

Part 1

SAMPLING TECHNIQUES AND ANALYTICAL METHODS

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1.- Introduction.

Trace metals in the marine environment have become a significant topic of concern to scientists because of the potential danger for man resulting from direct or indirect pathways of accumulation of metals in marine food.

Chemical analysis is the basis for studies of the diffusion, dilution, transport and sedimentation mechanisms of trace elements in the hydrosphere. But in order to better understand the exact forms, mechanisms of transport and residence times of the ultra micro amounts of metals in the marine environment, one is forced to develop improved technologies for the measurement of the concentrations of trace metals in environmental samples of sediment, plant and animal material, sea water (dissolved and particulate species), aerosols and volatile elements (especially Hg).

Continuing efforts have been made to increase the sensibility and the selectivity of methods, the precision and the accuracy of results and to simplify the procedures in order to avoid contamination especially during sampling and filtration.

In order to check the reliability of the adopted analytical methods, several intercalibration exercises at the international level have been performed. We have applied these validated analytical methods for routine determination of traces of Bi, Cd, Cu, Hg, Pb, Sb and Zn in the various environmental samples.

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2.- Sampling.

2.1.- Generalities.

In principle all stages in the preparation of a sample for the trace metal analysis should be submitted to a severe assessment of occasional or permanent sources of error, whatever the type of sample.

Sampling is one of the most important steps in marine heavy metals studies. If the sampling procedure is biased, the results of analysis will have little or no meaning and the interpretation will suffer as much. This is especially true in the case of trace heavy metals where the concentrations can go down to the sub-ppb levels.

One of the most serious problems in sampling is the choice of the appropriate type of sampler. It is essential that sampling should be performed with an awareness of all possible sources of contamination and preferably under the direction of an analytical chemist.

2.2.- *Sea water, samples (bulk water column) for the determination of dissolved and particulate heavy metals.*

Present knowledge in the distribution of metallic elements in water suggests that trace metals may exist in any or all of the following physicochemical forms :

true solution ($< 0.001 \mu\text{m}$)

as inorganic complexes such as chloro-complexes, hydroxides, carbonates ; as complexes with dissolved organic matter such as amino acids, humic material ; in organometallic compounds.

colloidal ($0.001 - 0.10 \mu\text{m}$)

adsorbed on colloidal matter and/or occluded within colloidal species.

particulate ($0.1 - 50 \mu\text{m}$)

adsorbed on fine mineral material or organic detritus or bound within plankton material or co-precipitated with the hydrated oxides of iron and manganese.

Metal analyses are generally reported both on suspended particulate matter and on the "soluble" metal fraction of sea water samples.

In unfiltered samples, trace metals ions can be liberated rapidly from, or taken up by, particulate matter. High bacterial concentrations associated with sedimentary material will also lead to changes in the distribution of chemical forms of metallic elements in solution. It is advisable to separate the suspended matter as soon as possible after sampling.

The heavy metals content of suspended matter can be determined in a sample collected after filtration.

According to a widely accepted convention in marine chemistry, filtration is defined as separation by a $0.45\ \mu\text{m}$ filter into a filter residue and a filtrate, which contains of course still the colloids and the fraction of very small particles of mineralogical and biological origin that have passed the pores of the $0.45\ \mu\text{m}$ filter if not clogged.

The two problems confronting the analyst are the representativity of the samples and their possible contamination. Besides the sampler, research ships reveal to be a main cause of trouble if not some other ships leaving behind themselves a cloud of Zn, Cu, Pb, ..., from propellers, zinc anodes, paint, engine exhausts among other sources. Airborne material, careless handling by unskilled experimenters, the depth at which the sample is taken versus the ship's draught, the location on board of sampling system, atmospheric conditions, inhomogeneity of the water, etc. are all possible sources of difficulties.

To make sampling more reliable we have designed a sampler adequate to work at moderate depth from a large or small research vessel, even in rough conditions (Gillain et al, 1980). The principle of this system is to continuously collect small samples of sea water from a very large volume screened from atmospheric pollution. A peristaltic pump draws continuously $6\ \text{l}\cdot\text{min}^{-1}$ at 5 meters depth through a PVC tube previously soaked for several days in 6N HCl, rinsed with ultra-pure water.

When sampling, it is necessary to avoid ship's discharges, antifouling paints, etc., in close proximity to the sampling point. The ship is best, left adrift and the tube is on the lee side so that it meets water masses not polluted by the vessel. A second peristaltic pump draws $0.5\ \text{l}\cdot\text{min}^{-1}$ from the main flow which is returned to the sea. The unfiltered sea water is kept in a 5 l polyethylene bottle and continuously stirred magnetically for the next operation : filtration.

The problem of representativity becomes more acute in the suspended matter matrix. This is especially true with the conventional separating system where the amount of suspended matter collected on the filter before clogging is generally very small. In this case, the heavy metal content of particulate matter collected on the filter will be lower than the filter blank. Consequently, the error introduced by filtration is then significantly larger than the bias arising from the dissolution of adsorbed material and from the analysis itself.

On the other hand, filtration is a slow process and the characteristics of the filter will change after the deposition of the first layer of particulate matter. To overcome all these problems and to minimize all possible external interference (dust-free conditions), we have employed a shipboard stirred pressure system in a purified nitrogen atmosphere.

The type of filtration apparatus used is made from a pyrex glass tube 120 mm long and 47 mm diameter fitted in between two PVC lids with silicone seals. A magnetic rod is suspended a few millimeters above the filter to accelerate filtration, retard clogging by vigorous stirring and collect the greatest possible amount of material.

The filtration apparatus is connected directly to the water sampler. The water drained from the sampler is replaced by pure nitrogen and filtered sea water is continuously collected in a polyethylene flask which is used to store the sea water sample.

There is practically no risk of contamination of the sample during transfer from sampler to storage bottle.

More detailed description and cleaning procedure can be found in a recent paper (Gillain et al, 1980). They are summarized below.

In samples containing zoo- and phytoplankton, we use a low constant pressure (0.3 kg/cm^2) in order to avoid desintegration of delicate organisms. This may lead to increase heavy metals concentrations in the filtrate and change heavy metal speciation.

Objections which can be raised against this sampling and filtration system are the adsorption and/or the contamination of samples by the PVC suction tube, filter unit, membrane filter, etc.

The contamination is prevented by cleaning the filters with 100 ml of a 10^{-2} DTPA solution and washing them with highly purified water before filtering the sea water.

On the other hand, the sampling and filtration kit is treated during one week with 6N HCl and large amounts of ultra-pure water is used for rinsing.

In any case there is little time for possible interaction between wall and water, since filtration is started immediately after collection and is usually completed within one hour.

The first 200 ml are needed to condition the membrane filter ; they are rejected.

