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Methods for analysis for trace metals in marine and other samples

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CONTENTS Page

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BACKGROUND

The Burnham-on-Crouch Laboratory of the Directorate of Fisheries Research (DFR) of the Ministry of Agriculture, Fisheries and Food (MAFF) has well-equipped analytical chemistry facilities and is called upon to provide a comprehensive range of analytical services. Although some of the requirements arise from customers elsewhere in DFR, these are usually single or small volume requests. The vast majority of the analyses undertaken stem from programmes undertaken at the request of MAFF Policy Divisions which are responsible for assessing the distribution and the impact, or potential impact, of many types of discharge other than radioactive discharges, on both marine and freshwater fisheries. Some of this work arises from Marine Environment Protection Division's special responsibility for the licensing, enforcing and monitoring of the impact of wastes dumped at sea. In addition, there is a major programme of general environmental quality monitoring which is used to back-up pollution prevention and environmental protection activities.

Over the years, analyses have been conducted on a wide variety of sample types, both freshwater and marine, environmental and non-environmental (effluents, mine tailings, drill-muds, dredgings) for a large range of contaminants. Many of the analyses are of a routine or semi-routine nature and set procedures have been developed for their conduct. These procedures have been thoroughly tested and proven by the Burnham-on-Crouch Laboratory and in most cases have been subjected to intercomparison tests with other laboratories and to quality assurance schemes involving the use of standards and reference materials.

This publication is one of a series relating to both non-radioactive and radioactive contaminants and concerns revised analytical methods for analysis of trace metals. Although fairly complete details are provided, the intention of the publication is to inform other analysts of the general procedures, apparatus and analytical instrumentation required. If difficulties in applying the methods are encountered, contact should be made with the authors from whom further details can be obtained. Where trade names are quoted this merely indicates these products have been found satisfactory for the stated purpose. The equivalent products of other suppliers may perform equally well.

INTRODUCTION

Trace metal analysis is carried out at the Directorate of Fisheries Research, Burnham-on-Crouch Laboratory for a number of major environmental programmes and for research into the fate of trace metals in the marine environment.

The publication is divided into two sections, the first is concerned with the methods of analysis for metals in sea water. The methods are used in studies designed to establish the environmental concentrations and fates of the trace metals. To this end, dissolved and suspended particulate metal concentrations are determined separately.

The second section is concerned with the methods of analysis of trace metals in biota, sediments and dredge spoils. Biota samples are used for general environmental quality monitoring as indicator species and for food quality assurance purposes. Sediment samples are also analysed as environmental quality indicators but particularly in connection with licensing of disposal of dredged material. The 'Total' metals analysis of sediments allows the determination of normalisation elements such as aluminium, lithium and rubidium. These can be used to distinguish between background concentrations of trace metals and elevated concentrations resulting from anthropogenic inputs.

SAMPLING, FILTRATION AND DETERMINATION OF TRACE METALS IN SEA WATER

1. INTRODUCTION

The seven dissolved and suspended particulate trace metals most commonly determined by the Burnham-on-Crouch Laboratory (Cd, Cu, Hg, Mn, Ni, Pb and Zn) are all anthropogenically important. Manganese is also important because of its redox chemistry and its interaction with other trace metals which in turn affects their environmental fates.

2. SAMPLING

Extreme care is necessary to avoid contamination of sea water samples for dissolved trace metal analysis. Sources of possible contamination include the research vessel, sampling equipment, atmosphere, analytical reagents, labware, and the analyst. All operations are therefore carried out in a 'Class 100' laminar flow cabinet, and disposable polythene gloves are worn. Equipment is precleaned by soaking in acid and rinsing with pure water (>10 megohm. cm) followed by a final thorough rinse with the liquid it is to hold. This reduces the chances of metal contamination or loss by adsorption onto solid surfaces.

To avoid contamination from the ship, samples are taken via a tube suspended from a buoy at least 3 m from the ship's side, and from a depth of about 2 m. Surface water samples are pumped through PFA tubing directly into the shipboard clean laboratory using a teflon bellows pump. Deep water samples (>5 m) are collected using teflon coated Go-Flo bottles (General Oceanics, Miami) deployed on a kevlar rope and triggered by teflon-coated messengers.

2.1 Surface water sampling

A pneumatically-driven, teflon bellows pump (Cole Parmer, Chicago, model 07152-70) is connected to 25 m of PFA tubing (8 mm o.d.), which is sheathed with reinforced pressure tubing (12 mm i.d.) for protection. Compressed air at about 60 psi is supplied to the pump from a compressor or gas cylinder. The pump can deliver the sample at a rate of 750 ml min^{-1}.

The buoy is constructed as shown in Figure 1. A buff, used for flotation, is held in a frame made from 20 mm o.d. 'Durapipe' PVC tubing. The frame is extended below the buff to a single vertical tube, weighted internally with a steel rod and lead shot, and securely sealed. The tubing from the pump is held to the buoy with tie-wraps and waterproof tape. Samples are drawn from 1 m below the bottom of the vertical tube to avoid contamination from the buoy.

The sampler is deployed, on a nylon rope, to the windward side of the ship. The pump is switched on and the system is allowed to flush through for 5-10 minutes before the sample is taken.

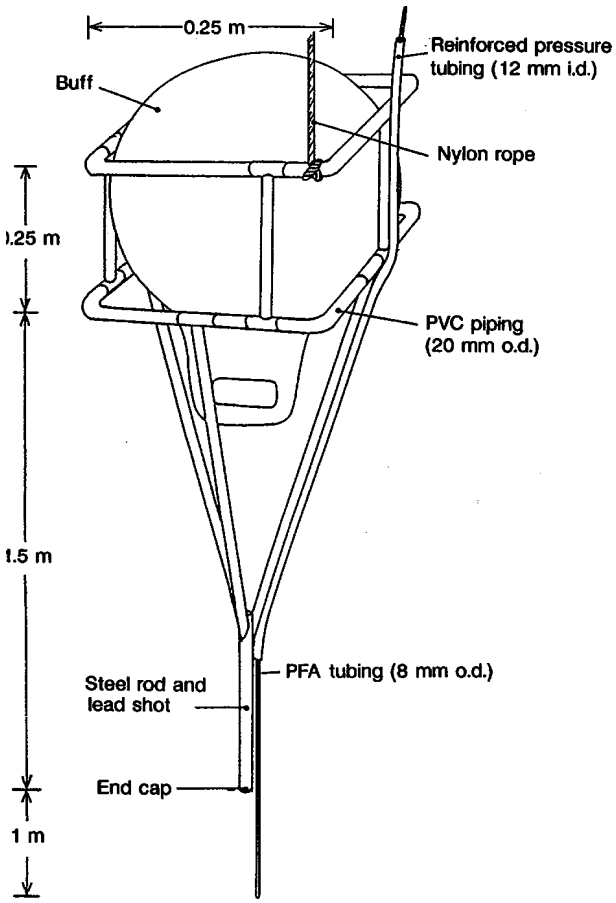

Figure 1. Diagram of the sampling buoy (Harper, 1987)

2.2 Sampling at depth

The Teflon coated Go-Flo bottles (2.5 or 5 l) are fitted with silicone 'O'-rings and teflon taps. Before use they are rinsed with pure water, filled with 0.1% nitric acid solution (Analytical grade) and stored in the closed position for at least five days. They are emptied, refilled with pure water and wrapped in polythene bags.

A kevlar rope is wound onto the winch, and a 10 kg weight, contained in two heavy duty plastic bags, is fastened onto the end of the rope. The Go-Flo bottles

are cocked, attached to the rope, and lowered to the required depths. They are allowed to remain at the sampling depth for five minutes to equilibrate with their surroundings before the messengers are released to trigger the bottles.

On recovery the bottles are handled with plastic gloves during transfer to the clean laboratory and for subsequent operations. A short length of acid soaked silicone tubing is fitted to the tap, and the sample is transferred to the collecting bottles.

3. COLLECTION, FILTRATION AND STORAGE OF SEA WATER AND SUSPENDED PARTICULATE MATTER FOR ANALYSIS OF TRACE METALS OTHER THAN MERCURY

3.1 Sample bottle preparation

Unused low density polythene bottles (500 ml) are initially rinsed with water and soaked for two days in 5% v/v solution of 'Decon 90' These, and previously used bottles, are then rinsed with deionised water, filled to the brim with 1% nitric acid (Analytical grade) and wrapped in resealable polythene bags. After seven days they are emptied, rinsed, and refilled with 0.1% nitric acid (ultra pure grade) and wrapped in polythene bags for a further seven days before use.

When required, each bottle is emptied in the shipboard clean laboratory, rinsed thoroughly with pure water (>10 megohm. cm) and then with the sea water sample. The bottles are filled to the brim, capped and stored in polythene bags. Samples are filtered as soon as possible after collection and certainly within 24 hours.

