement instruments are small and directly connected to the chemical preparation steps: here the entire analysis is performed in the clean lab.
The air within the entire clean lab is maintained dust-free down to approx. class 100 of US Federal Standard No. 209b. However, any human activity within the laboratory produces dust particles and disturbs the laminar air flow. For this reason, the really sensitive chemical preparation steps are performed within separate clean benches in the clean laboratories. They provide class 10 conditions.
Floors, walls and ceilings of the clean labs are covered by plastics and paints without heavy metal pigments etc., with smooth surfaces without cracks or joints.

Figure 3. Details of the features of the clean laboratories of the Bundesamt für Seeschiffahrt und Hydrographie (BSH) in Hamburg Sülldorf.



Clean laboratory containers

For the handling of samples on board the research vessels, two clean laboratory containers have been designed and constructed, according to our specifications, for trace metal work. They are both built according to international standards and regulations for maritime shipping and are mechanically sturdy, licensed and registered to allow maritime transportation by commercial container freight lines, virtually to any port around the globe.

In recent years, our containers have been used successfully on research cruises in the North Sea and the Baltic Sea as well as in various areas of the Atlantic Ocean. They have been shipped commercially, inter alia, to or from the ports of Cape Town (South Africa), Funchal (Madeira), Dakar (Senegal), Halifax (Canada), and Reykjavik (Iceland).

Features of the two individual containers that have proven advantageous as well as major applications demonstrated in our group are listed below for each of the two containers.

Clean laboratory container No. 1 (Fig:. 4):

Constructed and put into service in 1985; several modifications have been made according to experience gained.

Used predominantly for sampling, sample pretreatment, handling and cleaning of samplers.

Standard size 20 ft. container with 10 ft. overall height.

Container consisting of two separate rooms of equal size.

First or entry room is kept at semi-clean condition, providing access to the second room.

Second room is kept under clean room conditions similar to clean land-based laboratories.

First room provides ample space for large equipment, e. g. deep freezers, racks for large-volume GoFlo samplers.

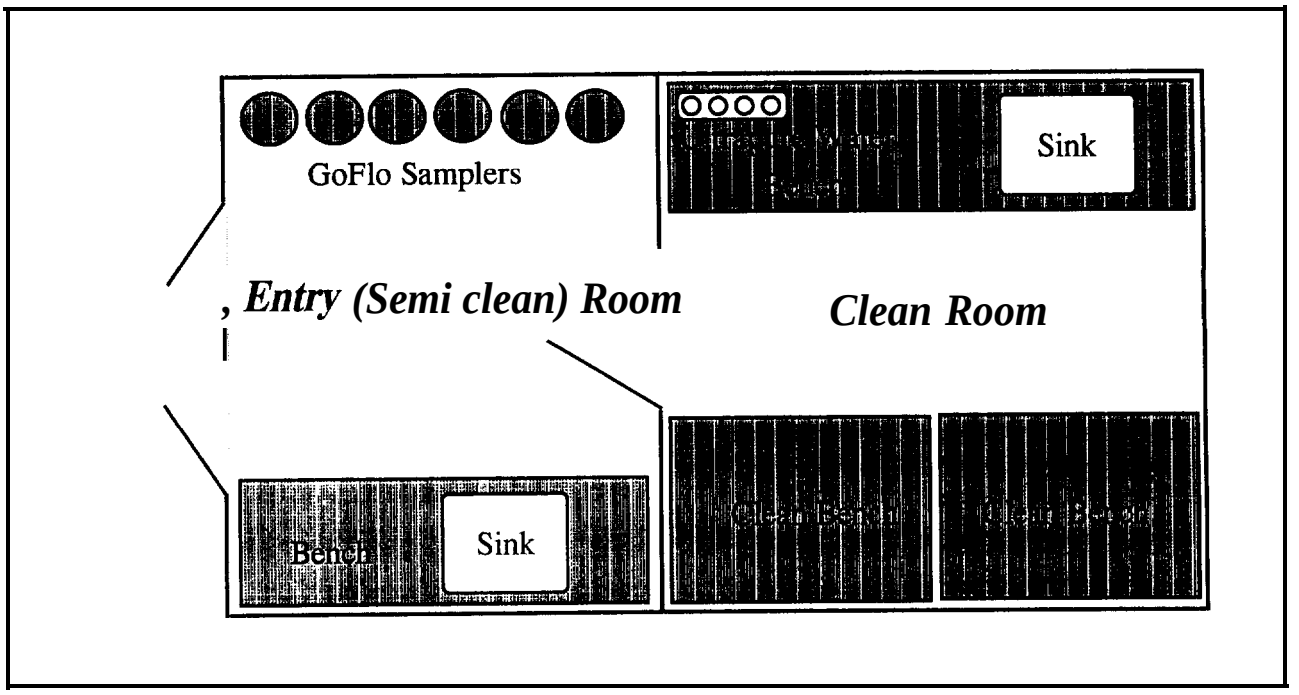
Second room contains two clean benches for chemical operations, filtration etc.

Ultra pure water preparation unit is mounted permanently in the second room.

Air filtering units suspended from the ceiling in both rooms; filtered air streams move from first to second laboratory.

Air conditioning and cooling unit was found necessary to reduce heat generated by equipment.

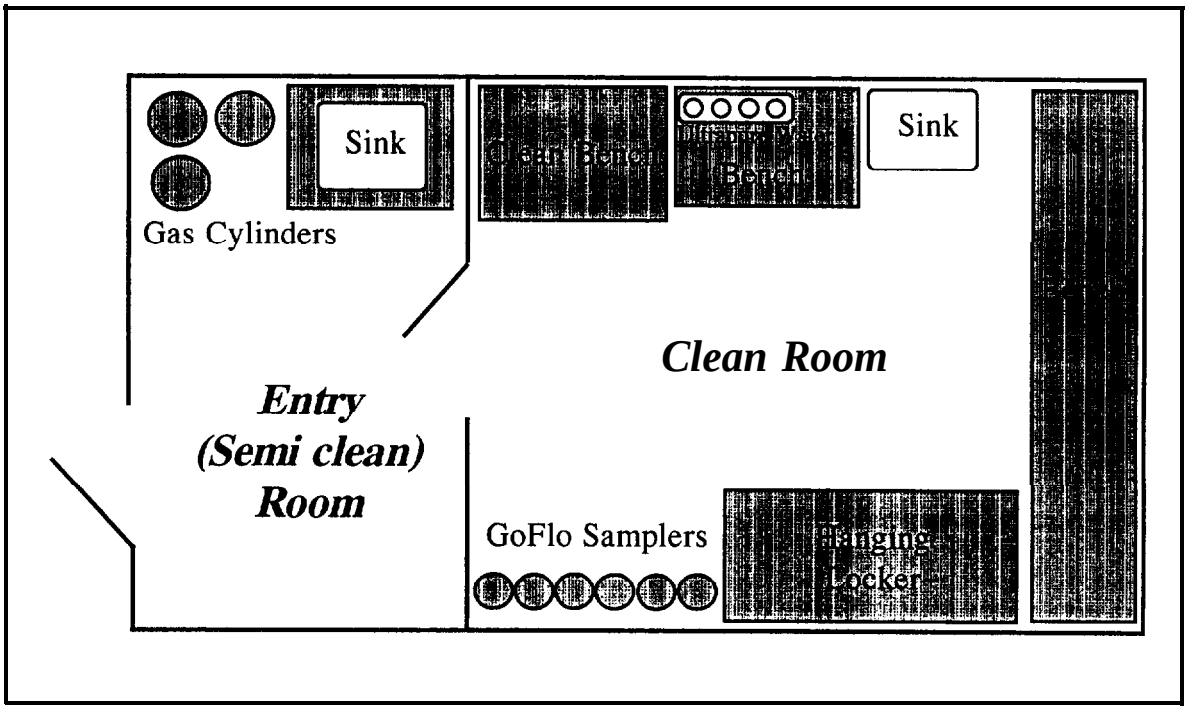
Figure 4. Clean laboratory container for sea water sampling.



Clean laboratory container No. 2 (Fig.5):

Constructed according to our specifications in 1987.
This container was mainly designed for on-board analysis.
Standard 20 ft. container with 9 ft. height.
First, entry room smaller than main, second, clean room.
Other features and equipment analogous or similar to those of clean lab container No. 1.
Both containers are operated under slight overpressure, similar to land-based clean laboratories.

Figure 5. Clean laboratory for on board analysis of sea water samples.



Uses and successful applications of both clean lab containers "clean vans".

Very frequent operation of our MERCOS sampler system for monitoring in the German Bight (North Sea): handling, cleaning, preparation, reconditioning of the samplers.

- Working with 30 1, 12 1, and 5 1 GoFlo samplers in series.
- Pressure filtration with our Sartorius system inside the clean bench.
- Pressure filtration directly on 30 1 GoFlo samplers.
- Partitioning, distribution, preparation of many individual samples for participants of a large multi-team cruise.
- Cleaning of bottles.
- Acidification and freezing of water samples.
- Chemical separation by micro-column chromatography for speciation analysis of chromium in sea water.
- Chemical separation and sample preparation by newly developed automatic chromatographic instruments for trace metal determination by TXRF.
- "Real time" on-board analysis of Pb and Cd by ASV.
- On-board analysis for Hg by CVAAS.
- Use of container as quasi-stationary, remote laboratory positioned on seaward pier in the outer harbour of Helgoland island for 4 weeks mesocosm tank experiments.
- Simultaneous participation of different members of our group in two separate large cruises in different sea areas at the same time was possible by using each of the two containers: IOC/GIPME Baseline Study for Trace Contaminants in the Open Atlantic Ocean, 2nd cruise, North Atlantic, and BSH/HELCOM baseline cruise in the entire Baltic Sea, both August 1993.

Conclusions

Almost five years ago 4 clean room laboratories were put into service and this has led to substantial improvement in analytical quality of results in ultratrace metal analysis of sea water.

8 years of experience with clean laboratory containers aboard modern research vessels have demonstrated the flexible applicability and the many advantages of this system. The introduction of similar containers by other trace metal analysts in marine institutions is strongly recommended by BSH.