Laboratory experiments performed on Reagent Grade NaCl 0.5 M solution (loss test) and on suprapure NaCl 0.5 M solution (contamination test) indicate that it is possible, in our conditions, to apply the described sampling and filtration system for the determination of Bi, Cd, Cu, Sb and Zn dissolved in sea water, without significant changes of their initial concentrations.

The concentrations of the elements were :

- (1) Reagent Grade Quality
NaCl 0.5 M solution (spiked)
Zn : $5 \text{ } \mu\text{g/l}$;
Cd : $0.1 \text{ } \mu\text{g/l}$;
Pb : $1 \text{ } \mu\text{g/l}$;
Cu : $1 \text{ } \mu\text{g/l}$;
Sb : $0.05 \text{ } \mu\text{g/l}$;
Bi : $0.05 \text{ } \mu\text{g/l}$.

(2) Suprapure Quality

NaCl 0.5 M solution

Zn : < 0.1 $\mu\text{g}/\ell$;
Cd* : 0.009 $\mu\text{g}/\ell$;
Pb* : 0.012 $\mu\text{g}/\ell$;
Cu* : 0.012 $\mu\text{g}/\ell$;
Sb* : < 0.005 $\mu\text{g}/\ell$;
Bi* : < 0.005 $\mu\text{g}/\ell$.

This sampling and filtration system has been tested and shown to give far more better results in our survey areas Belgian coast than usual systems which get easily out of control.

Better results (Zn : 0.70-5.00 $\mu\text{g}/\ell$; Cd : 0.020-0.180 $\mu\text{g}/\ell$; Pb : 0.20-2.00 $\mu\text{g}/\ell$ and Cu : 0.10-1.5 $\mu\text{g}/\ell$) mean that in some cases the trace metals concentrations are one order of magnitude lower than with the conventional sampling (Niskin bottle) and filtration methods (open air shipboard suction filtration).

The concentrations of the metals (Cd, Cu, Hg, Pb and Zn) in our sampling area are high compared with other oceanic zones (Bruland, 1980 ; Boyle et al, 1981). However, at the present time, our trace elements data are believed to represent as truthfully as possible the situation in the Belgian coastal area (Southern Bight).

The performance of our sampling and filtration system was tested on the Mediterranean sea in an area considered as uncontaminated (Corsica, Calvi). Our own study on trace metal in this region has revealed dissolved levels for Cd* in the range from 0.02-0.005 $\mu\text{g}/\ell$, for Pb* from 0.08-0.015 $\mu\text{g}/\ell$, for Cu* from 0.10-0.04 $\mu\text{g}/\ell$ and for Zn from 0.18-0.05 $\mu\text{g}/\ell$. All these data are in sharp contrast to those measured in Belgian coastal water and reinforce our confidence in the sampling and filtration system designed to minimize contaminations.

2.3.— Sea water microlayer samples for the determination of dissolved and particulate heavy metals.

The sea surface microlayer is often characterized by anomalously high concentrations of heavy metals (Wallace et al, 1975 ; Duce et al, 1972b ; Hoffman et al, 1974 ; Baier et al, 1974 ; Wade et al, 1975) and other trace substances thought to be associated with particulate matter and surface-active organic material.

As for the water column, metal analyses are generally reported both for particulate matter and for the "soluble" metal fraction.

* Determined by ASV on ring-disk glassy carbon electrode.

The methods used to collect the surface microlayer are extremely varied (Garrett, 1965 ; Harvey, 1966 ; Harvey & Burzell, 1972). In our case, the sea surface microlayer samples were collected using the Garrett-screen technique (Garrett, 1965). The sampler consists of a nylon screen with openings of 400 by 400 μm and a fabric thickness of 440 μm stretched in a plexiglas frame. The principal difficulty with the microlayer sampling is the airborne contamination resulting from the research vessel (smoke stacks, corrosion products, dirt and dust particles,...).

The only possibility to avoid this problem is to reach, by a small rubber boat, an uncontaminated zone for sampling. This sampling site is chosen upstream, several hundred meter from the research vessel. Under such circumstances we can effectively work only in calm weather conditions.

To prevent also the contamination by the small boat itself, we move it up-wind and sample water in front of the boat continuously moving into uncontaminated water. The operator, wearing polyethylene gloves, touches the sea surface with the screen in horizontal position. Because of surface tension a film of water is captured between the meshes of the screen and we collect approximately 50 mL of surface water, representing a water film of 0.200 mm. Sea water is then transferred from the collecting screen to prewashed polyethylene bottles. At each microlayer sampling site the water column is sampled by immersion of polyethylene bottles at -30 cm.

In the future, we intend to compare different sampling techniques with in view 1) efficiency (recovering any surface film and associated matter present), 2) representativity of microlayer (heterogeneous distribution of physical, chemical and biological constituents), 3) deficiency (time of sampling, deterioration and dilution of microlayer with bulk sea water).

After collection, the samples were transported aboard the ship. Particulate matter in the microlayer surface was then immediately isolated by the filtration processes using the same nitrogen pressurized system and precautions as described for the water column.

2.4.- Plankton samples.

The plankton was collected from the sea with a 100 μm mesh nylon net (Hecq, 1981). Contact with metal was avoided at all stages of the sampling ; the net employed and other parts of the sampling gear were constructed of plastic materials.

However, in our case, the magnitude and the extent of the sampling problems for heavy metals determination are not all resolved. These include 1) the difficulty to collect sufficient material for elemental analyses, 2) the difficulty to separate phytoplankton from zooplankton and the inherent difficulty to determine which group contains which element, 3) the contamination

due to ship paint and fall out of rust chips from almost all research vessels, 4) the contamination by mineral materials ; in shallow water, the resuspension of bottom sediments often contaminate the plankton samples, 5) the representativity of the sample, depending on the natural variability (varying with place, depth and time of sampling, species distribution).

In addition to the sampling problems the establishment of general elemental levels for phytoplankton and zooplankton is made difficult because of the complexity of the relationships between the metals and the organisms.

In order to collect and analyze plankton samples for a better estimate of their importance in the biogeochemical cycling of elements in the North Sea (Belgian coastal zone), it seems obvious that future work will be essentially directed to improve sampling techniques.

2.5.- Air samples.

The most practical way for a detailed study of atmospheric concentrations and possibly fluxes of pollutants at ocean level is to have a stationary sampling site in operation over a relatively extended period of time.

In our case, sampling of marine aerosols is performed aboard a light vessel anchored on the West-Hinder bank, at 20 miles from the Belgian coast.

The collection of atmospheric particles from the ship presents many problems : 1) contamination by the ship (chips of rust and paint, smoke), 2) contamination by the collecting equipment such as tube, pump, filter, etc., 3) difficulty of collecting a sufficient number of air samples to obtain any meaningful statistics at a given location.