3.2 Filtration equipment and procedure

Offline pressure filtration avoids committing sampling apparatus for lengthy periods of time when filtration is slow. The filtering apparatus (Figure 2) consists of four 500 ml polythene separating funnels calibrated at 50 ml intervals. The lids are modified to allow an airtight seal with silicone rubber tubing (6 mm i.d.), through which oxygen free nitrogen is supplied at a pressure of 1 to 2 psi.

All filtration apparatus is washed thoroughly before use by soaking for two days in 'Decon-90', rinsing with pure water and soaking for a further five days in 1% nitric acid (Analytical grade). After a final thorough

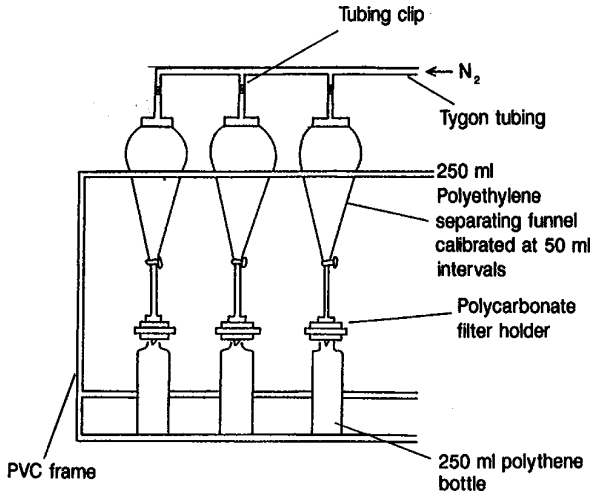

Figure 2. Filtration system for metals other than mercury

rinse with pure water the equipment is dried in a laminar flow hood, and wrapped in clean polythene bags or PVC-free cling film.

Pre-weighed 47 mm diameter 0.4 µm Nuclepore polycarbonate membranes are loaded into clean, numbered, polycarbonate filter holders (Swin-loc, Nuclepore) fitted with silicone 'O'-rings (supplied by Arco Ltd). The membranes are cleaned by sequentially passing about 50 ml 1% HNO_3, and pure water through the holders. This procedure does not affect the membrane weights. The membrane-loaded filter holders are stored wrapped in PVC-free cling film.

The equipment is set up as shown in Figure 2. All taps are closed and a pressure of about 1 to 2 psi is applied through the system. The sea water sample bottle is thoroughly shaken and the entire contents (about 585 ml) are poured into a separating funnel. The funnel lid is replaced and pressure is applied by opening the appropriate taps. The sea water sample is allowed to run back into the sample bottle until the water level in the separating funnel reaches the 500 ml mark. This first aliquot of sample is used to rinse the bottle, and is then discarded. The remaining 500 ml is collected in the bottle. (For offshore waters with low suspended particulate matter loadings it is necessary to filter up to 2000 ml of water for each station to obtain enough particulate material for trace analysis). After filtration the water sample is acidified with 500 µl concentrated nitric acid (ultra pure grade). The bottle is capped and stored in a resealable polythene bag prior to dissolved trace metal analysis.

The membranes are washed free from salt with about 50 ml pure water (>10 megohm. cm). They are then removed from the filter holders with teflon forceps, transferred to a sterile polystyrene Petri-dish, and left to dry in a laminar flow hood. After being reweighed to an accuracy of 0.01 mg, they are stored in sealed labelled Petri-dishes ready for particulate analysis.

4. ANALYSIS OF DISSOLVED TRACE METALS (OTHER THAN MERCURY)

A liquid/liquid extraction technique is used to remove the interfering effects of the major 'salt' elements, and to concentrate the dissolved trace elements in sea water. The method used is based on that of Danielsson *et al.* (1978), as modified by Statham (1985) and Tappin (1988). It is a multi-element technique, suitable for determining cadmium, cobalt, copper, iron, manganese, nickel, lead and zinc in sea or estuarine water. The metals are extracted from the water into 1,1,2-trichloro-trifluoroethane (Freon) using a mixed dithiocarbamate complexing agent (see Section 4.2). They are then back extracted into dilute nitric acid for analysis by graphite furnace atomic absorption spectrometry. Freon is used because it has a low toxicity compared with other suitable solvents, has a very low solubility in sea water, gives a rapid total phase separation and is readily purified. The reagents must be very pure to ensure that they do not become a source of contamination. Double deionised water (>10 megohm. cm) and ultra pure grade nitric acid are used. Freon is cleaned with the sub-boiling point distilled nitric acid, and the complexing agent is sequentially extracted with purified Freon. All reagents are stored in fluorinated ethylene propylene (FEP) bottles. The liquid/liquid extraction steps are mechanised by using specially designed rotating tables made from polypropylene. The tables are fixed inside the laminar flow cabinet, but the motor and other fittings are outside.

4.1 Apparatus

All apparatus is cleaned before use by soaking with 1% nitric acid for a week, rinsing with double deionised water and leaving to dry in a 'Class 100' laminar flow cabinet.

125 ml FEP separating funnels* (Nalgene) x 12
500 ml FEP separating funnels (Nalgene) x 2
125 ml FEP bottle (Nalgene) x 1
250 ml FEP bottles (Nalgene) x 4
1000 ml FEP bottle (Nalgene) x 1
2000 ml FEP bottle (Nalgene) x 1
10 ml polythene measuring cylinder x 1
100 ml polythene measuring cylinder x 1
10 ml FEP Oak Ridge centrifuge tubes (Nalgene)
1.5 ml polypropylene microcentrifuge tubes with hinged cap (Eppendorf)
Micropipettes and clear pipette tips (Finnpipette)
Rotating tables (see Figures 3, 4 and 5)
100 ml polypropylene volumetric flasks
Teflon autosampler cups (Perkin Elmer) and polypropylene lids.

* The lids of these separating funnels leak and must be sealed with teflon thread seal tape.

4.2 Reagents

4.2.1 Dilute nitric acid

Four per cent nitric acid is prepared from ultra pure grade concentrated nitric acid and double deionised water using acid cleaned apparatus. It is stored in a 250 ml FEP bottle, wrapped in polythene.

4.2.2 Ammonia solution

Ammonia solution (sg 0.800, Analytical grade) is diluted 1:1 with double deionised water and stored in a 125 ml FEP bottle wrapped in polythene.

4.2.3 Metal standards

Spectroscopic (or equivalent) grade 1000 ppm metal nitrate standards are used to make up a mixed element working standard of 0.1 µg l^{-1} Cu, Ni, Mn, Pb, and Zn and 0.01 µg l^{-1} Cd, in 4% HNO_3. This is stored in a 100 ml polypropylene volumetric flask.

4.2.4 Freon

About 250 ml Freon is placed in an acid cleaned 500 ml FEP separating funnel with 1000 µl concentrated nitric acid (ultra pure grade). This is rotated as shown in Figure 3 for five minutes. 100 ml of double deionised water is added to the funnel which is rotated for a further five minutes. The Freon is run off into a clean FEP bottle, and the aqueous phase discarded. The Freon is returned to the separating funnel and the procedure is repeated twice. The cleaning is completed by adding a further 100 ml of double deionised water to the Freon and rotating without any acid. Cleaned Freon is stored in a 2000 ml FEP bottle wrapped in polythene.

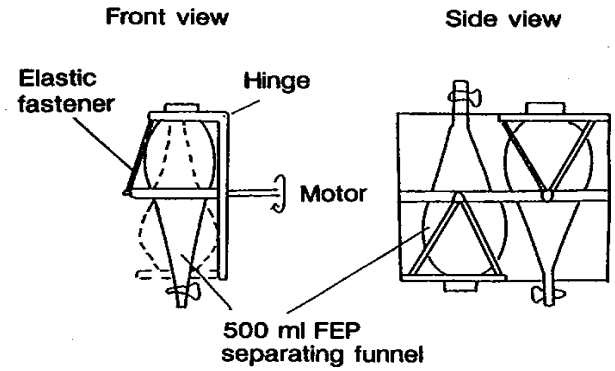

Figure 3. Rotating table for cleaning freon and complexant (after Statham, 1985)

4.2.5 Complexant

A 3.5% salt solution is made up by dissolving 8.75 g sodium chloride (Analytical grade) in 250 ml double deionised water. Five grams each of APDC (ammonium pyrrolidine dithiocarbamate, Analytical grade) and DDDC (diethylammonium diethyldithiocarbamate, Laboratory Grade) are weighed out and dissolved in the sodium chloride solution. The complexant is filtered through a Whatman No. 1 paper under vacuum, to remove any undissolved salts or precipitating metal-containing colloids, then stripped of trace metals by rotating for five minutes with four aliquots of clean Freon (25 ml) in the 500 ml separating funnel (Figure 3). The complexant is stored in a 250 ml FEP bottle in a refrigerator, and used within 24 hours.