The Role and Application of Independent Analytical Procedures in Trace Metal Analysis

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1. Introduction

To ensure the quality of analytical results and to control and prove the accuracy and precision achieved by different analytical methods, two ways have been well established and generally accepted in the marine community:

(1) Participation of laboratories in national and international intercalibration rounds,

(2) invitation of selected analysts (that had previously demonstrated the reliability of their determinations) to certification exercises for marine reference materials.

The Bundesamt für Seeschiffahrt und Hydrographie (BSH, Federal Maritime and Hydrographic Agency) is responsible for the determination of a large number of different pollutants in the marine environment especially in the North Sea and the Baltic Sea. The BSH is involved in different national and international marine monitoring programs. For the determination of vanadium, manganese, iron, cobalt, nickel, copper, zinc, lead and uranium in sea water BSH mainly uses the relatively new method of total reflection X-ray fluorescence analysis (TXRF) for its routine work.

The Community Bureau of Reference (BCR) of the Commission of the European Communities (EC) in Brussels operates a series of intercalibration exercises and certification campaigns for trace heavy metals in water of the open sea and coastal and estuarine areas. The results from these exercises have shown that many European laboratories use different modifications of atomic absorption (GFAAS) or voltammetric methods (ASV etc.). Details of the methods used by participants are listed in Table 1 and 2.

Table 1. Methods of final detection used for the certification of sea water reference material (CRM 403).

Method	Abbreviation	Laboratory Number
Atomic Absorption Spectrometric Methods		
Flame Atomic Absorption Spectrometry	FAAS	4, 12, 13
Electrothermal Atomic Absorption Spectrometry	ETAAS	4, 5, 9, 12
Zeeman Electrothermal Atomic Absorption Spectrometry	ZETAAS	1, 3, 13, 14, 15
Voltammetric Methods		
Adsorptive Differential Pulse Cathodic Stripping Voltammetry	ADPCSV	6, 8
Anodic Stripping Voltammetry	ASV	
Cathodic Stripping Voltammetry	CSV	5
Differential Pulse Anodic Stripping Voltammetry	DPASV	8, 10, 11
Flow Potentiometric Stripping Analysis	FPSA	10
Other Methods		
Total Reflection X-Ray Fluorescence Analysis	TXRF	18
Isotope Dilution Mass Spectrometry	IDMS	16

Table 2. Methods of final detection used for the intercomparison of trace metals in estuarine water.

Method	Abbreviation	Laboratory Number
Atomic Absorption Spectrometric Methods		
Flame Atomic Absorption Spectrometry	FAAS	3, 11, 17, 19, 23, 25
Electrothermal Atomic Absorption Spectrometry	ETAAS	4, 5, 11, 22, 29
Zeeman Electrothermal Atomic Absorption Spectrometry	ZETAAS	1, 3. 8, 10, 23, 25, 26
Voltammetric Methods		
Adsorptive Differential Pulse Cathodic Stripping Voltammetry	ADPCSV	6, 8
Anodic Stripping Voltammetry	ASV	30
Cathodic Stripping Voltammetry	c s v	30
Differential Pulse Anodic Stripping Voltammetry	DPASV	9
Flow Potentiometric Stripping Analysis	FPSA	10
Other Methods		
Total Reflection X-Ray Fluorescence Analysis	TXRF	12
Isotope Dilution Mass Spectrometry	IDMS	16
Inductively Coupled Plasma Atomic Emission Mass Spectr.	ICPMS	2

In examining the successes and failures of measurements by such methods, the organizers as well as the participants expressed a strong desire to have access to participants providing very different, truly "independent" analytical methods, preferably with a simultaneous multi-element determination capability. Only two analytical methods were found to meet these requirements: TXRF which was only provided by BSH, and isotope dilution mass spectrometry (IDMS) that was later offered by a few participants. Whereas IDMS results showed in some cases severe systematic errors that led to the exclusion of data using this procedure, TXRF was shown to perform well in the intercalibrations and certifications. To illustrate the situation, the results from the second trace metal intercalibration (associated with the certification of estuarine water from the Tejo river) revealed that too many participants offered AAS and ASV whilst IDMS (or ICPMS) methods were in short supply, whereas BSH was the only laboratory to use TXRF. During the course of these BCR exercises, TXRF was successfully established by our group as an independent analytical procedure.

The first intercalibration experiment presented below was a certification campaign associated with the production of sea water reference material (CRM). The second experiment was an intercomparison exercise of an estuarine water as a first step to produce an estuarine water reference material.

2. Experimental

2.1. Certification of sea water reference material

2.1.1. Sample preparation, homogeneity and stability

Approximately 2600 litres of sea water from the central part of the Southern Bight of the North Sea were collected in a precleaned linear polyethylene (LPE) storage tank. The sample was filtered using 0.45 μm membrane filter and then acidified to pH = 1.5 using nitric acid (purified by sub-boiling distillation). The 2 litre LPE sample bottles were directly filled from the LPE tank using a peristaltic pump.

Homogeneity and stability tests have been performed for Cd, Cu, Pb and Zn by four different laboratories prior to the certification campaign. For more details about sample preparation, homogeneity and stability tests see Quevauviller et al., 1992a.

2.1.2. Analytical methods for sea water analysis

Each of the 18 European laboratories which participated in the certification exercise were requested to make a minimum of five replicate determinations. Table 1 gives an overview of the different methods used by participants. BSH obtained four 2 litre bottles and made six replicate determinations on the water in each bottle.

2.2. Intercomparison on trace elements in estuarine water

2.2.1. Sample preparation, homogeneity and stability

Two batches of different salinities of estuarine water, from the River Tagus (Tejo) estuary, were collected in 300 litre linear polyethylene (LPE) tanks. Batch A had a salinity of 16.4 while batch B had a salinity of 7.4. The samples were filtered using 0.45 μm filter cartridges and acidified to about pH=2 using hydrochloric acid (Merck, suprapur). The 1 litre LPE sample bottles were directly filled from the LPE tank using a peristalic pump.

Homogeneity and stability tests have been performed for As, Cd, Cu, Pb and Zn by six different laboratories. More details about sample preparation, homogeneity and stability tests can be found by Kramer et al., 1991.

2.2.2. Analytical methods for estuarine water analysis

Each of the 3 1 European laboratories which participated in the intercomparison exercise were requested to make a minimum of five independent replicate determinations for batch A and batch B estuarine water samples. Table 2 gives an overview of the different methods applied.

Because BSH got 1 litre of each batch it was only possible to make four replicate determinations by TXRF. In this paper the results of batch B will be presented.

2.3. TXRF measurements

Apparatus

The TXRF spectrometer consists of an EXTRA II module (R.Seifert, D-2070 Ahrensburg) with double beam excitation (molybdenum and tungsten tubes), an X-ray generator (Iso-Debyeflex 1001), a Si(Li) detector, an automatic sample changer, and a computer controlled multichannel analyzer system (Link Systems AN 10000).

Preconcentration of trace metals

The trace metals are preconcentrated and separated from the sea water and estuarine water salt matrix by reverse phase chromatography described by Prange (1983) and Freimann and Schmidt (1989). 200 g of the water samples are spiked with 2.5 μg/kg Se (Merck, AAS-Standard solution) as internal standard, adjusted to pH = 4.8 with sodium hydroxide solution (Merck, suprapur) and acetate buffer (Merck, suprapur). The trace metals are complexed by the addition of a methanolic solution of sodium dibenzyldithiocarbamate (NaDBDTC, Fluka). The carbamate complexes are adsorbed on a reverse phase column (Chromosorb W, Merck).
After drying of the column, the complexes are eluted with 3 ml of a mixture of chloroform/methanol (purified by sub-boiling distillation). 100 μl of this solution are dried by means of a special device, as described in Prange and Knöchel (1985), to achieve a thin film on the silica glass sample carrier.
Using molybdenum excitation and a measuring time of 3000 seconds, vanadium, manganese, iron, cobalt, nickel, copper, zinc, lead, and uranium could be determined.

For analytical blanks 200 g of ultrapure water (MilliQ, Millipore), containing 500 mg sodium chloride (Merck, suprapur), were analyzed by the procedure for the determination of trace metals in sea water and estuarine water as described above.
All sample treatments are carried out in a laminar clean bench (Class 10, U.S. Federal Standard 209b) installed in a clean room laboratory (Class 100).

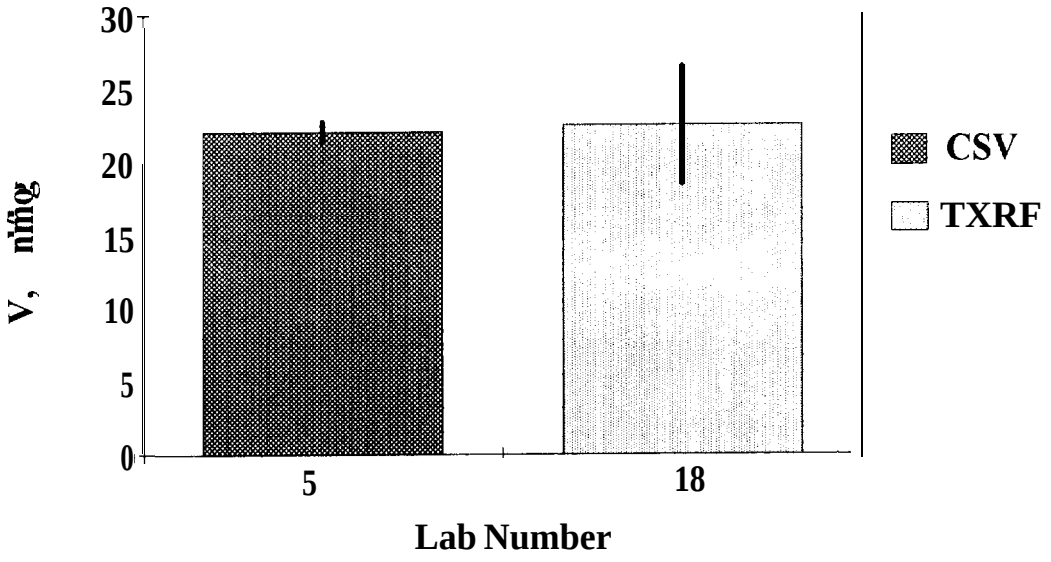
3. Results and Discussion

3.1. Trace elements in sea water

Vanadium
Only two laboratories measured this element. The results of the two laboratories which use cathodic stripping voltammetry and TXRF respectively agree very well (see Fig. 1).