The aerosol samples were collected as follows :

samples of total atmospheric particulate matter were collected by air filtration (200 to 300 m³) on Whatman 41 filters. Filters were mounted in all plastic filter holders and the sampling PVC head was separated from the Hi-vol sampler (GMW - 2000H) by 15m of supple PVC tubing to avoid contamination by the pump itself. Volumes filtered were determined by use of a calibrated gas volume counter.

Size fractionated aerosol samples were collected by 6 stages Hi-vol Sierra model 235 Cascade Impactors operating with a flow rate of about 55 m³.hour⁻¹. The particles were deposited on 5 consecutive Whatman 41 filter papers and finally filtered on a Whatman 41 back-up filter.

According to the manufacturer, the equivalent aerodynamic diameter (EAD) cut-offs at 50% collection efficiency for particles with a density of 1g cm⁻³ at the flow rate used, are as follows :

stage 1 : > 7,2 pm ; stage 2 : 7,2 - 3,0 pm ; stage 3 : 3,0 - 1,5 pm ; stage 4 : 1,5 - 0,95 pm ; stage 5 : 0,95 - 0,49 pm and back-up filters : < 0,49 pm.

A specific sampling device using Lo-Vol pumps is used for Hg which is present mainly in the gaseous phase (Fitzgerald et al, 1979). Gaseous Hg is collected by amalgamation on a gold column and up to 1 m³ of air is sampled.

Particulate Hg is collected on Whatman GF/C glass fiber filters by sampling up to 15 m³ of air.

All samplings were performed under controlled conditions of wind-direction and wind-velocity. Filter holders were positioned 5m ahead from the flying bridge at 8m above sea level and also on the bow of the ship.

2.6.- *Sediment samples.*

Sampling meets great problems at the level of contamination and maintaining the "in situ" conditions.

To avoid contamination of samples from the main frame of the apparatus we use a PVC core-liner tube (6.35 cm diam.).

Penetration of the core-line tube was restricted to the first 40 cm. Great care was taken in handling cores : all cores were sealed in situ by two PVC-caps to minimize disturbance and compaction and brought aboard ship in a vertical position.

Two cores were collected at each site.

3.- *Sample storage.*

3.1.- *Generalities.*

Problems of sample interaction with storage container material will be most evident and most severe because of the longer contact time (day or months) compared to the conditions discussed above concerning the sampling itself which implies times only of the order of minutes or hours.

At this step, we must consider two aspects : 1) container heavy metal content and sample contamination by different kinds of container materials, 2) losses by surface adsorption and bacterial activities.

The contamination risks have induced several authors to analyse a large number of container material for heavy metal content

(Robertson, 1968 ; Montiel et al, 1981 ; Robertson, 1972). On the other hand, the adsorption effects have also received considerable attention (Jenne, 1975 ; Batley et al, 1977 ; Eichholz et al, 1965 ; Issaq et al, 1974). The results depend on a variety of factors such as the characteristics of the solution (pH, salinity, complexing agents, dissolved gases,...), presence of suspended matter, microorganisms ; wall geometry, temperature, contact time, etc).

In addition, most of the results from storage experiments are often based on the addition of ionic metal or radiotracers detectable as spikes which will drastically disturb the solution equilibrium. This possibly explains the wide variation in the results and the data from stored material should therefore be interpreted with caution.

The most reliable experiments are those which attempt to directly measure metal concentrations as a function of time under different conditions (Mart, 1979).

Results generally support the conclusion that PTFE and high density polyethylene show little tendency to adsorb and release the above metals and consequently, these are preferred materials for storage containers.

These problems are of particular importance for mercury (Jenne et al, 1975). In addition to adsorptive losses of ionic mercury both on container walls and on colloidal and particulate matter, there are also 1) the large adsorption capacity of particulate matter for several organic mercury components, 2) losses to the atmosphere, 3) diffusion through the container surface (Baeyens et al, 1979), 4) the increase of dissolved mercury content stored in polyethylene bottles in areas of high atmospheric mercury content (Robertson, 1972).

3.2.- *Cleaning of storage bottles.*

In our experiments, 1000 ml high density polyethylene bottles have been used. New bottles were used exclusively. Polyethylene bottles are rinsed with pure water (deionized) to remove particles. They are then filled with a 6N HCl solution, analytical reagent grade, for 8 days. The acid is changed and the containers are rinsed several times with pure water. The acid procedure is repeated once again for another 8 days, but at this time, the acid is suprapure Merck (1N HCl). Some days before use the bottles are rinsed with ultra-pure water (Millipore-Milli-Q) and conditioned with sea water which has a very low level of heavy metals.

As we have discussed (Baeyens et al, 1979), the container cleaning procedure for mercury analysis is totally different. Some specific tests have shown that a pretreatment of the polyethylene container with a solution of KMnO_4 (2%) in H_2SO_4 (50%)

during 24 hours make a storage bottle completely mercury-free. Pyrex bottles (BOD type) soaked in boiling 3N HNO₃ and rinsed several times with Milli-Q water, can also be used.

It needs to be quoted that the efficiency of mostly used storage procedures for Hg is not subject to an unanimous quality judgement (Feldman, 1974 ; Fitzgerald et al, 1975).

3.3.— *Storage of samples.*

3.3.1.— *Sea water (water column).*

Our analyses (Zn, Cd, Pb, Cu) of natural filtered sea water samples stored over a long time period (6 months) in cleaned polyethylene bottles at pH 1 or unacidified at - 20°C in a deep-freezer showed no significant changes (total metal concentrations) within experimental error. However, at pH 8 and at room temperature, significant changes, depending on the element, occur after a few days. It is difficult to draw a conclusion about Sb and Bi because their concentrations in natural sea water often approach the determination limits.

Similar results depending on the storage pH and temperature conditions were also observed by other investigators (Mart, 1979 ; Batley et al, 1976).

When only a total dissolved metal analysis is required, it is usual to prevent changes by acidification of the sample after filtration to pH 1 with either HCl or HNO₃. Since we are interested in speciation studies, the samples were deep-frozen (-20°C), immediately after collection and filtration, without any additive.

With freezing, however, it should be noted that changes in metal speciation do occur, although the total metal concentrations remain unchanged (Florence, 1976).

Fabricant et al (1966), Batley (1976) and Fukai (1976) recommend refrigerator storage at 4°C, but this appears to be hazardous as a general practice for long time storage (Mart, 1979).

For determination of total mercury, we opted for a sample storage at -20°C after acidification to pH 1 (Decadt, 1980). This procedure is not recommended for speciation of mercury forms (e.g. inorganic mercury, methylmercury and alkylmercury compounds). In that case, mercury analysis aboard the ship immediately after sampling and filtration seems necessary.