4.3 Method

A typical batch consists of two reagent blanks, two double deionised water blanks, four standard additions, three commercial certified reference materials, and about 25 samples.

The extraction of the metals must be performed at a sample pH of 7 or higher to include the quantitative determination of manganese (Kinrade and Van Loon, 1974; Statham, 1985). The volume of ammonia solution necessary to neutralise the samples is determined before analysis. Small volumes of ammonia solution are added sequentially to a 50 ml sub-sample of water. The pH of the solution is monitored after each addition using a glass electrode, and the volume of ammonia required to bring the pH to 7 or 8 is recorded. This is repeated for three sea water samples per batch, and an average volume of ammonia necessary to neutralise 100 ml is calculated. The volumes required for the reference sea water, the double deionised water blanks and the additions are determined separately. The sub-samples used for these tests are discarded.

One hundred ml of each sea water sample is measured out using a clean 100 ml polythene measuring cylinder, and poured into a 125 ml separating funnel in the rotating table (Figure 4). The appropriate amount of ammonia solution is added using a micropipette and the funnel is shaken to prevent the formation of localised areas of high pH. For standard addition purposes a metal spike of a few tens of microlitres is added to duplicate samples. To each separating funnel 4 ml of Freon is added using a 10 ml polythene measuring cylinder followed by 3 ml of complexant using a micropipette. The separating funnels are then rotated for five minutes.

When the two phases are fully separated, the Freon is drawn off into a 10 ml screw-capped FEP centrifuge tube. Care is taken to avoid any transfer of the aqueous

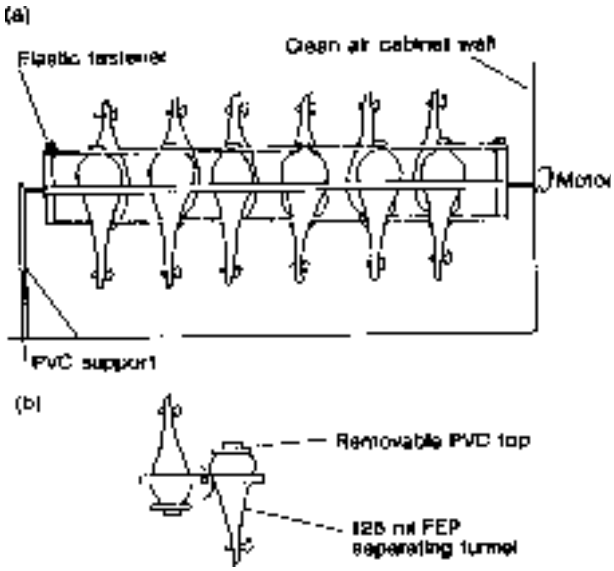

Figure 4. Rotating table for forward extraction (after Tappin, 1988). (a) Front view and (b) side view from inside clean air cabinet

phase since the salt would interfere with the subsequent GFAAS determination (Danielsson *et al.* 1978; Statham, 1985). Another 3 ml of clean Freon is then added to each funnel, and they are rotated for a further five minutes. The Freon is drawn off and added to the same centrifuge tube as previously.

The back extraction is carried out as follows, 20 µl concentrated nitric acid (ultra pure grade) is added to the Freon in the centrifuge tubes, and they are rotated for five minutes (Figure 5), 500 µl of double deionised water is added to the tubes and they are rotated for a further five minutes. The aqueous phase is then carefully drawn off using a micropipette and transferred to 1.5 ml snap-shut polypropylene micro-centrifuge tubes (Eppendorf). The back extraction is repeated, and the second aliquots are added to the first. The extracts are then analysed using a PU9200 furnace atomic absorption spectrometer, with a PU9380 autosampler (Unicam Instruments), under the furnace conditions listed in Table 1. Deuterium background correction is used throughout, and a matrix modifier, ammonium dihydrogen orthophosphate $NH_2H_2PO_4$, is used for Pb analysis. Part-ridged pyrolytically coated graphite tubes are used for the analyses.

To prevent carry over between samples, the 100 ml measuring cylinder is rinsed with each sample before use, and the separating funnels are cleaned before use and between each sample by rotating with 6 ml Freon and 3 ml complexant for five minutes. The micropipette tips used to transfer the aqueous phase to the micro-centrifuge tubes are rinsed with 4% nitric acid between each sample.

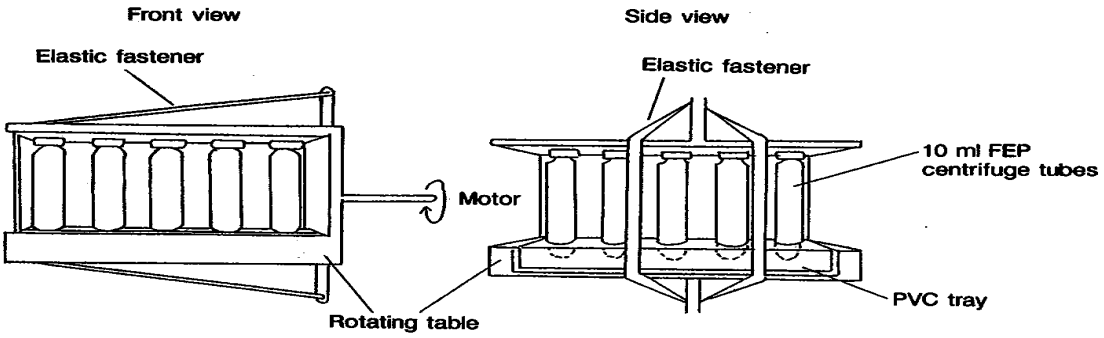

Figure 5. Rotating table for back extraction (after Tappin, 1988). Tray holds 25 centrifuge tubes and slides into the rotating table

Table 1. Furnace conditions for analysis of dissolved and suspended particulate metals in sea water

Element	Unit	Cd	Cu	Fe	Mn	Ni	Pb	Zn
Wavelength	nm	228.8	324.8	248.3	279.5	232	283.3	213.9
Bandpass	nm	0.5	0.5	0.2	0.2	0.2	0.5	0.5
Lamp current	mA	8	8	8	10	8	9	10
Ashing temperature	°C	300	800	1100	1000	1200	750	550
Ashing time	s	60	35	60	10	20	30	30
Atomisation temperature	°C	1200	2400	2100	2500	2600	1300	1400
Atomisation time	s	3	3	3	3	6	3	3
Gas flow	ml min^{-1}	0	0	0	1	0	0	3
Clean up	°C	2200	2800	2600	2800	2800	2300	2400

4.4 Analytical quality control

Detection limits have been defined as "the smallest concentration of a determinand for which we can be 95% confident that the determinand will be detected by the method" (Caulcutt and Boddy, 1983). The corrected reading corresponding to a limit of detection is equal to the criterion of detection (tb x sb) plus (td x sd), where:

sd = standard deviation of duplicate blanks from n batches
td = 95% critical value from t-distribution with n-1 degrees of freedom
sb = standard deviation of a set of m readings near the detection limit
tb = 95% critical value from t distribution with m-1 degrees of freedom.

Detection limits, and typical blank values, for 100 ml sea water samples are listed in Table 2, along with the long-term average and standard deviation of the reference material values (CASS-2, National Research Council, Canada) obtained for 28 samples from 14 batches extracted throughout a year. (No data are given for Pb, since the certified value is very close to the blank value for this element.) The relative standard deviations of ten replicate samples analysed in a single batch are also given in Table 2.

The relative standard deviations for manganese and zinc samples are higher than those for the other elements. This is because their higher extract concentrations require further dilution steps before they can be analysed by furnace.

Table 2. Analytical quality control parameters (1992-1993) for dissolved metal analysis based on 100 ml sea water samples

Metal	Detection limit (µg l^{-1})	Typical blank (µg l^{-1})	Observed CASS-2 Mean (µg l^{-1})	Observed CASS-2 Std. Deviation (µg l^{-1})	n	Certified CASS-2 Mean (µg l^{-1})	Certified CASS-2 Std. Deviation (µg l^{-1})	% RSD (n=10)
Cd	0.006	0.001	0.02	0.002	24	0.019	0.004	5.7
Cu	0.05	0.004	0.664	0.078	21	0.675	0.039	3.4
Mn	0.03	0.001	1.81	0.37	21	1.99	0.015	12.9
Ni	0.1	0.01	0.338	0.068	24	0.298	0.036	5.1
Pb	0.005	0.02				0.019	0.006	4.3
Zn	0.4	0.006	2.28	0.37	18	1.97	0.12	10.5

RSD = relative standard deviation

5. ANALYSIS OF SUSPENDED PARTICULATE TRACE METALS (OTHER THAN MERCURY)

The partial digestion technique described here leaches most of the heavy metals from the suspended particulate matter (SPM), but does not break down the mineral lattices of the particles. It has been previously described by Balls (1985).