Unfortunately, the number of data sets were not sufficient for the certification of vanadium.

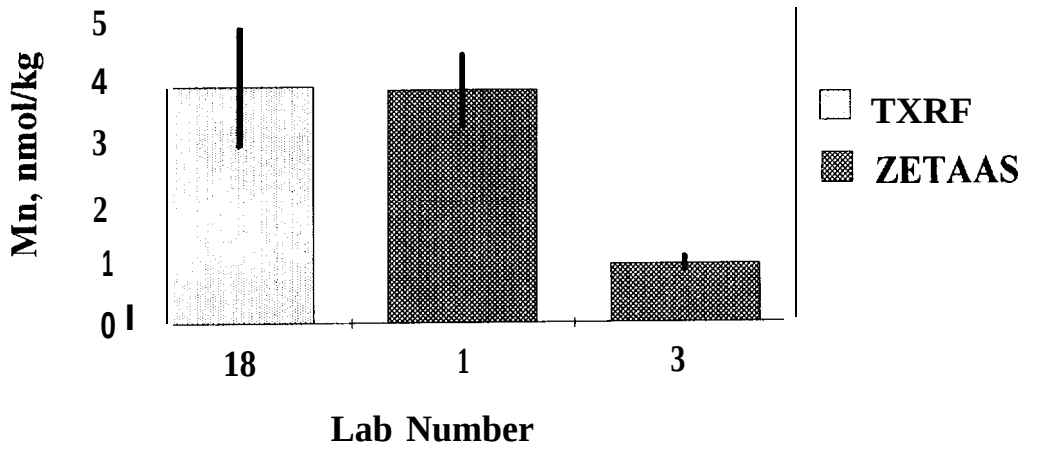
Figure 1. Comparison of the vanadium content of the sea water reference material measured by cathodic stripping voltammetry (CSV) and TXRF.



Manganese

Four laboratories measured manganese. The results of three laboratories were accepted by BCR (see Fig. 2), but the number of data sets were not sufficient for the certification of manganese.

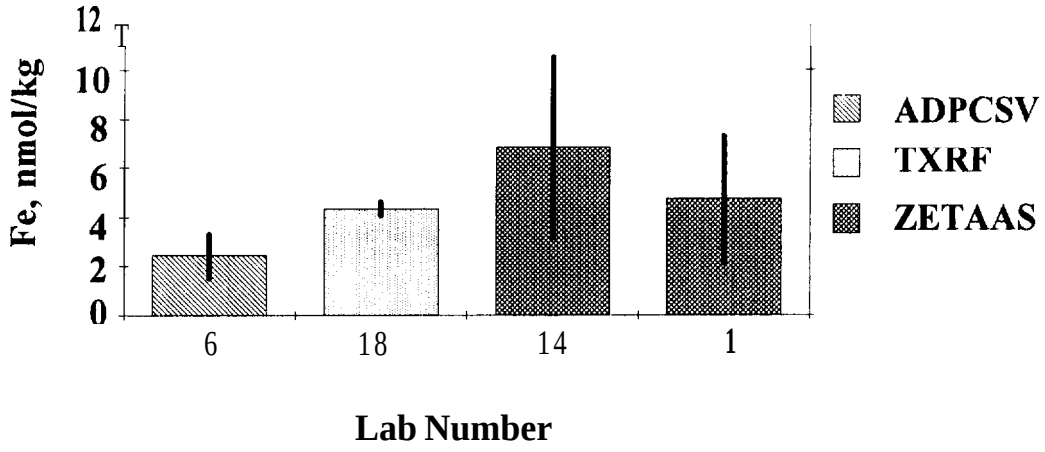
Figure 2. Comparison of the manganese content of the sea water reference material measured by Zeeman electrothermal atomic absorption spectroscopy (ZETAAS) and TXRF.



Iron

Four laboratories measured iron (see Fig.3). The standard deviations of the results are very high probably due to contamination problems. Therefore the iron content could not be certified. TXRF measurements showed that one of the four bottles obtained, seemed to be contaminated by iron.

Figure 3. Comparison of the iron content of the sea water reference material measured by adsorptive differential pulse cathodic stripping voltammetry (ADPCSV), Zeeman electrothermal atomic absorption spectroscopy (ZETAAS), and TXRF.



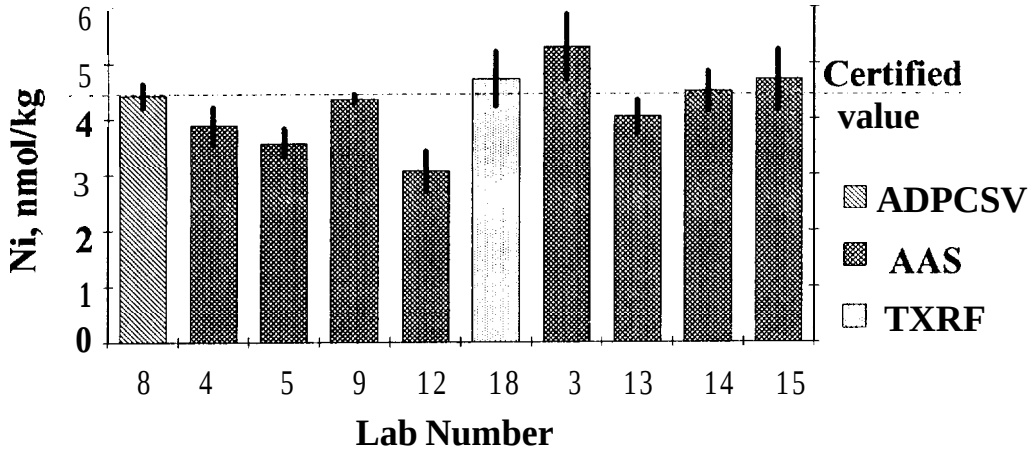
Cobalt

Only one laboratory (BSH) provided measurements of cobalt, and therefore a comparison of TXRF with other analytical techniques is not possible. BSH reported a concentration of 0,18 nmol/kg Co with a standard deviation of 0,03 nmol/kg (n=24).

Nickel

Eleven laboratories sent their Ni results to BCR from which ten were accepted (see Fig. 4). The Ni content was certified to 4.36 nmol/kg with a standard deviation of 0.36 nmol/kg.

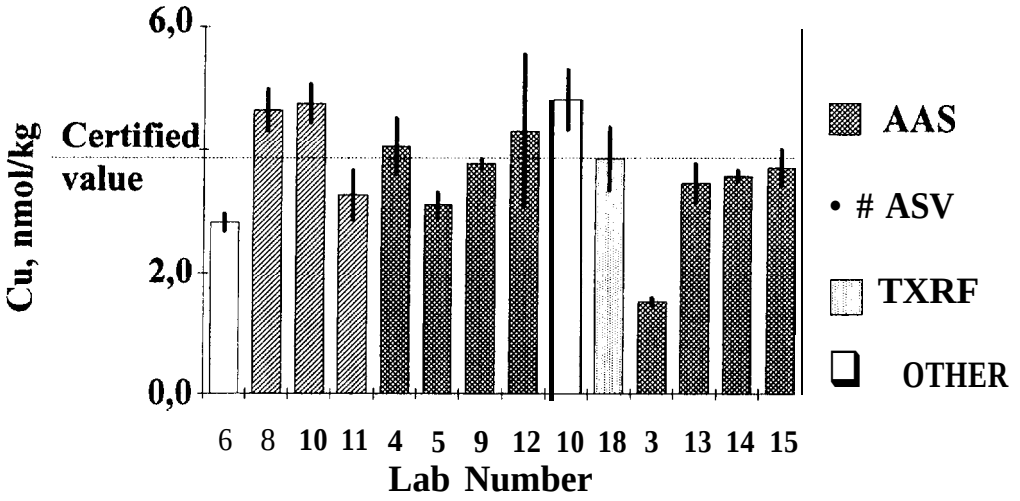
Figure 4. Comparison of the nickel content of the sea water reference material measured by adsorptive differential pulse cathodic stripping voltammetry (ADPCSV), atomic absorption spectroscopy (AAS), and TXRF. The certified value is marked by a dashed horizontal line.



Copper

Fourteen laboratories measured copper. BCR accepted thirteen data sets measured by seven different methods (see Fig.5). The Cu content was certified at 3.90 nmol/kg with a standard deviation of 0.37 nmol/kg.

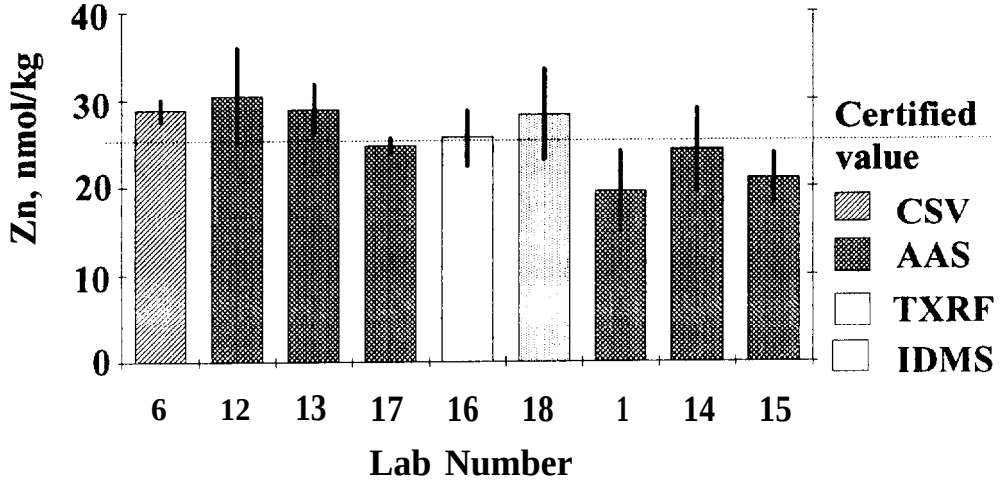
Figure 5. Comparison of the copper content of the sea water reference material measured by atomic absorption spectroscopy (AAS), differential pulse anodic stripping voltammetry (DPASV), TXRF, and other methods (ADPCSV, FPSA). The certified value is marked by a dashed horizontal line.