From what precedes, it looks advisable to choose storage methods selected for a particular research programme and we intend, in the future, to consider this problem in connection with speciation studies.

3.3.2.— *Suspende matter (water column).*

Suspended matter samples collected on precleaned Millipore membranes for either Hg or other metals such as Bi, Cd, Cu, Pb, Sb and Zn are stored in cleaned Petri dishes and maintained at -20°C prior to analysis.

3.3.3.— *Microlayer samples.*

Immediately after collection and filtration the solution and particulate samples are stored in the same conditions as described above for water column samples.

3.3.4.— *Plankton samples.*

Short term storage is made under conventional refrigeration at 4°C , but freezing at -20°C is the best method for long term sample conservation.

3.3.5.— *Sediment samples.*

The cores are best immediately deep-frozen to minimize alterations during storage and transport.

3.3.6.— *Aerosol samples.*

The aerosol samples are kept in acid cleaned plastic containers at low temperature (-30°C).

4.— *Pretreatments and analytical methods.*

4.1.— *Prevention of contamination in the laboratory.*

In addition to the sampling operation and sample containers, local laboratory dust, reagents and common laboratory equipment (glassware, cells, pipette, etc) all represent potential sources of contamination.

All materials whenever possible are precleaned by soaking in 6N HCl for several days before use. Only ultra-high purity reagents are used (suprapure Merck H_2O_2 , HF, HCl, HNO_3 , NaCl, ...). High purity water is prepared by using the Milli-Q system which consists of a prefilter and a charcoal absorption column followed by two mixed-bed ion exchange columns. After deionisation, water

passes through a microfilter (0.22 μm) to remove particulates. A typical analysis of this water yields the following results :

Zn < 0.05 $\mu\text{g}/\ell$;
Cd : 0.006 $\mu\text{g}/\ell$;
Pb : 0.008 $\mu\text{g}/\ell$;
Cu : 0.005 $\mu\text{g}/\ell$.

4.2.— Analytical methods used for accurate determination of trace heavy metals in environmental samples.

Atomic absorption spectroscopy (AAS) and anodic stripping voltammetry (ASV) are two of the most widely used method for trace metal analysis. The selection of the most appropriate technique is not always a simple matter. Such factors as specific element, the chemical forms of the metal to be determined (e.g., ionic as opposed to total), sensitivity, accuracy, ease and rapidity of analysis, potential run (ASV) for multi-element analysis, availability of instrumentation, cost, support the decision.

4.2.1.— Atomic absorption spectrophotometry (AAS).

a) The determination of Cd, Cu and Pb.

The principle of AAS is easily described. The wavelength of light from a cathode lamp is characteristic of the element being analyzed and the energy absorbed in the flame is related to the concentration of the element in the sample.

This technique can be used for the determination of most of the metallic elements.

When using the flame method, the detection limits are often unsuitable to make directly quantitative determinations of low level heavy metals in some environmental samples.

Recent developments of flameless atomizers such as the graphite furnace (GFAAS) have increased sensitivity by several orders of magnitude and therefore can 1) eliminate the need for prior concentration, 2) reduce possible contamination and 3) blank lower values.

However, matrix effects of concentrated salt solutions such as sea water are especially serious and preliminary treatments are unavoidable in most cases.

In addition, in sea water with very low trace element concentrations, separation techniques may serve to concentrate the element to be analysed. However, the great number of operations and reactivities used in manipulations such as complexation with APDC or DDDC, extraction with MIBK or chloroform, separation by centrifugation, wet digestion, evaporation to dryness, redissolution, etc. are not desirable steps for they greatly increase the possibility of loss, contamination and extend the analysis time.

For these reasons conventional methods were reviewed. We used the complexation-extraction method of Bruland et al (1979). Its main advantages, stated by Bruland et al are : 1) the combined use of the dithiocarbamates APDC and DDDC as chelating reagents for metals which was shown to work well over a wide pH range and allowed a good discrimination against the major cations in sea water, 2) the chelated metals are extracted with chloroform, since only small volumes of this solvent are required and since re-extraction of the metals in HNO_3 is possible, 3) the buffering of the solution is done with ammonium acetate, since it has a good buffering capacity at the optimum pH for extraction (pH 4) and since it was shown that for Cu and Zn no interference of acetate ion-metal complexes occurred.

Furthermore, contamination problems are minimized by a more rigorous pre-cleaning of the commercial reagents, utilization of pre-cleaned PTFE vessels and handling the samples in a controlled dust-free atmosphere.

In our experiments a Varian AA 275 spectrometer equipped with a graphite furnace was used for the determination of Cd and Cu in sea water after preconcentration and following the method of Bruland et al (1979). The overall recovery factor using the prescribed method for sea water spiked with Cd and Cu was 110% and 105% respectively.

The average blanks were $1,8 \mu\text{g/l}$ for Cd and $4,8 \mu\text{g/l}$ for Cu. After a 50:1 preconcentration by the described extraction methodology, the detection limits, based on 3 times the standard deviation of the blanks, were $0,6 \mu\text{g/l}$ for Cd and $0,73 \mu\text{g/l}$ for Cu.

The reproducibility of the solvent extraction method was observed to be 6% for Cd and 10% for Cu, as based on five replicate extractions performed on the same sample.

Determination of Cd, Pb and Cu in particulate matter (air, sea water, biota and sediments) are carried out with a JARRELL ASH 810 spectrometer using an air-acetylene flame after a wet digestion of the sample.

In general, atomic absorption techniques require a solution for analysis, but there have been some attempts to analyse solid samples directly by flameless atomic absorption. For example, Lord et al (1975) and Machiroux et al (1976) have determined a variety of metals in freeze-dried fresh water mollusks and in dried plankton samples. Two problems occur particularly with solid samples. One is the difficulty of obtaining a representative sample, since only milligram amounts are analysed. The other problem concerns the availability of an adequate calibration procedure which takes into account the solid matrix composition.

Of considerable current interest is the form in which a metal exists in solution. In the case of atomic absorption it is assumed that the high temperatures involved eliminate the direct possibility of discriminating between species ; only total metal is determined. However, speciation is eventually possible in some cases by coupling the atomic absorption spectrometer with a gas chromatograph which serves to separate various volatile metal species.

b) The determination of mercury

The mercury ion is usually determined by flameless (cold vapour) atomic absorption spectrophotometry with or without a pre-concentration process.

This technique is rapid and simple to use and has the advantage of very high sensitivity and in many cases complete freedom from interference due to other elements.

In a coastal marine environment, an important part of the mercury components is associated with the particulate matter.