5.1 Apparatus

30 x 10 ml PTFE beakers (Azlon)
Teflon forceps (Cowie Scientific)
1-10 ml acid dispenser (Volac)
Enamel topped hotplate (Corning)
Micropipettes and clear pipette tips (Finnpipette)
30 ml Universal polystyrene vials (Nunc)
Acid cleaned PTFE autosampler cups (Perkin Elmer) and polypropylene lids.

5.2 Reagents

5.2.1 Dilute nitric acid

Twenty per cent (v/v) acid is prepared from ultra pure grade nitric acid and double deionised water. It is stored in a 2.5 l glass bottle.

5.2.2 Working standards

Spectroscopic grade (or equivalent) 1000 ppm metal standards are used to make up a mixed element working standard of 0.01 mg l^{-1} Cd and 0.1 mg l^{-1} Cu, Mn, Ni, Pb and Zn in 20% nitric acid. The standard is stored in a 100 ml polythene volumetric flask.

5.3 Method

A typical batch consists of about 20 samples, four membrane blanks, two reagent blanks, and three standard additions. The 10 ml PTFE beakers are numbered and washed in distilled water. They are thoroughly cleaned as follows; 5 ml concentrated nitric acid (Analytical grade) is added to each beaker from a glass dispenser. The beakers are placed on an enamel topped hotplate at 160°C in a 'Class 100' laminar flow cabinet, discharging into a fume hood. After at least one hour (but before the acid boils dry) the beakers are removed from the hot plate and allowed to cool. The excess acid is discarded and the beakers are rinsed with double deionised water.

The SPM loaded sample membranes are removed from their Petri-dishes with teflon forceps, folded, and placed in the appropriate numbered PTFE beakers. Four unused membranes are added in the same way, as blanks. A 1-5 ml variable pipette and clear pipette tips are used to add 4 ml concentrated nitric acid (ultra pure grade) to each crucible. The crucibles are returned to the hotplate and left at 160°C as before. They are periodically swirled to ensure continued total immersion of the membrane. After boiling dry the samples are removed and left to cool. Redissolution is achieved by adding 2 ml 20% HNO_3 to each beaker using a micropipette. They are left to stand for at least five minutes and swirled so that all the metals are taken up into the acid. A micropipette is used to transfer 1 ml of each sample to a labelled universal polystyrene pot for subsequent dilution and analysis by flame AAS (Varian SpectrAA-400) using an air-acetylene flame (elements Mn and Zn). The rest of the sample is analysed immediately by flameless atomic absorption spectrometry using a PU9200 spectrometer with PU9380 autosampler (Unicam Instruments). A matrix modifier, $NH_2H_2PO_4$, is used for the analysis of Cu, Pb, Ni, (and Mn and Zn if analysed by graphite furnace AAS), but no modifier is required for Cd analysis. The furnace conditions are the same as those listed in Table 1.

5.4 Analytical quality control

Detection limits for SPM analyses were calculated according to the definition of Caulcutt and Boddy (1983) (Section 4.4), using blank membranes from five batches analysed over several months. Since no reference material is included with each batch of analysis, an estimate of sample variability at concentrations close to the detection limit has been calculated

Table 3. Analytical quality control parameters (1992) for suspended particulate metal analysis based on 1 mg sediment samples

Metal	Detection limit (µg g^{-1})	Typical blank (ng membrane^{-1})	Observed BCSS-1 Mean (µg g^{-1})	Observed BCSS-1 Std. Deviation (µg g^{-1})	n	Certified BCSS-1 Mean (µg g^{-1})	Certified BCSS-1 Std. Deviation (µg g^{-1})	% RSD (n=10)
Cd	0.04	0.00006	0.27	0.04	10	0.25	0.4	13
Cu	2	0.05	19.4	4.7	9	18.5	2.7	24
Mn	2.2	0	246	22	9	229	15	9
Ni	8	0.00002	56.4	66	8	55.3	3.6	12
Pb	5	0.0002	19.5	2.4	10	22.7	3.4	8
Zn	3	0.0002	151	17	9	119	12	11

RSD = relative standard deviation

from replicate samples of BCSS-1 reference sediment, digested with Nuclepore membranes. The detection limits for 1 mg SPM samples, along with results from 10 replicate samples of BCSS-1, are listed in Table 3.

The nitric acid digestion technique described here does not attack the silicate lattices of the particle structures. It is therefore expected to give lower concentrations than the certified values of the reference materials. The values obtained from the BCSS-1 sediment are, however, very close to the certified values. This may partly be due to the nature of the particulate material (low silicate content) and partly to the small mass of sediment digested. These small masses may explain the fairly high coefficients of variation observed here. Lower coefficients of variation are expected for larger sample masses.

6. COLLECTION, FILTRATION AND STORAGE OF SEA WATER FOR ANALYSIS OF MERCURY

6.1 Introduction

This method divides the mercury content of sea water, estuarine water or fresh water into three fractions; 'reactive dissolved' mercury, 'total dissolved' mercury and 'total' mercury. 'Reactive' mercury is that which can be determined without oxidation. It includes ionic and weakly complexed mercury but not the organic or strongly complexed inorganic species. 'Total dissolved' mercury is that which can be passed through a 0.45 µm membrane. 'Total' mercury is determined after oxidation of all the mercury species present by potassium bromate/bromide solution. This releases about 90% of the suspended particulate mercury.

6.2 Cleaning procedures

6.2.1 Reagents

(a) Potassium bromate/bromide solution
Ten grams of potassium bromide (Analytical grade) and 2.8 g of potassium bromate (Analytical grade) are dissolved in 1000 ml double deionised (> 10 megohm. cm) water. The solution is stored in a 1 l ground glass-stoppered bottle wrapped in polythene.

(b) Hydroxylammonium chloride solution
One hundred grams of hydroxylammonium chloride ($HONH_3Cl$) (Spectrosol [low in mercury] grade, or equivalent) are dissolved in 1000 ml double deionised water. The solution is stored in a 1 l ground glass-stoppered bottle wrapped in polythene.

6.2.2 Cleaning of labware

To eliminate contamination from organic or inorganic mercury, all equipment is soaked for 24 hours in a 5% solution of 'Decon 90', rinsed with double deionised water, and immersed in a bath containing 10 ml l^{-1} brominating solution and 20 ml l^{-1} hydrochloric acid (Analytical grade). After at least 24 hours, any residual bromine in the bath is reduced by adding 4 ml l^{-1} hydroxylammonium chloride solution. The equipment is then rinsed with double deionised water, and soaked in a bath containing 1% nitric acid (Analytical grade) for at least a week. The equipment is rinsed with double deionised water, dried in a 'Class 100' laminar flow cabinet and wrapped in polythene bags until required.

6.2.3 Cleaning of filters

Glass fibre filters (47 mm) Whatman GF/F are heated in a muffle furnace at 450°C for 24 hours, and immersed in brominating solution for at least 24 hours. Again any excess bromine is reduced with 4 ml l^{-1} hydroxylammonium chloride solution, and the filters are rinsed with double deionised water then stored in 1% nitric acid.

6.2.4 Sample bottle preparation

Borosilicate glass bottles with ground glass stoppers are used to collect and store mercury samples, since plastic bottles are permeable to mercury vapour. The 1000 ml glass bottles are initially soaked for two days in 5% v/v solution of 'Decon 90'. They are then rinsed and filled with about 1000 ml deionised water. In a fume cupboard, 20 ml of concentrated hydrochloric acid and 10 ml brominating solution are added to each bottle. The bottles are topped up with deionised water, stoppered, wrapped in polythene bags and left to stand.

After at least 24 hours, the excess bromine is reduced by adding 4 ml of 10% hydroxylammonium chloride solution to each bottle, which is then shaken well. This cleaning solution is poured away in a fume cupboard, and the bottles are rinsed with deionised water. The bottles are then filled with 1% (v/v) nitric acid (Analytical grade), wrapped in polythene bags, and stored in plastic boxes until required.

Each bottle is emptied in the shipboard clean laboratory, and rinsed twice with the sea water before the sample is taken. The bottles are filled, stoppered and stored in polythene bags. Samples for total dissolved or reactive dissolved mercury analysis are filtered as soon as possible after collection and certainly within 24 hours.

6.3 Filtration procedure

Filtration of samples for subsequent determination of dissolved mercury is carried out in a 'Class 100' laminar flow cabinet. The filtration equipment and

membranes are pre-cleaned as described in Sections 6.2.2 and 6.2.3. A filter is placed in a PTFE holder connected to a 1000 ml PTFE pressure reservoir (Cowie Scientific) via FEP tubing (Figure 6). A pressure of 1-2 psi of oxygen free nitrogen is applied via a gold trap. The sample is thoroughly shaken, and about 100 ml of sample is used to rinse the pressure reservoir, filter and collection bottle. This sub-sample is discarded. The remaining sample is filtered, and the filtrate is acidified with 6 ml l^{-1} nitric acid (ultra pure grade). The bottle containing the sample is stoppered and wrapped in polythene until required.