Zinc

Zinc has been measured by twelve laboratories. BCR accepted nine data sets measured by six different methods (see Fig. 6). The Zn content was certified at 25.7 nmol/kg with a standard deviation of 2.9 nmol/kg.

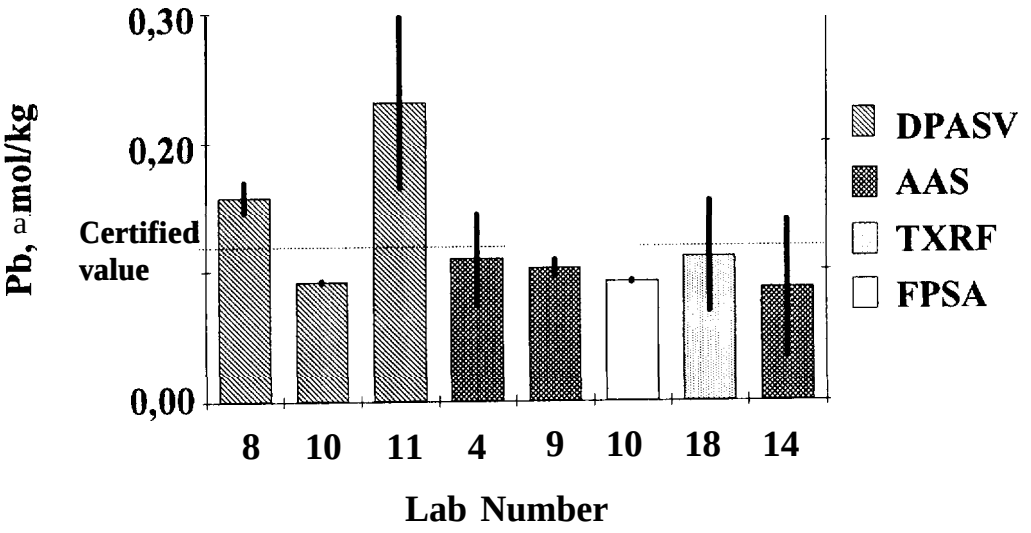
Figure 6. Comparison of the zinc content of the sea water reference material measured by cathodic stripping vohammetry (CSV), atomic absorption spectroscopy (AAS), TXRF, and isotope dilution mass spectrometry (IDMS). The certified value is marked by a dashed horizontal line.



Lead

Ten laboratories sent their Pb results to BCR from which nine data sets have been accepted (see Fig. 7). The Pb content was certified at 0.117 nmol/kg with a standard deviation of 0.025 nmol/kg.

Figure 7. Comparison of the lead content of the sea water reference material measured by differential pulse anodic stripping voltammetry (DPASV), atomic absorption spectroscopy (AAS), TXRF, and flow potentiometric stripping analysis (FPSA). The certified value is marked by a dashed horizontal line.



Uranium

Only one laboratory (BSH) reported results for uranium, and therefore a comparison of TXRF with other analytical techniques is not possible. BSH found a uranium content of 14.0 nmol/kg with a standard deviation of 1.3 nmol/kg (n=24).

3.2. Trace elements in estuarine water (batch B)

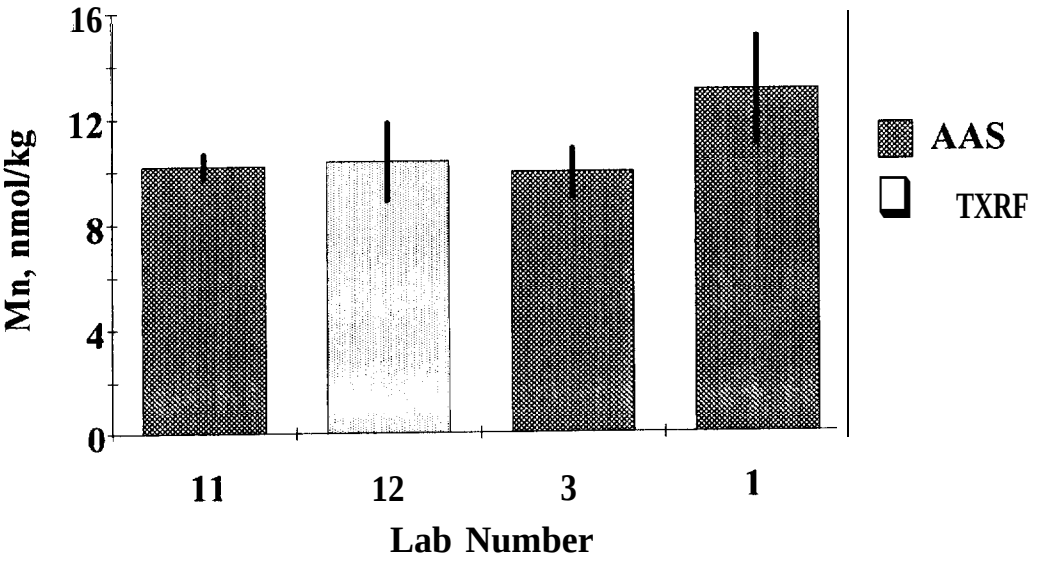
Vanadium

Only one laboratory (BSH) reported results for vanadium and therefore a comparison of TXRF with other analytical techniques is not possible. BSH found a vanadium content of 25.3 nmol/kg with a standard deviation of 0.6 nmol/kg (n=4).

Manganese

Five laboratories measured manganese using FAAS, ZETAAS and TXRF. The results of four laboratories were accepted by BCR (see Fig. 8).

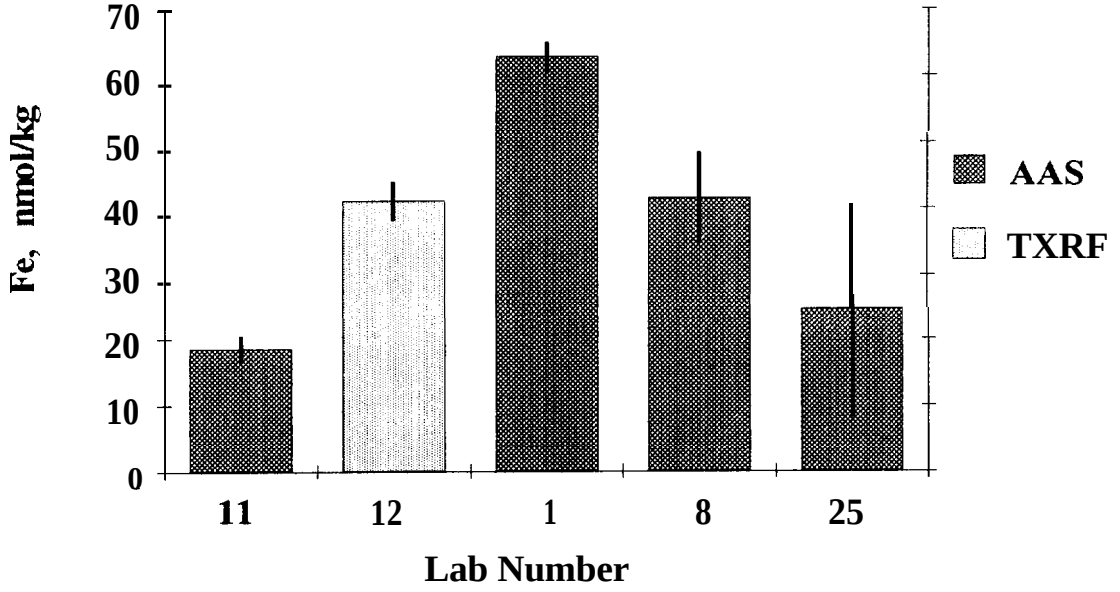
Figure 8. Comparison of the manganese content of the estuarine water sample measured by atomic absorption spectrometry (AAS) and TXRF.



Iron

Seven laboratories sent iron results to BCR. Two data sets were rejected by BCR due to extremely high values. The remaining five data sets are presented in Fig. 9. It is readily apparent that in two cases blank problems leads to high standard deviations of the mean values.

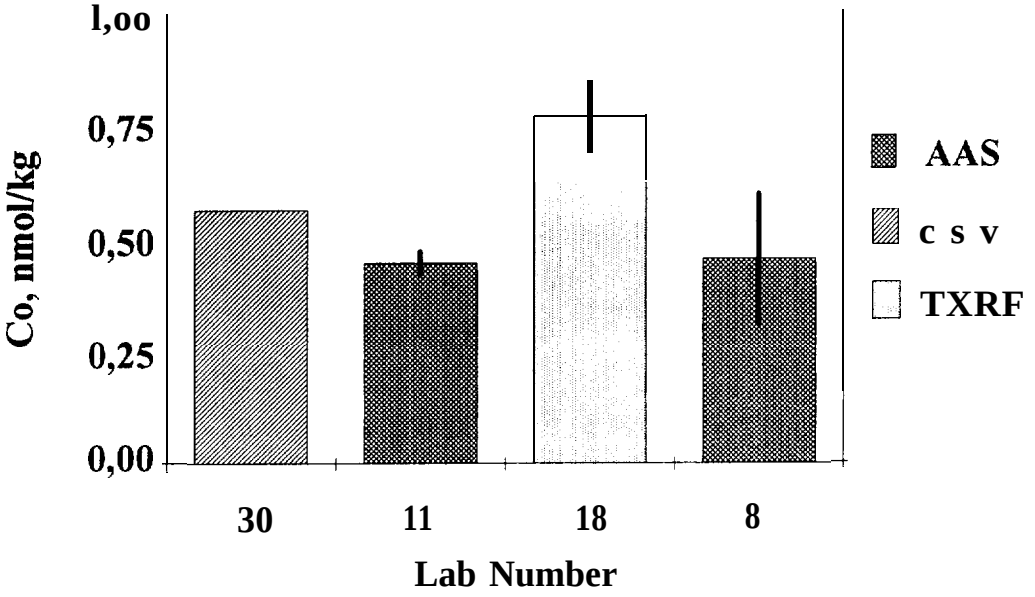
Figure 9. Comparison of the iron content of the estuarine water sample measured by atomic absorption spectrometry (AAS) and TXRF.