For the total mercury (particulate and dissolved) in sea water, we use a method based on the complete conversion of all mercury components present to $\text{Hg}^{(II)}$ and subsequent reduction of this form to $\text{Hg}^{(0)}$ aeration of the solution and spectrophotometric determination with the MAS-50 (Baeyens et al, 1979). It is, therefore, important to choose an appropriate digestion method for the complete oxidation to $\text{Hg}^{(II)}$ such as KMnO_4 + acid or the H_2O_2 method (see below).

We compared both digestion methods, but no systematic difference could be shown. For the dissolved mercury concentration in sea water which is in most cases close to the detection limit, a pre-concentration step is necessary. An adapted method such as a reduction-aeration Au-amalgamation procedure, or a pre-concentration on an exchange column has been tested in order to obtain a better estimate of the concentration of dissolved mercury.

After modification of the single-beam standard apparatus type MAS-50 into a double-beam and the operating conditions being optimized, a detection limit of 5 ng Hg l^{-1} is obtained.

The relative standard deviation obtained in replicate determinations of standard solutions is about 5% at mercury levels of 20 ng Hg l^{-1} .

In order to evaluate our method, we participated in an inter-calibration exercise of Hg in sea water, organized by Olafsson (1979).

From these results we deduce that 1) the precision of our method for the measurement of very low mercury levels (5 ng Hg l^{-1}) is approximately 15%, 2) the recovery of the added quantity of Hg is 102% for 21 ng l^{-1} .

The mercury content in solid matrixes such as particulate matter (air, sea water), biota and sediments were determined by the same method after digestion (see analysis procedure).

A certain number of supplementary studies such as speciation will be carried out in the future ; it has been found possible to distinguish more species of mercury compounds (inorganic mercury, arylmercury compounds, alkylmercury compounds) by changing the chemical reduction system (Goulden et al, 1980).

4.2.2.— Anodic stripping voltammetry (ASV).

The determination of Bi, Cu, Pb, Sb and Zn.

Numerous anodic stripping voltammetry procedures have been reported with direct current (DCASV) applied to a mercury film electrode (TFE) or a hanging mercury drop electrode (HMDE).

Essentially, ASV is a two-step technique, the first step of which involves the electrolytic deposition of a portion of the metal ions in solution into the HMDE or TFE, to preconcentrate the metals in the form of a dilute amalgam. In the second step, the metal in the amalgam is reoxidized to the corresponding ion by the application of a fast linear potential sweep. Current and potential measurements are made during the second step. The position and height of stripping peaks are characteristic of the type and concentration of the metal ion in solution.

In order to increase the signal to background ratio, various voltammetric techniques have been developed. The differential pulse anodic stripping voltammetry method (DPASV) has extended downwards the limits of detection compared to direct current anodic stripping voltammetry (DCASV) and thereby has made this technique very practical.

The voltammetric techniques are only applicable to a relatively small group of metals compared to AAS.

However, it has recently received considerable attention from analytical chemists specialized in water analysis because of the following advantages : 1) the direct determination of heavy metals in natural sea water without the complication of the presence of matrix components (alkaline salts), since they are rather welcome in this case, as the sample contains the necessary supporting electrolyte, 2) for all sorts of aqueous samples (sea water, estuarine, river, lake, rain,...) and other solid matrixes (particulate matter, biota, sediments, ...) stripping voltammetry enables simultaneous determination of several toxic trace metals such as Bi, Cd, Cu, Pb, Sb and Zn after a minimum of sample pretreatment and pH adjustment. The amount of acid required to do this is small and with the ready availability of highly purified acids (suprapure Merck), the need to acidify is not a significant source of contamination, 3) the possibility to discriminate between various oxidation states or such categories as "free" and "bound" metals without modifying the medium, 4) the relative simple and inexpensive instrumentation and its very high sensitivity without preconcentration, 5) the possibility to study the interactions between heavy metals and organic matter (stability constants) and it might be used to determine the concentrations and surface active properties of some organic compounds (e.g. humic substances) in water.

However, some remarks may be made about the interference effects. The most common type of interference is the overlapping stripping peaks (e.g. Sb-Bi). The next most common interference obscures DPASV results because inter-element compound formation

on/in the electrode (e.g. Zn-Cu). The third type of interference encountered is the formation of an organic film over the electrode surface which inhibits the metal deposition and/or stripping processes.

In our experiments a Bruker E 310 modular polarographic analyser with a Hewlett-Packard 7040 A X-Y recorder was used for differential pulse anodic stripping voltammetry on a hanging mercury drop electrode (DPASV-HMDE). A three electrode system was employed. A Metrohm E 410 hanging drop mercury electrode (HMDE) was used as the working electrode. Potentials were measured in reference to a saturated calomel electrode (SCE). An auxiliary platinum electrode was used to minimize I.R. drop effects across the electrolysis cell.

We overcome the overlapping stripping peaks problem by changing the supporting electrolyte and the stripping conditions. An example of that is clearly demonstrated in the simultaneous determination of Bi, Cd, Cu, Pb, Sb and Zn (Gillain et al, 1979). On the other hand, we use the HMDE because this type of electrode is less susceptible to inter-element compound formation than the mercury film electrode (MFE). In our experimental conditions, no intermetallic formation between Zn-Cu and Cu-Sb was found. However, Zn can be expected to interfere with Cu ($\text{Cu} = 80 \mu\text{g/l}$) if it is present at a level exceeding $500 \mu\text{g/l}$ (f.e. in some plankton samples). In that case, to achieve the independent determination of Zn and Cu, it is necessary to add gallium ions to the solution.

Finally, natural water contain tensio-active substances which have a strong tendency to be adsorbed at the electrode surface. This may lead to various effects depending on the voltammetric technique, the type of electrode used and the nature of adsorbed species. Very strong effects have been observed in our simultaneous determination of Bi, Cd, Cu, Pb, Sb and Zn in sea water such as depression of stripping peaks as a result of film formation on the electrode and appearance of anomalous stripping peaks in the vicinity of Pb, Sb and Bi peaks.

However, despite these difficulties, the DPASV technique proves to be a valuable tool for studying metal species dissolved in filtered sea water since some forms are not reductible at the electrode (Florence et al, 1977 ; Gillain et al, 1977 ; Figura, 1980) whereas other forms are. Under *in situ* (neutral) conditions, the DPASV results include ionic as well as some easily dissociable organic and inorganic metal complexes. By changing experimental conditions such as decreasing the pH, we obtain more metal available to the electrode during the reduction step. The metals are apparently made available through a number of processes : 1) protonation of inorganic complexing anions such as carbonate, sulfate, bicarbonate and hydroxide, 2) dissolution of hydrous iron oxide which adsorbs metals, 3) protonation of organic complexing agents (e.g. weak-organic acids such as amino acids) for which complexation strength increases with pH.