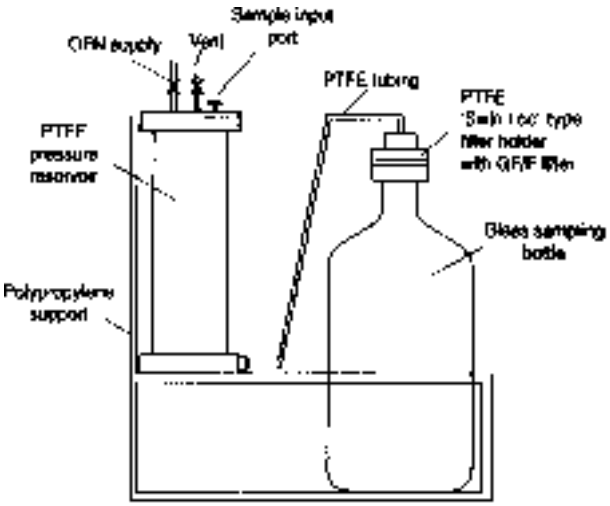

Figure 6. Filtration system for mercury in sea water

7. ANALYSIS OF MERCURY SPECIES IN SEA AND ESTUARINE WATER

7.1 Introduction

For reactive dissolved mercury the mercury in the filtered sample is reduced to elemental mercury by the addition of tin (II) chloride solution. The mercury is purged from solution with zero grade argon and trapped as an amalgam on gold chips. The trap is heated to drive the mercury off as a pulse of vapour. The mercury is then analysed by atomic fluorescence spectrometry.

For total (or, if filtered, total dissolved) mercury, all mercury species are converted to mercury (II) by adding hydrochloric acid, potassium bromide and potassium bromate solutions. After 90 minutes, the bromine is reduced with excess hydroxylamine chloride solution. The sample is then analysed as for reactive mercury.

7.2 Reagents

7.2.1 Water

All water used for reagent preparation must be as pure as possible. Double deionised water (>10 megohm. cm) is used at the Burnham-on-Crouch Laboratory, but may be replaced by water of equivalent purity.

7.2.2 4% Nitric acid

Dilute nitric acid is made up by adding 4 ml of ultra pure grade HNO_3 to 96 ml water, and is stored in a 100 ml ground glass stoppered conical flask. This reagent is used to rinse each pipette tip before use, thus avoiding sporadic contamination by dust etc.

7.2.3 Tin (II) chloride solution

A 50% hydrochloric acid solution is made up by adding 50 ml hydrochloric acid (ultra pure grade), to 50 ml double deionised water in a ground glass stoppered 100 ml conical flask. Twenty grams of tin (II) chloride (Spectrosol grade, or equivalent) is dissolved in the 50% acid solution. About 5 g of granulated tin (Analytical grade) is added to the flask to slow down the oxidation of the tin (II) chloride. The reagent is purged for 15 minutes with zero grade argon at 600 ml min^{-1} to remove any mercury present. This reagent is stored in a 100 ml ground glass stoppered conical flask, and is stable for at least five days.

7.2.4 Potassium bromide solution

An excess of potassium bromide (Analytical grade) is heated in a muffle furnace in a covered silica crucible, at 350°C for 24 hours. One gram of this is weighed out and dissolved in 100 ml double deionised water. The potassium bromide solution is stored in a 100 ml ground glass stoppered conical flask, and is prepared weekly.

7.2.5 Potassium bromate solution

An excess of potassium bromate (Analytical grade) is heated in a muffle furnace in a covered silica crucible, at 350°C for 24 hours. 0.28 g of this is weighed out and dissolved in 100 ml double deionised water. The potassium bromate solution is stored in a 100 ml ground glass stoppered conical flask, and is prepared weekly.

7.2.6 Hydroxylammonium chloride solution

Twenty grams of hydroxylammonium chloride (Spectrosol, low in mercury) are dissolved in 100 ml of

double deionised water. 500 µl of the tin (II) chloride reagent are added, and the solution is purged for 15 minutes with argon at 600 ml min^{-1}. The hydroxyl-ammonium chloride solution is stored in a 100 ml ground glass stoppered conical flask. It is only stable for a few days and should be made up regularly.

7.2.7 Anhydrous calcium chloride

Anhydrous calcium chloride (12-24 mesh, Laboratory Grade) is heated in a muffle furnace at 450°C for 24 hours. It is stored in a 100 ml glass screw-capped bottle.

7.2.8 Silica wool

Silica glass wool is heated in a muffle furnace at 450°C for 24 hours. It is stored in double wrapped resealable polythene bags.

7.2.9 Gold

Gold chippings (prepared from 0.25 mm diameter gold wire, Aldrich) are regularly washed with deionised water, dried with tissue, and then heated in a muffle furnace overnight at 450°C. The gold is always handled with forceps and may be stored in a universal pot until required.

7.2.10 Mercury standards

A stock standard of 10 mg l^{-1} inorganic mercury is made up by diluting 1 ml of 1000 mg l^{-1} mercury nitrate standard (Spectrosol) to 100 ml with 4% v/v nitric acid. A working standard of 10 ng l^{-1} is made up by diluting 100 µl of the stock standard to 100 ml with 4% v/v nitric acid. The stock standard is stored in a 100 ml ground glass stoppered volumetric flask. The working standard is transferred to a 100 ml ground glass stoppered volumetric flask, and is renewed weekly.

7.3 Apparatus

The apparatus is shown diagrammatically in Figure 7. Zero grade argon passes through one of two flow meters set at 600 and 400 ml min^{-1} and then through gold traps set into each line. The traps consist of silica glass tubes (6 mm o.d. and 50 mm long) packed with gold chips held in place by silica wool. The traps remove any mercury contamination from the gas. All tubing used after these traps is Teflon (6 mm o.d., Cowie Scientific).

Argon from the 600 ml min^{-1} flow meter is led via a tap to a 125 or 250 ml two necked round bottomed sample flask. The teflon tubing entering the flask is held in place by a ground glass screw adapter. It extends to the bottom of the flask, enabling the gas to be bubbled through the sample. Gas leaves the sample flask

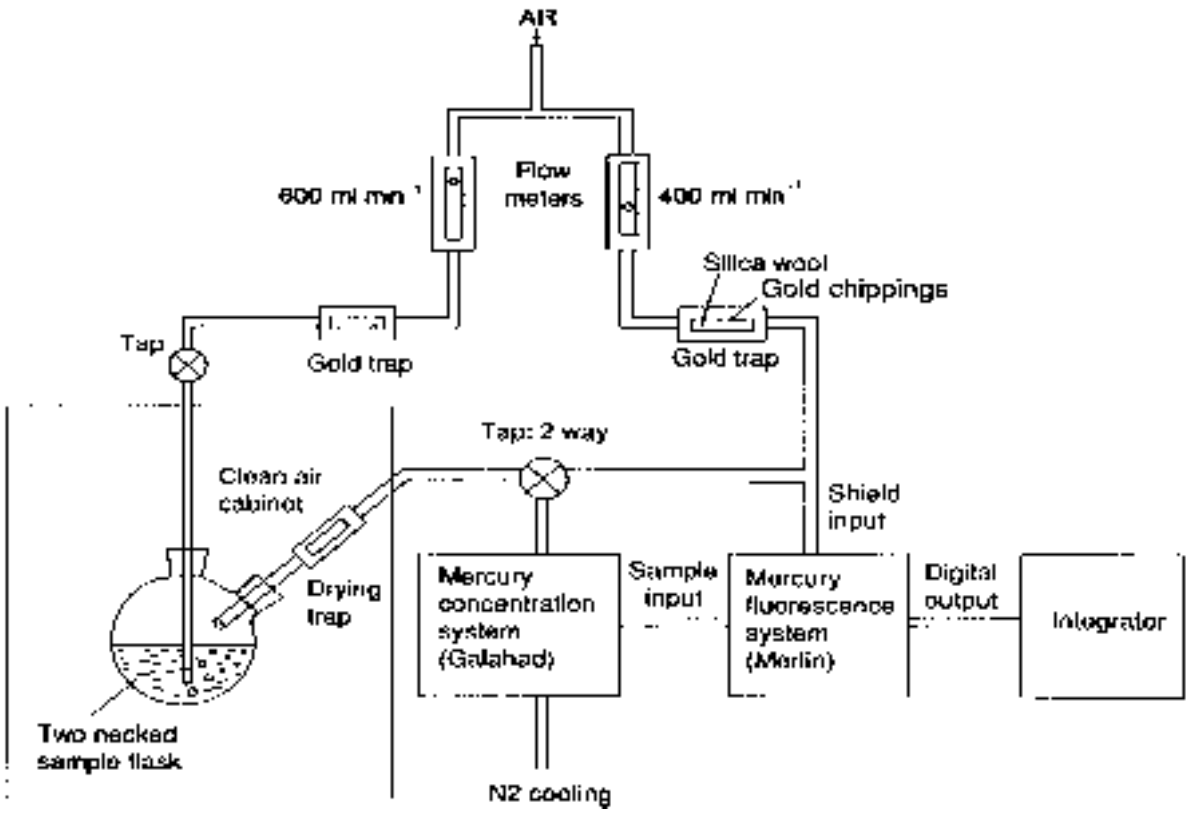

Figure 7. Apparatus used in the determination of mercury in sea water

through tubing attached to the second neck with a screw adapter. The gas is then carried to a drying trap consisting of a PTFE tube (8 mm o.d., 50 mm long, Cowie Scientific) containing 12-14 mesh anhydrous calcium chloride, held in place with silica wool. The dried gas is taken (via a two way tap) to a platinum/gold trap (Galahad, model 10.501, P.S. Analytical). Nitrogen gas, at 50 psi, is used to cool the gold trap. The trap is connected via teflon tubing to the mercury analyser.