Cobalt

Five laboratories measured this element. BCR accepted four data sets measured by CSV, ETAAS, ZETAAS, and TXRF (see Fig. 10). The TXRF data seem to be too high compared to the other analytical techniques.

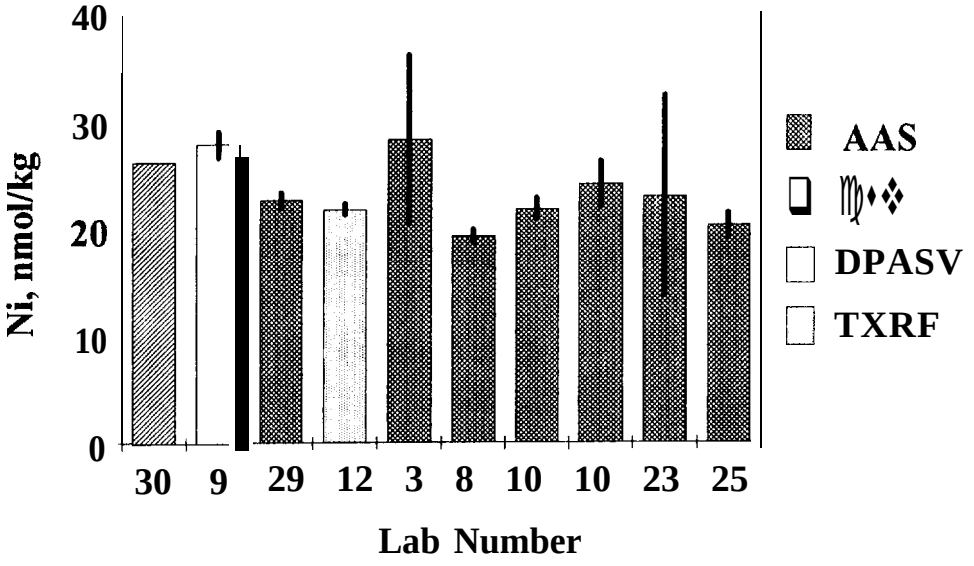
Figure 10. Comparison of the cobalt content of the estuarine water sample measured by atomic absorption spectrometry (AAS), cathodic stripping voltammetry (CSV), and TXRF.



Nickel

Thirteen laboratories sent their nickel results to BCR, from which three data sets were rejected due to extremely high values. The remaining ten data sets are presented in Fig. 11. The methods applied were CSV, DPCSV, ETAAS, ZETAAS, and TXRF.

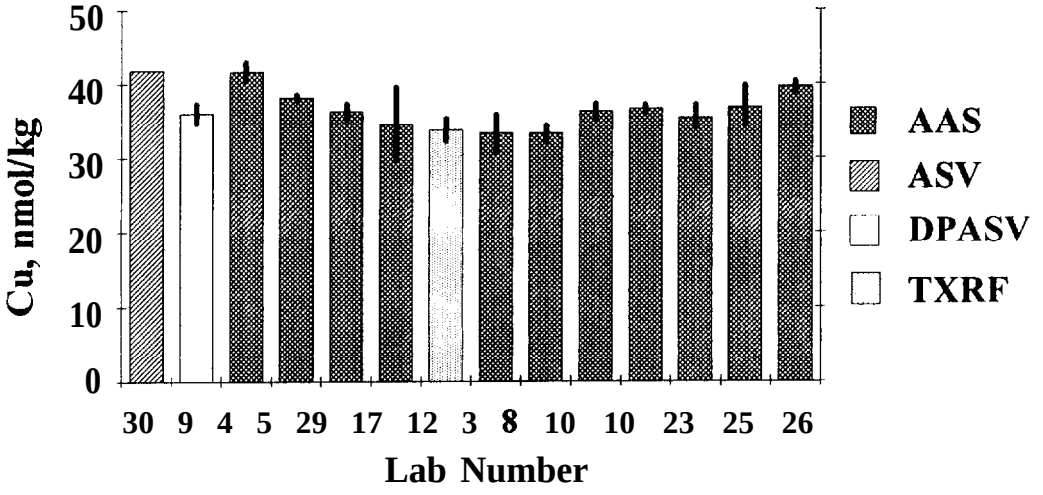
Figure 11. Comparison of the nickel content of the estuarine water sample measured by atomic absorption spectrometry (AAS), cathodic stripping voltammetry (CSV), differential pulse anodic stripping voltammetry (DPASV), and TXRF.



Copper

Seventeen laboratories measured copper. Two extremely high data sets and one extremely low data set were rejected by BCR. The remaining fourteen data sets are presented in Fig. 12. Seven laboratories measured copper by ZETAAS, three by ETAAS, and one each by ASV, DPASV, FAAS, and TXRF.

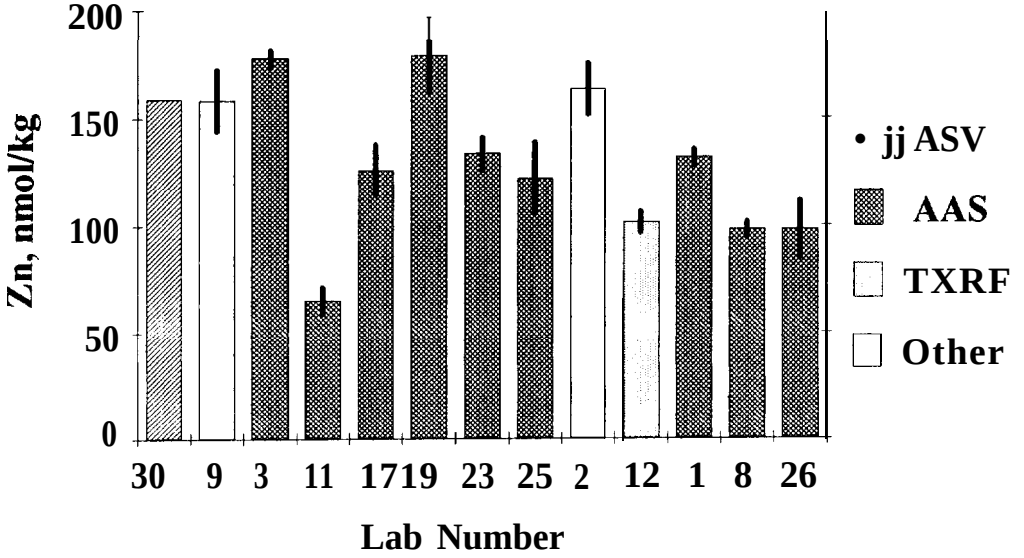
Figure 12. Comparison of the copper content of the estuarine water sample measured by atomic absorption spectrometry (AAS), anodic stripping voltammetry (ASV), differential pulse anodic stripping voltammetry (DPASV), and TXRF.



Zinc

Fourteen laboratories sent their zinc results to BCR, from which one data set was rejected due to the extremely high value (mean: 2459 nmol/kg). The remaining thirteen data sets show a relatively large scatter between 65 and 178 nmol/kg (see Fig. 13) in comparison to the relatively low scatter of the zinc data in the certification campaign for sea water. This effect may be explained by methodical problems due to the high dissolved organic matter content of the estuarine water samples. The methods applied were ASV, DPASV, FAAS, ICPMS, ZETAAS, and TXRF.

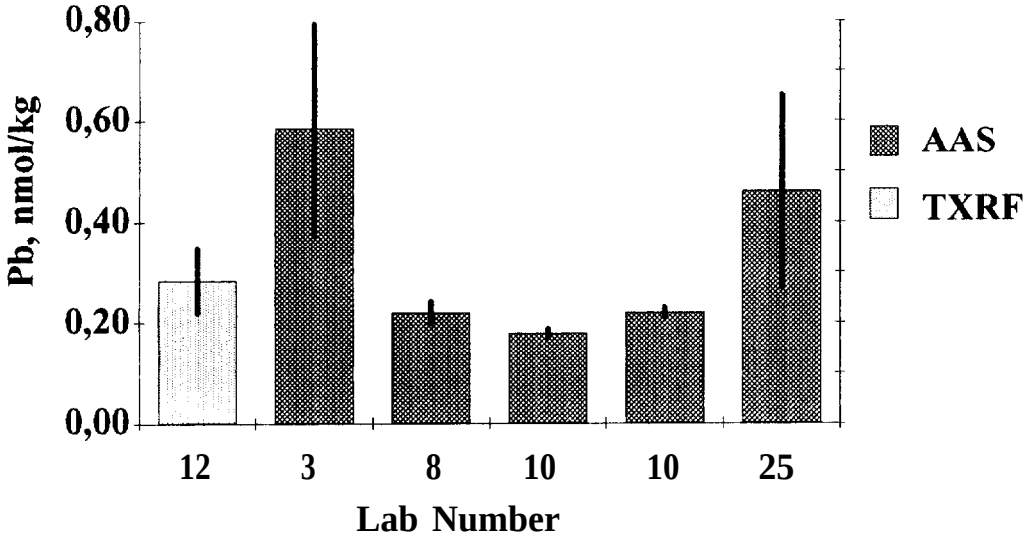
Figure 13. Comparison of the zinc content of the estuarine water sample measured by atomic absorption spectrometry (AAS), anodic stripping voltammetry (ASV), TXRF, and other methods (differential pulse anodic stripping voltammetry, and inductively coupled plasma atomic emission mass spectrometry).



Lead

Ten laboratories measured lead, from which three extremely high data sets and one extremely low data set were rejected by BCR. The results of the remaining six laboratories are presented in Fig. 14. The two highest values in the figure show the highest standard deviations. This effect may be explained by contamination problems.

Figure 14. Comparison of the lead content of the estuarine water sample measured by atomic absorption spectrometry (AAS) and TXRF.



Uranium

Uranium has only been measured by TXRF and therefore a comparison to other analytical techniques is not possible. We found a uranium content of 4.57 nmol/kg with a standard deviation of 0.43 nmol/kg (n=4).