With the ultra-violet irradiation step, we destroy organic matter and analyse the "total" dissolved heavy metal content.

The limit of detection, arbitrarily defined as three times the standard deviation of the noise level for the simultaneous determination of Bi, Cd, Cu, Pb, Sb and Zn are 0.02, 0.05, 0.10, 0.03, 0.02 and 0.01 $\mu\text{g}/\ell$ respectively under the following conditions: electrolysis time 60 min, stirring 450 r/min and electrode diameter 0.760 mm.

The precision is estimated to be 5 % for 5.2 $\mu\text{g}/\ell$ Zn; 10 % for 0.6 $\mu\text{g}/\ell$ Cd; 11 % for 6.2 $\mu\text{g}/\ell$ Pb; 9 % for 4.3 $\mu\text{g}/\ell$ Cu; 15 % for 0.4 $\mu\text{g}/\ell$ Sb and 13 % for 0.3 $\mu\text{g}/\ell$ Bi.

4.3.— *Drying, homogenization and ashing of samples with solid matrixes samples (particulate matter, biota, sediments).*

The determination of chemical trace elements in solid matrixes such as suspended matter, plankton and sediment presents many methodological difficulties: drying, homogenization and ashing.

4.3.1.— *Drying.*

A freeze drying method, when carried out close to dry ice temperature, under high vacuum and with cold traps is used (lyophiliser Leybold-Heraeus GT2).

The freeze drying process has the advantage to minimize environmental contamination and involves much less risks of loss of constituents than oven drying.

Prior to drying the sample is frozen but this is not always required for solid material containing not much water (sediments, suspended matter).

The procedure for relatively rapid drying of sample, depending on the nature of product to be dried, is established by trial and error. The total time for complete drying is determined by pressure control.

4.3.2.— *Homogenization.*

The dry residue is very heterogeneous. When small quantities are used for each analysis (plankton, sediment), we reduce the particle size by grinding the sample in a cleaned mechanical agate mortar.

We have tested the homogeneity of the samples and the conclusion is that the gross sample obtained by our method of preparation can be considered as homogeneous (Gillain et al, 1977).

4.3.3.- Ashing.

a) Low temperature ashing (LTA) [Gillain et al., 1982].

A stream of low pressure oxygen excited by a radio frequency discharge is used to decompose organic substances prior to trace element analysis.

The sample is inserted in a closed borosilicate glass reaction chamber. Variable power (0-400 watts) is supplied by a conventional radio frequency oscillator.

Low temperatures (70°C - 100°C) can be maintained by using a low excitation power.

This LTA method minimizes sample handling, is easier to perform as it avoids the use of reagents and gives very low blank values : Zn : 0.030 ; Cd : 0.003 ; Pb : 0.020 ; Cu : 0.020 µg/g° (Sb and Bi not detected).

We have shown that the LTA method does not introduce any noticeable losses of Cd, Cu, Pb and Zn. The ashing was conducted under the following conditions : power 200 watts, pressure 1 mmHg, oxygen flow 40 ml/min and time 10-15 hours.

Nevertheless, under these conditions the mineralization of a standard sample (NBS, n° 1571) has shown that losses of Sb can appear (recovery 25-35%) chiefly in the presence of chlorides.

Because of the dependence of temperature and oxydation rate at the applied power, different powers and ashing times have been tested on a standard solution of SbIII. The results show that quantitative recuperation of Sb is obtained only for a low power (< 150 watts) and very short lapses of times (< 1 hr).

The conclusion is that the volatilisation losses cannot be prevented in the conditions previously cited necessary to a complete mineralization of marine samples (power 200 watts, time 10-15 hours).

Consequently, the low temperature ashing appears to be an acceptable method, except when dealing with volatile compounds.

b) Digestion bomb.

We use a home-made PTFE digestion bomb as described in a recent paper (Gillain, 1982).

We have determined the volume of acids (HNO₃ or HNO₃-H₂SO₄ 1:1) necessary to dissolve completely 300 mg samples (ASV) or 1 g samples (AAS) at a temperature such as to obtain in the bomb an internal pressure lower than 10 atmospheres with a heating time of 6 hrs.

From the experiments it was concluded that the samples with a high organic content were easily decomposed but not completely mineralized when these acids were used. Consequently, we decided to use the HNO₃-H₂O₂ 1:1 mixture (biological samples) + concentrated HF (geological, mineralogical samples).

The efficiency of the digestion has been checked with a NBS sample (Orchard Leaves, 1971).

We also use a commercial PTFE digestion bomb (Techni-Verre, Paris) with a larger inner volume of about 100 ml. The bomb should be heated above 80°C and the internal pressure must not exceed 2-3 atmospheres.

A definite advantage of the wet ashing in a PTFE bomb is that no significant loss of volatile metals such as Hg and Sb was observed (Decadt et al, 1980 ; Gillain, 1982).

Nevertheless the blank values are somewhat higher than those obtained by LTA ashing

Zn : 0.12 µg/g* ;
Cd : 0.01 µg/g* ;
Pb : 0.12 µg/g* ;
Cu : 0.06 µg/g* ;
Sb and Bi : not detected.

4.4. - Procedure for analysis.

4.4.1. - Sea water samples (water column, microlayer).

- a) *Direct and simultaneous determination of Zn, Cd, Pb, Cu or Zn, Cd, Pb, Cu, Sb, Bi dissolved in sea water by differential pulse anodic stripping voltammetry with a hanging mercury drop electrode. An approach to speciation.*

Direct and simultaneous determination of Zn, Cd, Pb and Cu.

30 ml of sea water previously filtered through a membrane (0.45 µm) is placed in the electrolysis cell. The sample is stirred magnetically (450 rpm) and oxygen is removed by bubbling pure nitrogen for 30-40 min. Electrolysis is carried out at a potential of -1.200 V (V.S. SCE) for 10-20 min while nitrogen is introduced into the space above the surface of the solution. One minute before the end of the electrolysis, the stirrer is stopped and the current-potential curve is then recorded from -1.200 to 0 V with an appropriate current sensitivity with scan rate 2 mV.s⁻¹ and pulse 35 mV.

Direct and simultaneous determination of Zn, Cd, Pb, Cu, Sb and Bi.

30 ml of filtered sea water are introduced into the electrolysis cell and adjusted to a 2M NaCl concentration with suprapure NaCl. Reagents blanks subjected to this procedure gave no problems.

* Dry weight ashed.

The solution is stirred, purged and deposition of six elements was made at -1.200 V (V.S. SCE) for 15-20 min in the same conditions as described above.