Argon from the 400 ml min^{-1} flow meter is divided to act both as a shield for the fluorescence detector and to carry the pulse of mercury vapour released from the gold trap to the detector. A Merlin Fluorescence Detector (model 10.023, P.S. Analytical) is used to analyse the mercury in the vapour and the signal is interpreted by a Hewlett-Packard integrator (3392A). These systems are used in accordance with the manufacturer's instructions.

7.4 Methods

7.4.1 Determination of 'reactive' mercury

The filtered acidified samples are left to stand for 24 hours, before being analysed for 'reactive' mercury. The analytical procedure, which also forms the basis of the 'total dissolved' or 'total' mercury, is carried out as follows.

Before each analysis, the drying trap is renewed by adding new clean calcium chloride. Both the amounts of calcium chloride and silica wool used in the drying trap are kept to a minimum to prevent adsorption of mercury vapour. Similarly the volume of the round bottomed sample flask is chosen according to the volume of sample required in order to minimise the amount of glass surface in contact with the vapour. A 250 ml flask is generally used for off-shore samples, where mercury concentrations are expected to be low, while a 125 ml flask is used for more contaminated near-shore waters.

The sample bottle, and a clean glass measuring cylinder are rinsed with a small aliquot of the sample. Using the measuring cylinder, an appropriate volume of sample is added to the sample bottle, and the volume is recorded. The flask is fitted to the apparatus and the ground glass joint made firm. A clear polypropylene pipette tip is fitted to a 400 µl micropipette (coloured tips give sporadic mercury contamination). The tip is rinsed with dilute acid, and then used to pipette 400 µl of tin (II) chloride solution into the reaction vessel. The ground glass adapter of the reaction vessel is replaced immediately to prevent escape of mercury vapour. The appropriate taps are turned on, and the sample is purged with zero grade argon at 600 ml min^{-1} for ten minutes. During this time, the vapour is carried to the Galahad unit and amalgamated on the gold trap.

When the purging is complete the 600 ml min^{-1} argon flow is switched off and the amalgam unit and integrator are turned on. During this stage the mercury is released as a pulse from the heated gold trap, and carried by argon at about 200 ml min^{-1} to the fluorescence detector. The peak is recorded by the integrator.

7.4.2 Determination of 'total' mercury

Filtered and unfiltered samples are used to determine total dissolved and total mercury respectively. The analysis can be performed immediately. A 125 ml sample bottle is usually adequate for 'total' mercury analysis, though 'total dissolved' determinations may require a 250 ml sample bottle.

The sample bottle, and a clean glass measuring cylinder are rinsed with a small aliquot of the sample. Using the measuring cylinder, an appropriate volume of sample is added to the sample bottle, and the volume is recorded. Using clear rinsed pipette tips 7 ml hydrochloric acid, 2 ml potassium bromate solution and 2 ml potassium bromide solution are added to the sample flask. Both necks of the flask are firmly stoppered to prevent the leakage of toxic bromine gas into the laboratory. The flask is wrapped in cling film and labelled with the sample number, time of bromination and volume of sample. It is left to stand for 90 minutes. During this time, the free bromine developed in the reaction vessel converts all forms of mercury to mercury (II). After the allotted time, the excess bromine is reduced with 1.2 ml of hydroxylammonium chloride solution. The sample is then analysed as for reactive mercury described above (Section 7.4.1).

7.4.3 Calibration

Calibration is by the method of standard additions with a blank sea water sample. The sea water is stripped of all mercury by repeated analysis as described above (Section 7.4.1). This is then analysed as a blank. The sample is then spiked with known amounts of inorganic mercury standard. They are analysed, and a calibration graph of peak height against amount of mercury (ng) is drawn.

7.4.4 Blanks

Reagent blank values for total mercury can be estimated by adding 7 ml hydrochloric acid, 2 ml potassium bromate, 2 ml potassium bromide and 1.2 ml hydroxylammonium chloride solutions to a sample stripped of mercury. The sample is then analysed in the normal way.

DIGESTION AND DETERMINATION OF TRACE METALS IN SOLID AND SEMI-SOLID SUBSTRATES AND INDUSTRIAL WASTES

8. INTRODUCTION

The aim of all of the many methods available for the digestion of solid samples is to convert all the metal present into a form available for quantification by the final method of analysis.

9. DIGESTION OF FISH AND SHELLFISH TISSUES FOR THE DETERMINATION OF TRACE METALS

The digestion method described below has been developed for the determination of trace metals in marine fauna using flame or furnace atomic absorption spectrometry (AAS/GFAAS), atomic fluorescence spectrometry (AFS) and inductively-coupled plasma mass spectrometry (ICP-MS).

9.1 Reagents

9.1.1 Nitric acid

Ultra pure grade nitric acid (sg 1.42) is used.

9.1.2 Double-distilled water

A Fisons bidistillation still is used to produce double-distilled water (DDW) which is stored in a 10 l high-density polyethylene aspirator.

9.2 Apparatus

9.2.1 Microwave oven

A CEM MDS 2000 microwave oven rated at 650W with pressure and optional temperature control is used (CEM Microwave Technology Ltd., Buckingham).

9.2.2 Digestion vessels

A set of twelve CEM lined digestion vessels with single use safety relief discs with pressure release valves, are used. One vessel contains a modified cap to allow connection for pressure and temperature monitoring.

9.3 Method

9.3.1 Cleaning procedure

Outer digestion vessels do not come into contact with the sample and therefore do not require specialist cleaning. The inner liners are soaked in Pyroneg detergent for 4 h then rinsed with DDW to remove all the detergent. They are then soaked in a 10% nitric acid bath for 4 h before being copiously rinsed with DDW, the liners are then dried at 50°C. The vessels are stored wrapped in domestic grade cling film until used.

9.3.2 Digestion procedure

The outer and inner vessels are labelled and the inner vessel is weighed to a precision of 0.01 g and weights recorded accurately (W1). The sample is well mixed with an acid rinsed plastic spatula and approximately 2 g of mixed shellfish tissue or fish liver or 4 g of fish muscle is accurately weighed into the liner (W2). The sample is dried at 102°C for 12 h, allowed to cool and is then reweighed and the weight recorded (W3). The total solids content is calculated as follows:

$$\text{total solids (\%)} = \{ (W3-W1)/W2 \} \times 100$$

For microwave digestion the dry weight (W3-W1) used in the vessels must not exceed 1 g. DDW (4 ml) is added to the vessel, followed by 4 ml of ultra pure nitric acid (sg 1.42). The vessels are then sealed according to the manufacturer's instructions ensuring that a safety release disc is present. The digestion vessels containing the samples are placed in the microwave, one vessel is connected via a modified cap to a pressure line and temperature probe. The samples are digested using the conditions in Table 4.

Table 4. Microwave settings used for digestion of fish and shellfish tissue

Stage	1	2	3	4
Power	25%	50%	75%	100%
Pressure(psi)	40	60	80	120
Time(min)	5.00	5.00	5.00	10.00
Temperature	80°C	100°C	130°C	170°C

After the digestion is complete the vessels are allowed to cool and the pressure to drop to <30 psi. The vessels are then carefully vented in the microwave. **The operator should wear a full face visor and protective gloves.** The digestion vessels are then removed to a fume cupboard and opened. The contents are then quantitatively transferred to labelled graduated polystyrene containers and made up to 25 ml with DDW.

Note: The microwave conditions are quoted for a set of 12 samples with weights and acid volumes as described above. If the number of samples, weight or volume are changed, different microwave settings may be required.

9.3.3 Blanks and quality control

One reagent blank and one commercial certified reference material is included in every batch (12 vessels).