4. Conclusions

TXRF has successfully participated, as a relatively new method, in the certification exercise for copper, nickel, zinc, and lead in sea water reference material (CRM 403), Quevauviller et al. (1992b). For other elements, which could not be certified, TXRF results show a good agreement to other analytical techniques. In the intercomparison exercise for trace elements in estuarine water TXRF shows its performance (accuracy and precision) in comparison to other analytical techniques. For more details see Freimann et al. (1993).

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State of the Art in the Analysis of Organic Contaminants

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Introduction

Measurement of trace quantities of organic contaminants in marine samples involve a separation procedure for each compound in combination with a specific detector to quantify the level of each compound.

Separation of organic compounds can be achieved by gas chromatography (GC) or liquid chromatography (HPLC, TLC). For GC analysis the flame ionisation detector (FID) and the electron capture detector (ECD) offer sufficient sensitivity for trace analysis. HPLC is mainly used in combination with a fluorescence detector which is specific for aromatic compounds.

In all chromatographic methods compounds are identified by comparing their retention time with those of well defined standards.
However, during the separation stage of the analysis of environmental samples it is possible for a number of compounds to co-elute within the window of retention time of a standard. Unless this problem is addressed, over-estimates of the compound of interest can occur. This is an increasing problem, particularly with decreasing concentrations of organics in samples that has not clearly been recognized by the organizers responsible for the monitoring guidelines.

This paper reviews the procedures for the analysis of organic contaminants which have been requested under the HELCOM BMP Guidelines.

Monitoring Guidelines

In the BMP Guidelines (HELCOM, 1988), the DDT group and the PCB group of compounds are oblinatory organic parameters to be monitored in the muscle of herring and liver of cod. In addition to the above mentioned compounds, analysts are also asked to report and quantify HCB and α- and y-HCH, since this analysis requires little extra effort and because “such information is useful”.

“Tentative organic contaminants” are chlordanes and dieldrin because "... more information is needed".

In the future: Polychlorinated camphenes (PCCs) and dibenzo-dioxins and furanes (PCDDs and PCDFs) and Polyaromatic hydrocarbons (PAHs) should be included in the monitoring programme.

In addition to measurements of these contaminants in organisms they should also be investigated in sediment and water on a tentative basis.

Analysis

Currently, the method of analysis for all these investigations is gas chromatography in temperature-programmed capillary columns.

The method offers excellent separation power and for chlorine containing-compounds there is an excellent and specific detector, the Electron Capture Detector (ECD), which is unresponsive to many other compounds.

When analysing the obligatory Σ DDT, PCBs and the tentative chlordanes and dieldrin (and some others, too), an almost apolar column is advised - preferably SE 54 or equivalent. (Compare Table 1).

Table 1. Chromatographic Conditions requested for ICES/IOC/OSPARCOM-Intercalibration

Column: 25 - 50 m,	ID = ≤ 0,25 mm,	Film: ≤ 0,25 µm
Stationary Phase:	SE 54 or equivalent For confirmation: other polarity	
Flow Gas:	H_2 - 30 - 50 cm/sec ≙ 1,2 ± 0,3 ml/min He - 25 - 30 cm/sec ≙ 0,75 ± 0,15 ml/min	
Injection:	Splitless:	automatic if possible
	Injector:	ca. 270 °C 1 minute closing time minimum
	on column:	no further advice

A length of 50 m, and ID of 0,2 mm and a film thickness < 0,25 µm of the stationary phase are the prerequisites for adequate separation. Flow gas velocity can be critical with these column dimensions, so hydrogen rather than helium is the preferred carrier gas because of its lower viscosity (Boer and Meer, 1993).

However, the range of reported flow gas velocities is large and every analyst has to find out by optimization which flow gas velocity is best for his apparatus and field of application. For optimization the analyst could analyze a highly chlorinated PCB-mixture, e. g. Arochlor 1254 or Clophen A60 with their large number of peaks, and compare the separation success as a function of flow gas velocity and temperature programme.

The starting temperature of the oven programme has to be set near the boiling point of the solvent. The programme can be constructed with differing rates of temperature increase. However, rates below 2 °/min can result in poor reproducible retention times, so it is better to replace them with isothermal intervals of appropriate duration.

However, it may be necessary to compromise since the analyst cannot achieve "best" separation along the entire chromatogram. This is illustrated in the following scheme:

GC-Analysis means compromise

1)	Film thickness: 3 μm (5 μm) 1,0 μm 0,5 μm 0,2 μm 0,1 μm	increase:	increased capacity increased temperature (good for volatiles) decreased separation
		decrease:	decreased capacity decreased temperature (good for high boiling compounds) increased separation
2)	Column diameter: 0,5 mm 0,3 mm 0,2 mm 0.1 mm	increase:	high capacity low separation
		decrease:	better separation low capacity low flow gas velocity
3)	Column length: 50 m (100 m) 25 m 10 m	longer:	increased separation and time and cost and temperature and decreased lifetime
		shorter:	low cost low time low separation
	Optimize:	Injector temperature Closing time of splitter Temperature programme Purge gas velocity	
	Set:	Linear gas flow	

The flow of purge gas through the detector is less important but eluting peaks should still be checked for good shape.

For stable organochlorine compounds splitless/split injection is adequate and is preferred to "on column" injection because non-evaporating residues remain in the injector from where they can be removed and do not spoil the column.

The advantages of an "on column" injector do not apply for analysis of stable organochlor ine compounds.

Confirmation

In GC analysis, conclusive evidence is only given if a compound does not appear during analysis and it has been shown that it would appear if it were present.

If you find a peak in your chromatogram at the appropriate place you still have to show that it is the compound you expect because it could be another substance co-eluting within the window of retention time.

A large analytical signal is seldom completely different from what is expected, but for quantification purposes you have to check how much of the signal is given by the calibration compound.

To solve the problem, confirmation tests are required. An old-fashioned way is chemical conversion, often used in the days of packed column GC.
Example: DDT → DDE (KOH in EtOH).
This method, however, can only be applied occasionally (e. g. not for PCBs) and a more general procedure is needed.

The advanced way of confirmation is the use of a mass spectrometer (MS) as a detector in GC analysis. However, an MS is not always at hand and the sensitivity of an MS could be insufficient at some concentrations of organics found in marine samples. Therefore, the next best approach is to use the ECD in combination with another column with a polarity different from the SE 54 standard low polarity. (Table 2).

Changing the polarity of the stationary phase will change the elution sequence of peaks in the chromatogram. On a non-polar column, the elution sequence follows the vapour pressure (i. e. ~ boiling point). On a polar column the polarity of the molecule additionally influences the elution sequence.

As we are dealing with high-boiling compounds and need excellent separation, there is not much choice left other than the OV 17 medium polar group of stationary phases because of their temperature resistance.

When comparing quantitative results from different polarity columns, the lower results will be the more reliable results as higher ones are almost always due to co-eluting compounds.

In coastal water samples we have occasionally found significant levels of the pesticide Aldrin on the SE 54 column, but this was never confirmed on a medium polarity column.

In the Baltic Sea water, a 10fold HCB concentration was observed on one column but not confirmed on the other column.

Table 2. Comparison of stationary phases used in GC analysis

Polarity %		Equivalent (similar)	Elution sequence
0	squalane		
1	SE 30 $(CH_3)_2$ PSO (Polysiloxane) Tmax	. :	DB 1, BP 1, OV 1 RTx 1 Ultra 1, SPB 1 300 / 350
5	SE 54 94 % (CH,), 5 % 0, 1 % vc T max	: :	DB5, BP 5, SPB 5, RTX 5 Ultra 2, SE 52, OV 73, Si 18 007-2, OV 5 300 / 350
50	ov 17 50 % CH, 50 % 0 T max	: :	DB 17, RTX-50, RSL 300, 007-17 (BP 10, Sil 19, OV 210, RTX-200) 300 / 360 (260/280): (F, Prop - PSO)
100	OV 275 CN-Prop. T max	: :	SP 2390, SP 2340, RTX 2330 CPS 2 (DB 23, BPX-70, Sil 88) 240 / 280

Another tool to investigate co-eluting compounds is two-dimensional capillary chromatography which also makes use of columns of different polarity (Duinker et al., 1988).

Several papers have been published on PCB investigations in marine media with elucidating results, but so far two dimensional chromatography proved too sophisticated to be included in an international monitoring programme.

Clean-up procedures

Clean-up procedures are needed to reduce interferences from co-extracted material. This becomes increasingly difficult with decreasing concentrations. This is well reflected in the cumbersome clean-up procedures described in methods for PCDD or PCDF analysis (Crummet, 1983).

A straightforward, but coarse, method is the reaction of the extract with concentrated sulphuric acid. In this reaction, every co-extracted natural semipolar or nonpolar compound is either degraded or dissolved in the sulphuric acid layer and only nonpolar refractory compounds like PCBs, HCHs, DDT group, HCB, Mirex, Methoxychlor and a few others remain unchanged in the solvent. Dieldrin and all the modern, less persistent pesticides are removed from the samples. This method has a very limited range of application, but it is foolproof and provides a good basis for international monitoring purposes.

Other clean-up procedures involve adsorption chromatography with Al_2O_3, SiO_2, Florisil ($MgO-SiO_2$) and Carbon (C) of different grain size and activity. Because activity is not easily controlled, and because it is also influenced by the amount of interfering compounds, these methods are not always easy to reproduce, nor are they foolproof.

There are some promising papers about the application of Gel-Permeation and High Precision Liquid Chromatography (HPLC) on Reversed Phases. Both methods can be done automatically, thus ensuring greater reproducibility than that obtained for many manual methods. However, the equipment is expensive and sophisticated.

HPLC also provides a reasonable control method, as liquid chromtography is quite different in the separation process from gas chromatography.

Liquid chromatographic clean-up procedures permit additional confirmation tests if the solvent is analyzed in fractions and the compound appears in the proper fraction.

The confirmatory tests can be sorted according to the weight of evidence of the methods applied. An example in consecutive order is given as follows:

C O N F I R M A T I O N

Normal confirmation:

1) proper retention time on one column plus specific molecular fragments

2) proper retention time on two columns of different polarity (e.g. SE 54 and OV 17)

Advanced confirmation:

1) proper retention time on one column plus mass of molecule

2) proper retention time on two columns of different polarity plus proper HPLC fraction

3) proper retention time on three columns of different polarity.