One minute before the end of the electrolysis, the stirrer is stopped and the current-potential curve is then recorded from -1.200 to -0.350 V with scan rate 2 mV.s^{-1} and pulse 35 mV .

The potential is held at -0.350 V with stirring for 15-20 min. At the end of this plating time, the stirring is stopped again and the resulting current-potential curve is recorded down to a potential of 0 V with scan rate 0.5 mV.s^{-1} and pulse 10 mV .

b) Speciation of Bi, Cu, Pb, Cd and Zn.

Determination of free and "very labile" metals.

Ionic and "very labile" metals were determined in sea water at its natural pH (pH = 8).

Determination of "moderately labile" metal.

Direct analysis is performed immediately after addition of suprapure concentrated HCl to about pH 1.

"Moderately labile" metal concentration is defined as the difference in concentration under acidic conditions (pH 1) and under in situ (neutral) conditions.

Determination of total metal and "very weak labile" + "inert" fractions.

Total metal concentrations were determined after exposing acidified sea water (containing 0.05 mL of 30% H_2O_2 per 30 mL) in quartz tubes to UV light with rotative stirring for one night (Gillain 1980).

The difference between the total concentration and concentration under acidic (pH 1) conditions represents the amount of "very weak labile" + "inert" metal.

c) Speciation of Sb.

Determination of Sb^{III} .

Direct analysis of acidified (pH 1) sea water are performed immediately after adjusting to a 2 M NaCl concentration.

In these conditions, we determine free metal ions + ASV "very labile" and ASV "weakly labile" organic and inorganic complexes of Sb^{III} . Sb^{V} gave no stripping peak in our experimental conditions ($[\text{Cl}^-] = 2 \text{ M}$, pH = 1).

Determination of $\text{Sb}^{\text{III}} + \text{Sb}^{\text{V}}$

The determination of $\text{Sb}^{\text{III}} + \text{Sb}^{\text{V}}$ is performed on acidified sea water (pH 1) after reduction of Sb^{V} to Sb^{III} by bubbling SO_2 through the solution.

Excess of SO_2 was removed from the solution by bubbling pure nitrogen through the cell (Brihaye, 1979).

The efficiency of the reduction procedure was tested on a standard solution and it was found that total Sb^{V} could be completely reduced by this technique (Brihaye, 1979).

Determination of total Sb.

Total antimony can be determined on a sea water aliquot after acidification to pH 1, UV irradiation and reduction of Sb^{V} to Sb^{III} are carried out by the same procedure as described above.

The flow chart for the speciation method is presented in figure 1.

d) Determination of Cd and Cu dissolved in sea water by atomic absorption spectrophotometry with graphite furnace.

The metals were determined after using an organic extraction technique. In this particular case it involves chelation with ammonium 1-pyrrolidine-dithiocarbamate (APDC) and diethylammonium diethyldithiocarbamate (DDDC), an extraction with chloroform and back-extraction with nitric acid (Bruland et al, 1979).

About 250 ml of sea water (filtered through a 0.45 μm membrane) was buffered to pH 4 with 2-4 ml of ammonium acetate; 1 ml of a solution containing 1% (w/v) each of APDC and DDDC (stabilized in 1% ammonium hydroxide solution and purified by chloroform extraction) and 8 ml of chloroform were added. The mixture was then shaken vigorously for 2 min in a separation funnel.

After 5 min to allow phase separation, the chloroform fraction was drained into a 125 ml separation funnel, and 4 ml of 7.5 M HNO_3 were added to initiate degradation of the dithiocarbamates.

The original sea water sample was then re-extracted with an additional 6 ml chloroform and the chloroform fractions were combined. The funnel containing the chloroform and the acid phases was then shaken for 2 min. After phase separation, the chloroform fraction was discarded and the acid fraction collected in a PTFE container.

The separation funnel was rinsed with another 2.5 ml of 7.5 M HNO_3 and the acid fractions were combined. The acid extract was then evaporated to dryness and the residue was redissolved in 5 ml of 1% HNO_3 for analysis by graphite furnace atomic absorption spectrophotometry.

The procedure yields a 50:1 concentration increase.

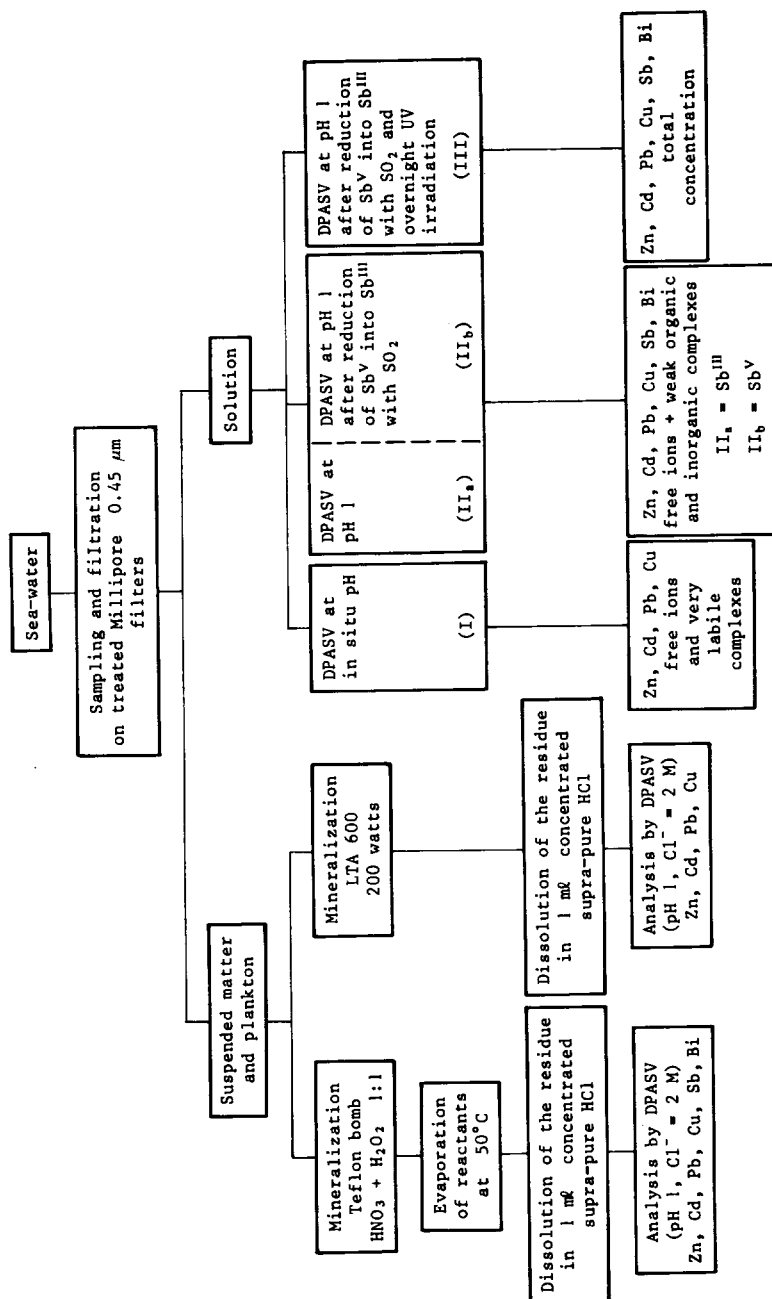


fig. 1.