10. PREPARATION OF SEDIMENTS USING *AQUA REGIA* FOR THE DETERMINATION OF TRACE METALS

The digestion method below describes a partial extraction of a sediment. It does not break down the more refractory matrices, such as silicates, nor does it dissolve some refractory minerals such as chromite (see Section 15, Total digestion of sediments). The *aqua regia* method has been used at the Burnham-on-Crouch Laboratory for more than 10 years, and thus provides comparability between recent and historic data.

10.1 Reagents

10.1.1 Nitric acid

Analytical grade nitric acid has been found to be of sufficient purity for flame or cold-vapour atomisation atomic absorption spectrophotometry (CVAAS). If electrothermal atomisation is to be used, ultra-pure acid is recommended. A 0.1 M nitric acid solution is prepared by dilution of 60 ml of nitric acid (sg 1.42) to 10 l with DDW and is stored in a high-density polyethylene aspirator.

10.1.2 Hydrochloric acid

Purity as for Nitric Acid (Section 10.1.1).

10.1.3 Double-distilled water

A Fisons bidistillation still is used to produce DDW which is stored in a 10 l high-density polyethylene aspirator.

10.2 Apparatus

10.2.1 Beakers

'Pyrex' glass or PTFE beakers (50 ml) are used.

10.2.2 Watch glasses

'Pyrex' or PTFE watch glasses are used.

10.2.3 Hot plate

A hot plate, (30.5 x 61 cm) incorporating a thermostat capable of achieving 320°C, is used. Up to 50 samples can be digested simultaneously on one hot plate.

10.3 Method

10.3.1 Cleaning procedure

Beakers and watch glasses are soaked in a 5% v/v Pyroneg detergent solution for 24 h. The detergent is subsequently rinsed off and the glassware is placed in a bath of 20% v/v nitric acid in DDW. After soaking for at least 24 h, the acid is removed by copious rinsing with DDW and the glassware is dried at 50°C. It is stored wrapped in cling film until used.

10.3.2 Digestion procedure

The beakers are labelled and weighed to 0.01 g and the weights are recorded (W1). They are given a final cleaning by adding 20 ml of nitric acid (sg 1.42), covering with a watch glass, and heating to 50°C for 2 h. After cooling, the acid is poured out (and retained for re-use in cleaning) and the glassware is rinsed well with DDW. Watch glasses are kept under DDW in a large, covered glass beaker, and the beakers are kept face down on a plastic covered tray.

The sample is well mixed with an acid-soaked plastic spatula and approximately 2.5 g is accurately weighed into a cleaned beaker (W2). The sample is dried at 80°C for 24 h, allowed to cool and is then weighed, the weight being noted (W3). The 'total solids' content can now be calculated:

$$\text{total solids (\%)} = \{ (W3 - W1)/W2 \} \times 100.$$

Nitric acid (sg 1.42) (18 ml) is added to the sample very slowly to avoid spillage from excessive frothing which may occur with carbonate or organic-rich sediments. The effervescence can be controlled, if necessary, by the addition of a few drops of DDW. Once the initial reaction has subsided, 2 ml of hydrochloric acid (sg 1.18) are added and the sample is covered and allowed to react for 24 h. The sample is heated to 50°C for a further 24 h, after which the watch glass is removed and the volume is reduced to about 5 ml. DDW is added to

make the volume up to about 20 ml which is reduced back to about 5 ml again. This reduces the acid content of the final solution after the sample has cooled, the volume is made up to 40 ml with 0.1 M nitric acid. This is performed on a balance using the equation:

top-up weight (g) = W3 + 42.5.

10.3.3 Blanks and quality control

One reagent blank and two certified reference materials are included for every 9 samples analysed.

11. PREPARATION OF EFFLUENT AND DREDGED MATERIAL SAMPLES FOR THE DETERMINATION OF TRACE METALS

These samples are collected for licensing, inspection and enforcement purposes under the Food and Environment Protection Act, 1985 (Great Britain-Parliament 1985).

A nitric acid/hydrogen peroxide method is used instead of the nitric acid or *aqua regia* procedures described above. This method was adopted in 1983 because it was considered to be easier to use with no loss in performance and to give a better approximation to a total digest.

11.1 Reagents

11.1.1 Nitric acid

Analytical grade nitric acid has been found to be of sufficient purity. A 0.1 M nitric acid solution is prepared by dilution of 60 ml of nitric acid (sg 1.42) to 10 l with DDW and is stored in a high-density polyethylene aspirator.

11.1.2 Double-distilled water

A Fisons bidistillation still is used to produce DDW which is stored in a 10 l high-density polyethylene aspirator. DDW is used throughout all of the digestion methods used at the Burnham-on-Crouch Laboratory.

11.1.3 Hydrogen peroxide

Analytical grade hydrogen peroxide (100 volume) is used.

11.2 Apparatus

11.2.1 Beakers

Short-form 'Pyrex' glass beakers (50 ml) are used.

11.2.2 Watch glasses

'Pyrex' watch glasses are used as appropriate.

11.2.3 Hot plate

A hot plate, (30.5 x 61 cm) incorporating a thermostat capable of achieving 320°C, is used. Up to 50 samples can be digested simultaneously on one hot plate.

11.3 Method

11.3.1 Cleaning procedure

Beakers and watch glasses are soaked in a 5% v/v Pyroneg detergent solution for 24 h. The detergent is subsequently rinsed off and the glassware is placed in a bath of 20% v/v nitric acid in DDW. After soaking for at least 24 h, the acid is removed by copious rinsing with DDW and the glassware is dried at 50°C. It is stored, wrapped in cling film, until used. A final cleaning step is included just before digestion is carried out, as described in Section 11.3.2.

11.3.2 Digestion procedure

The sample is thoroughly mixed by stirring with an acid-washed plastic spatula or shaking depending on the form of the material.

To determine the 'total solids' content of the homogenised sample approximately 10 g (W1) is accurately weighed into a pre-weighed foil tray or, if corrosive, into a glass beaker (W2). The sample is dried at 105°C for 24 h, cooled and reweighed (W3). The 'total solids' content is calculated according to the following equation:

total solids (%) = { (W3 - W2)/W1 } x 100.

Approximately 4 g (W4) of the mixed, wet sample is accurately weighed into a pre-weighed beaker (W5). Nitric acid (sg 1.42) (10 ml) is added, drop by drop, to avoid excessive frothing. The reaction can be controlled, if necessary by adding a few drops of water or cooling in an ice bath. When the reaction has subsided, 10 ml of hydrogen peroxide (100 volume) is slowly added. The sample is covered with a watch glass and allowed to react at room temperature for 24 h. Periodic inspection is necessary during this stage, as violent

frothing can occur. This may be controlled as described above. The sample is then heated to 50°C for 24 h, after which the watch glass is removed and the volume is reduced to about 5 ml by further heating The volume is then made up to about 20 ml with DDW and once more reduced to about 5 ml by further heating. The sample volume is now made up to 40 ml by weight with 0.1 M nitric acid using the equation:

top-up weight (g) = (W4 x total solids/100) + W5 + 42.5

11.3.3 Blanks and quality control

One reagent blank and two certified reference materials are included for every 9 samples analysed.

12. ANALYSIS OF *AQUA REGIA*, NITRIC ACID AND ACID/PEROXIDE DIGESTS FOR MERCURY

Mercury is analysed using a Merlin fluorescence system (P S Analytical, Orpington, Kent). Inorganic mercury in the digest is reduced to mercury vapour by the addition of tin (II) chloride solution. The mercury vapour is then swept through a drying tube to remove water and then into the mercury-specific fluorescence detector.

12.1 Reagents

12.1.1 Double-distilled water

See Section 11.1.2.

12.1.2 Tin (II) chloride stock solution

Analytical grade tin (II) chloride (200 g) is dissolved in 500 ml of analytical grade hydrochloric acid, and diluted with 500 ml of DDW. It is stored in a glass stoppered bottle and will remain stable for at least two weeks under normal laboratory conditions.

12.1.3 Tin (II) chloride working solution

A tin (II) chloride working solution (4% w/v) is prepared by diluting the stock solution to five times its volume with DDW.

12.1.4 Wash solution

The wash solution (0.1 M nitric acid in DDW) is prepared by adding 60 ml of ultra pure nitric acid (sg 1.42) to 1 l of DDW and making the volume up to 10 l with DDW. It is stored in a high-density polyethylene aspirator.

12.1.5 Hydroxylammonium chloride

8 g of hydroxylammonium chloride low in mercury is added to 1 l of DDW. The resulting solution is stored in a plastic bottle.

12.2 Apparatus

The following equipment is used:-

Merlin fluorescence detector, model No 10.023
Hydride generator, model No 10.003
PSA random access multiple tray autosampler
Perma drying tube
Gilson autosampler, model No 222.