Conclusion

The aim of international monitoring programmes is to determine and make an inventory of spatial and temporal trends of contaminants.
We have experienced that the spatial trends in offshore waters are low and the temporal changes are even lower.
Contributions to international monitoring programmes have to be comparable to the greatest possible extent.
This can only be achieved with methods that are straightforward and foolproof. However, there are only a few questions left today that can be answered using simple methods.

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External Quality Assessment of Chlorobiphenyl Analysis

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Abstract

While PCB-contamination has been one of the major environmental problems during the last 25 years, the comparability of PCB determinations among laboratories has been very poor. In order to improve that situation in 1988 a stepwise intercomparison exercise on the analysis of individual chlorobiphenyl (CB) congeners in marine media was initiated by ICES, IOC and the Oslo and Paris Commissions. During this intercomparison study, which is now in its last phase, detailed advice was given to the participants on calibration, gas chromatographic analysis, clean-up and extraction of CBs from marine sediments and biota. In contrast with single stage intercomparisons, in this study it was demonstrated that for a large group of laboratories the level of performance could considerably be improved. The majority of the participating laboratories is now considered to be able producing reliable results in common studies.

Introduction

Since the first observation of the presence of polychlorinated biphenyls (PCBs) in the environment by Jensen (1972) there has been a continuous development in the determination of these compounds. Because of the complex character of this type of compounds, forming a group of 209 different congeners, initially attempts were being made to quantify the total PCB concentrations after chlorination to decachlorobiphenyl or dechlorination to biphenyl (Lang, 1992). Because these techniques often produced false positive results, alternatively total PCB determinations were carried out by gas chromatography (GC) with electron capture detection(ECD). On packed GC columns patterns of technical PCB mixtures (Aroclor, Clophen, etc.) were compared with PCB patterns in environmental samples. Because of weathering effects and metabolism patterns in sediments and biota looked different from those in the technical mixtures. The determination was therefore not better than a rough estimation of the PCB concentration. Besides, as with the chlorination and dechlorination techniques, no information was obtained about concentrations of the individual congeners. Because gradually more evidence was found that different congeners showed a different toxicity, knowledge about concentrations of individual congeners was needed. The introduction of capillary GC columns in the mid 1970s enabled the determination of individual CBs. Until then intercomparison on PCB determination in environmental samples had not been successful, showing large relative standard deviations (rsds) (Uthe et al., 1981 Musial and Uthe, 1983, Reutergårdh and Litzen, 1992).

In the beginning of the 1980s the Community Bureau of Reference (BCR) of the European Community started some intercomparison studies on individual CBs within a group of expert laboratories, leading to the certification of reference materials for CB analysis. During those studies the various stages of the CB analysis like calibration, GC analysis, clean-up and extraction were studied in detail. If necessary intercomparisons were repeated in order to enable participants to apply new knowledge (Wells et al., 1988, 1992a).

During these years the comparability of PCB data in international studies in the marine environment like the Joint Monitoring Programme and the ICES 1985 Baseline study on contaminants in fish and shellfish (ICES, 1988) was very poor. Some laboratories still used total PCB concentrations, other determined individual CBs without a thorough quality control. In order to improve this situation in 1988 the ICES/IOC/OSPARCOM intercomparison exercise on the analysis of chlorobiphenyl congeners in marine media was initiated according to the model of the BCR intercomparison studies. The objectives of this exercise were:

1) To determine the variation in results of the analysis of chlorobiphenyls among the participating laboratories;
2) To identify the sources that cause that variation; and
3) To reduce this variation by means of a learning process through a step-by-step organised intercomparison exercise.

The following stages of the CB analysis were studied:

1.	(1988-1990)	GC analysis (standard solutions)
2.	(1990-1991)	Calibration and analysis of cleaned samples
3a.	(1991-1992)	Long term precision
3b.	(1992-1993)	Calibration and clean-up
4.	(1993-1994)	Extraction, complete analysis

Materials and methods

The matrices used were seal blubber and marine sediment and in step 4 also a fish tissue. The following CBs were selected:

CB 28	-	2,4,4'	- trichlorobiphenyl
CB31	-	2,5,4'	- trichlorobiphenyl
CB 52	-	2,5,2',5'	- tetrachlorobiphenyl
CB 101	-	2,5,4,2',5'	- pentachlorobiphenyl
CB 105	-	2,3,4,3',4'	- pentachlorobiphenyl
CB 118	-	2,4,5,3',4'	- pentachlorobiphenyl
CB 138	-	2,3,4,2',4',5'	- hexachlorobiphenyl
CB 153	-	2,4,5,2',4',5'	- hexachlorobiphenyl
CB 156	-	2,3,4,5,3',4'	- hexachlorobiphenyl
CB 180	-	2,3,4,5,2',4',5'	- heptachlorobiphenyl

About 50 laboratories, mainly from western-Europe, Canada and the USA participated in all steps of this study. Participants were given about 4 months time for the analysis of standards and samples that were distributed as ampouled solutions for each step.

Statistics

Because of the complex character of the information resulting from these exercises, with more than 50 laboratories, 10 CBs, different samples and columns, principle component analysis (PCA) was chosen as the statistical technique for evaluation of the results. The advantage of the technique that PCA presentations are able to summarise in one picture the performance of all participants, illustrating outlying laboratories etc., this in contrast to e.g. bar graphs which only can show results for one parameter.

Because the error in PCB analysis shows a relative character a model with a multiplicative error structure was assumed. This means that logarithms of the CB concentrations were used in the statistical analysis. Standard deviations for the reproducibility and repeatability were expressed as relative instead of absolute values. Further, the statistical evaluation was mainly based on the international standard IS0 5725 for interlaboratory tests.

Results and discussion

Optimisation of GC analysis

The first step of the study focused on GC analysis. A standard solution and an unknown solution were requested to be analyzed. Emphasis was laid on optimisation of the various parameters. During the whole study participants were requested to use two GC columns for the analysis of samples and standard. This because no column is able to separate all 209 CBs. During the study it appeared that coelutions of the CBs studied with other CBs occurred more frequently than initially was assumed. Table 1 shows the possible interferences for the CBs studied on columns of different polarity. The two columns should be selected in such a way that all CBs can be quantified free from any interference on at least one column. The following advice on optimisation was given: use of hydrogen or, if not available, helium, as a carrier gas, injection of a fixed volume (1 μl or less), optimisation of detector and injector temperatures and splitter closing times when using splitless injection, optimisation of initial column temperature for on-column injection, use of isooctane as a solvent for all dilutions, use of internal standard and determination of linear range of ECD. Especially the linearity of the ECD appeared to be a problem which most participants were not ware of. All ECDs show a very poor linearity in the area below 50 pg. Because the ECD is just intended to measure concentrations of halogenated substances in this low picogram area, the user must be aware of a continuous linearity problem. By plotting the peak height divided by the injected mass vs. the injected mass (Fig. 1) this problem is clearly visualized. In order to overcome this problem standards and sample extracts should be concentrated, so that absolute amounts injects arrive in the linear range or, alternatively, a multiple level calibration should be carried out.

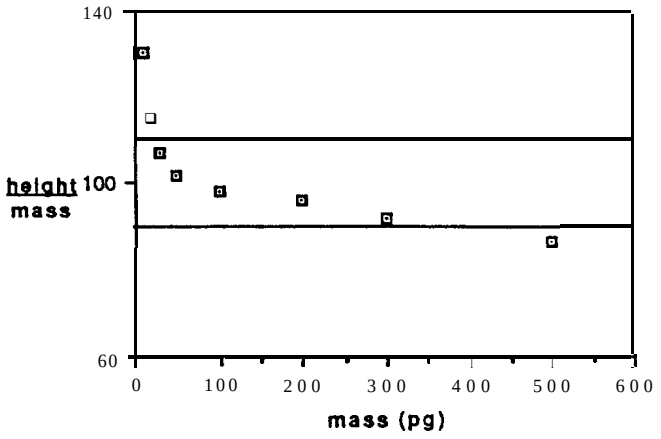
The first exercise of the study resulted in between laboratory rsds (relative standard deviation) of 1.1 1- 1.13 for CBs. Eleven laboratories were identified as an outlier. For this group the exercise was repeated. Reproducibility was worse for results based on peak areas. This was explained by inaccurate integration. Therefore only peak heights were used in further steps. Background contamination was a serious problem for many laboratories. Positive identifications of CBs, sometimes up to 60% of the CBs present

in the standard solution were found, where extraction and clean-up was even not applied.

Table 1. **Possible coeluting CBs.**

Column:	SE-54/CP-Sil 8/etc.	OV-1701/CP-Sil 19/etc.SP-2330/CP-Sil88/etc.
CB	CB	CBCB
28	31	3116
31	28	28
52	73	49
101	84, 90	55
105	132	141129, 174
118	149	81, 123, 200
138	160, 163	158, 160, 163, 176
153		110
156	171, 202	
180		157, 197197

Figure 1. Example of typical response curve of the electron capture detector for CB 153, which shows the relationship between the ratio of the detector response/mass to the mass of the injected CB.



Calibration

The second step of the study involved a study on calibration and the injection of cleaned samples. To check the ability of the participants to prepare a standard solution, an unknown solution was distributed. Participants were requested to analyze this solution with their own standard. This exercise leaded to rsds of 1.16-1.33 for only a selected group of 39 laboratories (Table 2).

Table 2. Standard deviations obtained in the ICES/IOC/OSPARCOM CB intercomparison.