Analytical protocol to analyse Zn, Cd, Pb, Cu, Sb and Bi in suspended matter, plankton and to study speciation of metals in solution in the water column.
LTA = low temperature ashing (activated O₂).

e) *Determination of total and dissolved Hg in sea water by cold vapour atomic absorption spectrophotometry.*

To 150 ml of sea water sample one adds 5 ml of KMnO_4 (5 %) + 5 ml of H_2SO_4 (1:1) + 5 ml of HNO_3 (1:2).

The digestion lasts 16 hours at 60 °C. The excess KMnO_4 is reduced with 1 ml hydroxylamine (5 %) and the volume in the BOD-bottle is brought to 180 ml. After reduction by 1 ml of NaBH_4 (1 %), mercury determination is carried out with a MAS-50.

The results obtained will be defined as dissolved Hg for the filtered and total Hg for the unfiltered samples.

4.4.2.- *Solid matrixes (particulate matter in sea water and air, biological samples and sediment samples).*

a) *Direct and simultaneous determination of Zn, Cd, Pb, Cu or Zn, Cd, Pb, Cu, Sb and Bi present in particulate matter (sea water, air), sediments and biological samples by differential pulse anodic stripping voltammetry.*

Wet digestion procedure.

The Millipore membrane filters or 300 mg of lyophilized material are, in a laminar flow clean box, inserted into a PTFE vessel. 4 ml of concentrated HNO_3 , 4 ml of H_2O_2 (30 %) and 1 ml of concentrated HF were added to the bomb which was then closed and heated to approximately 160 °C for 6 hours.

The digested material was subsequently evaporated to dryness and a further 1 ml of suprapure concentrated HCl was added. The digestion was allowed to proceed overnight and the digest was then brought to pH 1. The final volume was 100 ml of which 30 ml were transferred to the polarographic cell for DPASV analysis by the same procedure as for sea water samples.

A blank solution containing 4 ml concentrated HNO_3 , 4 ml H_2O_2 (30 %) and 1 ml concentrated HF was evaporated in the same way.

LTA procedure.

The Millipore membrane filter samples or 300 mg of solids are, with precautions against airborne contamination (clean box), introduced into a quartz beaker. The filter or the samples are then destroyed either by low temperature ashing in an oxygen plasma (< 200 °C) with the following conditions:

O_2 flow	50 ml/min,
RF power	200 watts,
Time	24 hours.

After dissolution of the ashed sample in 1 ml suprapure concentrated HCl during a night, the solution was quantitatively transferred to 100 ml volumetric flasks (final acid concentration: 0.1 N HCl) for DPASV analysis

b) Cd, Pb and Cu determination in particulate suspended matter, aerosol and sediment by atomic absorption spectrophotometry.

Particulate matter and sediment.

The membrane filter or 1g of finely ground sample are transferred into the PTFE bomb with concentrated HNO_3 + HCl + HF 4:1:1. The bomb is sealed and heated to 80°C for 24 hours.

The bomb is then cooled in a deep-freezer and its content is evaporated to dryness. Purified nitrogen was passed over the acid to accelerate the evaporation and to avoid contamination by dust. The residue was redissolved in 25 ml of 1% HNO_3 .

Depending on the concentration, the measurements of Cd, Pb and Cu are carried out using air-acetylene flame (FAAS) or graphite furnace atomic absorption spectrophotometry (GFAAS).

Aerosols.

Whatman filter samples are cut into quarters and inserted into the PTFE bomb with 10 ml concentrated HNO_3 , 5 ml concentrated HCl and 2.5 ml concentrated HClO_4 .

The digestion is carried out at 80°C for 4 hours. After cooling in a deep-freezer, the bomb is opened and 5 ml concentrated HNO_3 is added. Then the bomb is recapped and allowed to stand for 5 hours at 80°C . After cooling, the sample is evaporated to 1 ml and quantitatively transferred in 25 ml containers using ultra-pure water. The samples can then be analysed directly for the element of interest by flame atomic absorption spectrophotometry (FAAS).

c) Determination of mercury content in particulate suspended matter, sediments and biological materials.

Pyrolysis procedure.

10 mg of weighed lyophilized material are pyrolyzed under H_2 flux at 800°C . Complete reduction of pyrolysis products was ensured by passage through a tube packed with a catalyst (Ni) and the mercury set free was fixed on a Au-column.

By heating in an electric mini-oven (500°C), the amalgam formed is then decomposed and the released mercury is swept by the transporting gas at a flow rate of $750 \text{ ml} \cdot \text{min}^{-1}$ into the MAS-50 measuring cell at 253.7 nm into 75 s.

Aerosols collected on a GF/C-filter preheated at 500°C prior to the sampling, can be inserted directly into the pyrolysis tube.

The calibration curve is obtained by injections of known amounts of mercury saturated nitrogen. The coefficient of variation is about 1%.

Blanks were run frequently and consisted in the ultra-pure water and acids and unused, decontaminated GF/C-filters. No signal above analytical noise was detected.

Wet digestion procedure.

Sediments.

1 g of lyophilized material is weighed directly into the PTFE digestion bomb. 5 ml mercury-free (less than $5 \cdot 10^{-7}$ %) concentrated nitric acid and 5 ml concentrated fluohydric acid are added.

The digestion is carried out at 110 °C during 16 hours. After cooling the volume is brought to 180 ml with ultra-pure water. After addition of 1 ml of NaBH_4 (1 %), to reduce the oxidized mercury to its atomic state, mercury concentrations were determined using a Coleman MAS-50 mercury analyser.

Biological material.

1 g of undried or lyophilized material is weighed into a 250 ml Erlenmeyer flask with ground neck. 2 mg vanadium pentoxide, 10 ml concentrated nitric acid and 4 ml sulfuric acid are successively added.

The sample is gently refluxed at 80 °C during about 10 minutes. 20 ml of KMnO_4 (5 %) are added dropwise while the reflux is continued during 15 minutes. After cooling, 15 ml of hydroxylamine (5 %) is added and the cooler is washed down. The sample is quantitatively transferred to a 250 ml volumetric flask and the mercury content of this solution is determined using a MAS-50.

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