Matrix		sd (range/mean)	no. labs	CBs
Unknown standard solution	(2)	1.16-1.33/1.22	3 9	all
" " "	(3b)	1.11-1.22/1.14	3 7	all
Clean-up seal blubber	(2)	1.24-1.62/1.36	35	8 CBs, no 28, 31
" "	(3b)	1.08-1.61/1.33	3 6	8 CBs, no 28, 31
Cleaned-up sediment	(2)	1.31-1.56/1.66	2 8	6 CBs, no 28, 31, 105, 156
" "	(3b)	1.16-1.48/1.28	2 5	9 CBs, no 52
Uncleaned seal blubber	(3b)	1.22-1.49/1.34	3 5	8 CBs, no 28, 31
" sediment	(3b)	1.36-1.69/1.46	3 4	8 CBs, no 52, 156

This result showed that calibration was one of the major sources of error in CB analysis. The following recommendations on preparation of CB standards were given:

Do not use commercial standard solutions, unless they are checked against weighed solid standard. Several commercially available standard solutions showed to be not reliable.
Use solid materials of known purity > 95 % , preferably certified standards.
Weigh at least 5 mg directly into a measuring flask.
Do not use any weighing papers or weighing glasses.
The balance should have a precision of at least 0.01 mg.
Care should be taken for a balanced temperature in the weighing room. Draft should be avoided.
Electrostatical problems should not hinder the weighting procedure.
Deionization sources are available nowadays to prevent electrostatical problems (e.g. Zerostat, Mettler).
The balance should be serviced regularly by the manufactures engineer and calibrated.
A single solvent of known purity should be used.
Solvent quantities should also be weighed.
Two standards solutions should be prepared simultaneously and independently from each other.

A check should be made with another laboratory.
Standard solutions should be ampouled prior to storage. If this is not possible, standards should be stored in an isooctane atmosphere and evaporation losses should be checked and corrected for regularly.

Further advice on calibration, gathered during BCR-studies, can be found in Duinker et al. (1991) and Wells et al. (1992b). The calibration was checked by a similar test in step 3b. Now the rsds ranged 1.1 l-l.22 for 37 laboratories (Table 2), which was a considerable improvement since the first exercise and an acceptable basis for further common studies.

Analysis of cleaned-up samples

The analysis of cleaned samples was studied in step 2 and, in combination with uncleaned samples, in step 3b. The first results from step 2 showed that, even with an optimized GC and acceptable chromatograms of standards, major difficulties can be encountered when analysing samples. The first results showed rsds of 1.24-1.62 for only 8 CBs for a selected group of 35 laboratories for cleaned-up seal blubber and rsds of 1.3 1- 1.56 for only 6 CBs for a selected group of 28 laboratories for a cleaned-up marine sediment extract (Table 2). Especially for the CBs 28, 31, 52, 105 and 156 the results were very poor. This exercise very clearly demonstrated the need for improvement in comparability and quality control. Several sources of errors could be clearly identified. Although it was recommended to use capillary columns with minimum lengths of 50 m and maximum internal diameters of 0.25 mm, many participants still used 25 m x 0.25 - 0.32 mm columns. It was agreed, therefore, that results produced on columns shorter than 590 m and with internal diameters broader than 0.25 mm would not be accepted any longer. Reduction of the internal diameter to 0.20 mm was strongly recommended. Secondly several participants still refused to use on or more internal standards. Similarly it was agreed that results produced without the use of internal standards would not be accepted any longer. BCR intercomparisons had demonstrated the improvements made by the use of internal standards.

Emphasis was laid on the selection of appropriate stationary phases, in order to prevent coelution (Table 1). In order to enable the determination of the CBs present in low concentrations like 28, 3 1, 105 and 156, the sensitivity should be improved. Sometimes more extracts needed to be injected twice, in different concentrations.

The results obtained in step 3b showed an improvement compared with step 2. For a cleaned-up seal blubber extract rsds of 1.09 - 1.20 for all CBs except 28, 31, 105 and 156 were found and of 1.09 - 1.61 for all CBs with exclusion of 28 and 31 (Table 2). The latter two CBs are metabolized in seal and will always remain very difficult to be analyzed, due to their extremely low concentrations. For a group of 17 laboratories, who obtained good separation for the CBs 105 and 156 rsds ranging from 1.09 - 1.47 were obtained. For a cleaned-up sediment extract the rsds ranged 1.16 - 1.48 without CB 52 (Table 2) with a mean of 1.28, considerably lower than the mean rsd of 1.66 obtained in step 2.

Long term precision

During the first two steps of this study it was shown that the variation due to the bias (accuracy) was always much larger than the variation due to precision. This suggested that a repeated determination within a short period of time would always have only a minor contribution to the total variation. Because samples analyzed for intercomparison exercises are always handled within a short period of time, this could cause an unrealistic high precision and, on the other hand, could cause a poorer intercomparability. Therefore, in step 3a an exercise was planned to obtain information about the long term precision of CB analysis at the different laboratories. Participants were requested to analyze six time a certified reference material cod liver oil for 3 CBs (52, 153, 156) with intervals of at least one week between the analyses. Rsds for the repeatability of 7-8% were found for the CBs 52 and 153 with rsds for the reproducibility of 19% for both CBs. This meant a relative increase of about 100% of the importance of the repeatability in comparison with step 2. The long term precision therefore gives a more realistic impression of a laboratories performance. Duplicate analyses carried out at the same moment only have a very limited value.

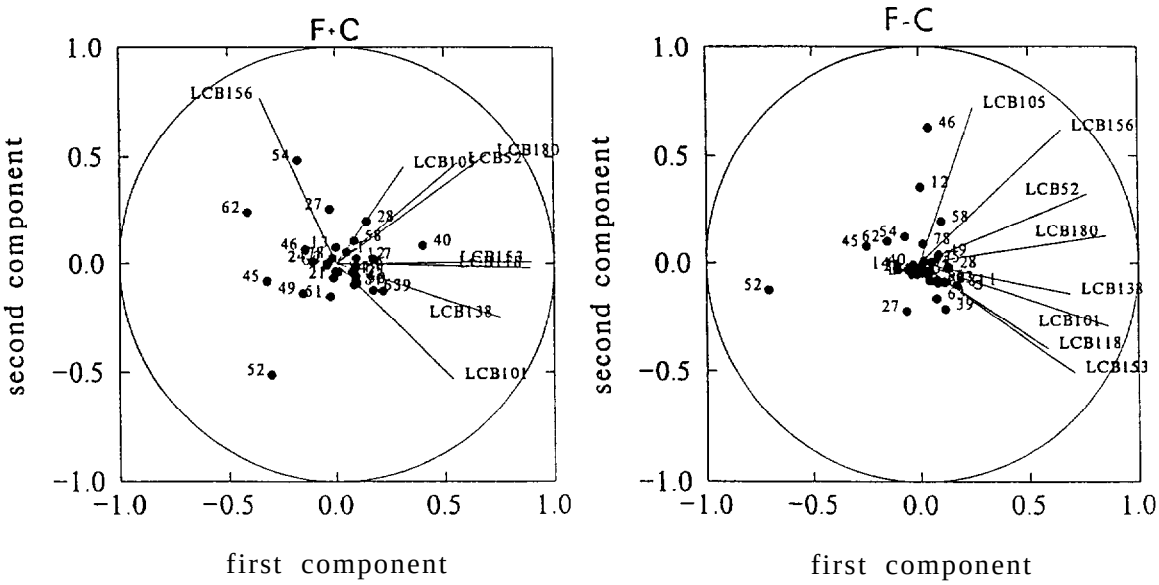
Clean-up

In step 3b the clean-up of seal blubber and marine sediment extracts were studied by comparing the CB determination in cleaned-up and uncleaned extracts of these samples. Participants were free to choose a method for the clean-up of the uncleaned extracts. Combination of alumina and silica columns, use of sulphuric acid and use of florisil columns were the most frequently applied methods. From the sediment extracts the sulphur had to be removed. This was done mostly by shaking with tetrabutylammonium sulphite (TBA) or by inserting copper wire into the extracts.

Both for the seal blubber extracts and the sediment extracts no significant differences between results of the cleaned-up samples and those of the uncleaned samples were found for the group as a whole. For the seal blubber 7 laboratories were advised to reconsider their clean-up methods due to significant differences. For the sediment extract 6 laboratories were given the same advice. The PCA-plots of the results of cleaned-up and uncleaned sediment is given in Fig. 2.

This exercise confirmed that major errors in the CB determination are normally not made in the clean-up step. Mean rsds found for uncleaned seal blubber and sediment were 1.34 and 1.46, respectively. Major difficulties were, similar to those for cleaned-up extracts, separation and sensitivity problems for the CBs 28, 31, 52 and 156. The deviating results of some laboratories show that also here optimisation and testing of the procedure is a prerequisite.

Figure 2. Biplots of a principal component analysis, showing the differences due to clean-up (F-C) of a sediment extract and the bias (F+C). LCB = log of the CB concentration. The lengths of the LCB vectors indicate the reliability of the approximation.



Extraction

The last exercise of the study, focusing on extraction of CB from seal oil, cod muscle tissue and marine sediment is now under way. Based on experiences from BCR studies and other literature data (de Boer, 1988) advice has been give to use a combination of a polar and a non-polar solvent of the extraction of CBs from fish tissue and sediment. Alternatively saponification is recommended, provided that moderate conditions are chosen to prevent decomposition of higher chlorinated CBs (van der Valk and Dao, 1988). Supercritical extraction may be an efficient method, but until now several difficulties have been met with this technique. Supercritical extraction of CBs from sediment seems to be more promising than form fish, because of coextraction of lipids. The final evaluation of this last step will take place in 1994. Together with this exercise on CBs, an intercomparison study on lipid determination will be carried out.

Conclusions

In comparison with other intercomparison exercises on the analysis of CBs the ICES/IOC/OSPARCOM intercomparison study has proved that considerable improvement in comparability among laboratories can be obtained. The stepwise design of the study, together with ample, directive, advice on optimisation of methods and instruments has been the basis for the improvements obtained. Another prerequisite for success was the willingness of laboratories to invest in appropriate GC columns and suitable instruments. The determination of CBs on a ppb-level needs a high level of well equipped, up-to-date analytical instruments and methods as well as highly qualified staff.

For calibration and for the analysis of major CBs in uncleaned environmental samples a sufficient basis for common studies of the participating laboratories is now obtained, with standard deviations ranging 1.1 - 1.2 for these items. Improvement is needed in resolution and sensitivity for the analysis of CBs present in lower concentrations like CBs 28, 31, 52, 105 and 156. Clean-up methods used do not cause any major errors in the determination of CBs. Extraction methods are now under study. Results will come available in the beginning of 1994.

Developments in super critical extraction and analytical instrumentation (GC, GC/MS, HPLC) may bring about a further reduction in between laboratory standard deviations in CB analysis. Rather than the analytical error, errors made in sampling may than become the determining factors for the total error.

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