12.3 Method

The equipment is set up according to the manufacturer's instructions. For fish/shellfish digests samples are diluted five times with 0.8% hydroxylammonium chloride using a Gilson autosampler. The samples are then heated at 40°C for thirty minutes in an ultrasonic bath to remove excess nitric acid. For other digests, samples are diluted five times with 0.1 M nitric acid and then placed on the PSA autosampler tray together with a set of calibration samples made from a 10 $\mu g\ ml^{-1}$ mercuric nitrate standard. The sample and wash solutions are pumped to the gas/liquid separator cell through 0.8 mm i.d. silicone tubing and are switched using a two way valve, the tin (II) chloride is pumped continuously to the gas/liquid cell through 0.4 mm i.d. silicone tubing. The autosampler and valve are controlled by computer which is also used for data capture and data processing. The sample is agitated in the gas/liquid separator by bubbling argon into the cell, mercury in solution is reduced to mercury vapour by the action of the tin (II) chloride and is swept by the argon through the drying tube and on into the mercury fluorescence detector. The detector is calibrated using external standards. The detection limit for the instrument is 0.02 $\mu g\ l^{-1}$ with a linear range up to 2000 $\mu g\ l^{-1}$.

12.4 Performance characteristics

Table 5 shows results achieved using this method for measurement of total mercury in three certified reference materials of marine origin. The method gives concentrations close to the certified results, with the exception of DORM-1 which is slightly lower and similar to results achieved by our old cold vapour AAS method. Table 6 compares results achieved for this method with the previous method of cold vapour atomic absorption spectroscopy, the concentrations achieved are very similar between methods.

Table 5. A comparison of data achieved by this method with certified values (All results in ppm)

Reference material	DOLT-1	DORM-1	MAA2
	0.21	0.64	0.43
	0.21	0.64	0.42
	0.17	0.67	0.45
	0.19	0.65	0.41
	0.20	0.69	0.47
	0.23	0.63	0.40
	0.22	0.70	0.49
	0.22	0.69	0.42
	0.21	0.74	0.43
Number	9	9	9
Mean	0.207	0.672	0.436
Standard deviation	0.018	0.036	0.029
Certified result	0.225	0.798	0.47
+/-	0.037	0.074	0.02

Table 6. A comparison of data for the certified reference materials. CVAAS method and the new CVAFS method (All results in ppm)

Reference material	DOLT-1	DORM-1	MAA2
CVAFS (new method)	0.24 ± 0.04	0.69 ± 0.09	0.44 ± 0.04
n	70	92	12
CVAAS (old method)	0.20 ± 0.02	0.70 ± 0.04	0.47 ± 0.03
n	43	36	28

13. ANALYSIS OF *AQUA REGIA*, NITRIC ACID AND ACID/PEROXIDE DIGESTS FOR ALUMINIUM, CADMIUM, CHROMIUM, COPPER, IRON, LEAD, MANGANESE, NICKEL AND ZINC

13.1 Reagents

13.1.1 Standards

Standards should be made up to reflect the expected concentration in the sample. Composite standards, of all of the metals to be measured within a particular concentration range, are especially useful.

13.2 Apparatus

Both a Varian 400 atomic absorption spectrometer, (equipped with deuterium continuum background correction) and a VG Plasmaquad 2+ inductively coupled plasma-mass spectrometer (ICP-MS) are used.

13.3 Method

13.3.1 Atomic absorption analysis

For atomic absorption samples are aspirated into an air/acetylene flame for metals other than aluminium and chromium, which require a nitrous oxide/acetylene flame.

The determination of digests by flame atomic absorption is a well-documented field. The response to different metals in different matrices varies from instrument to instrument. The instrumental parameters, therefore, should be as set out in the manufacturer's guide. Care must always be taken, especially with calibration curves.

13.3.2 ICP-MS analysis

For analysis by ICP-MS the samples are diluted in 2% nitric acid to reduce total dissolved solids to <0.4%. One or more elements can be added as internal standards e.g. In, Y. Calibration is by external composite standards. The sample is introduced to the plasma from a Gilson autosampler into a V-groove (de Galaan) nebuliser. The instrumental parameters are as set out in the manufacturer's guide.

14. THE DETERMINATION AND ANALYSIS OF TOTAL ARSENIC IN FISH TISSUES

14.1 Introduction

Organic arsenic compounds found in fish tissues are very stable. They must, therefore, be oxidised to inorganic arsenic before a meaningful analysis for total arsenic can be performed using hydride generation/atomic absorption spectrophotometry. To achieve the required oxidation, the arsenic must be brought into solution and then treated with a strong oxidising agent, in the presence of a catalyst.

14.2 Reagents

14.2.1 Nitric acid

Analytical grade nitric acid (sg 1.42) is used throughout.

14.2.2 Sulphuric acid

Ultra-clean sulphuric acid (sg 1.84) is used during the digestion stages, and a 10% v/v solution is used during the hydride generation stages and for making standards.

14.2.3 Potassium permanganate solution

A 5% w/v solution of potassium permanganate (analytical grade) is prepared in DDW.

14.2.4 Ammonium metavanadate solution

A 2% w/v solution of ammonium metavanadate is prepared in DDW. Heating may be necessary to complete dissolution.

14.2.5 Hydrogen peroxide solution

A 20% v/v solution of 100 volume hydrogen peroxide (analytical grade) is prepared with DDW.

14.2.6 Sodium tetraborohydrate solution

The solution used for the reduction of inorganic arsenic to the hydride is made up of 2% w/v sodium tetraborohydrate in 0.4% w/v sodium hydroxide solution.

14.2.7 Standards

All standards are made up in a 10% v/v solution of sulphuric acid in DDW. A 10 mg l^{-1} stock solution is made from a 1000 mg l^{-1} 'Spectroscopic' grade standard. This is then used to make up 0.05, 0.10, 0.20, 0.30, 0.40 and 0.50 mg l^{-1} working solutions.

14.3 Apparatus

14.3.1 Digestion block

An automatic digestion system (Tecator, 1012), capable of achieving 450°C, is used with 250 ml borosilicate glass tubes.

14.3.2 Atomic absorption spectrophotometer

A Varian 400 atomic absorption spectrophotometer is used according to the manufacturer's recommendations.

14.3.3 Hydride generation

A Varian continuous-flow hydride generation unit is used.

14.4 Method

The sample (approximately 1 g dry weight or 5 g wet weight) is accurately weighed into the digestion tube and 1 ml of ammonium metavanadate solution is added. The sample is digested with 5 ml of nitric acid at 125°C, until any frothing stops (approximately 20 min). (3 ml) sulphuric acid is added and the mixture is heated to 140°C until charring or discoloration occurs. If there is no reaction, 1 ml of nitric acid is added and the temperature is increased to 160°C. If there is still no reaction, another 1 ml of nitric acid (sg 1.42) is added and the temperature increased to 180°C. A further addition of 0.5 ml of nitric acid (sg 1.42) is made and the temperature increased to 200°C for 20 min followed by another 0.5 ml of nitric acid (sg 1.42), when the temperature is raised to 220°C for 20 min.

The excess nitric acid is now boiled-off at 240°C with the addition of 5 ml of DDW. Hydrogen peroxide solution (4 ml) is added and the digest is held at 240°C for 20 min. Excess peroxide is fumed-off by adding 5 ml of a 0.4% v/v solution of nitric acid in DDW and heating to 240°C.

The digestion is completed by the addition of potassium permanganate solution (3 ml) and boiling to dryness at 300°C. The residue is allowed to cool to room temperature and is then dissolved in 2.5 ml of sulphuric acid (sg 1.84) at 180°C. After cooling, the solution is made up to 25 ml with DDW.

The arsenic concentration is then determined by hydride generation/atomic absorption spectrophotometry using the equipment as recommended by the manufacturer.

Note: It is possible to measure arsenic in the microwave nitric digestion described earlier if ICP-MS is used. However corrections must be made for argonchloride interferences.

15. 'TOTAL' DIGESTION AND DETERMINATION OF TRACE METALS IN MARINE SEDIMENTS

15.1 Introduction

The Fisheries Laboratory at Burnham-on-Crouch provides data for various national and international bodies such as the Oslo and Paris Commissions (OSPARCOM) and the Advisory Committee on the Marine Environment (ACME) of the International Council for the Exploration of the Sea (ICES), on the contribution of anthropogenic inputs to the concentrations of metals currently found in the marine environment. Until recently the method used to analyse the sediment was based on *aqua regia* acid extraction (Harper, *et al.,* 1989). This extraction process does not determine the total metal content of the sediment as the silica lattice which may contain metals is not broken down. The Marine Sediment Working Group (MSWG) of ICES have therefore decided to promote the use of a digestion technique involving hydrofluoric acid (HF), which allows the total metal content of the sediment to be determined. This recommendation has been accepted by the Marine Pollution Monitoring Management Group (MPMMG) as the technique to be adopted for the National Monitoring Programme (NMP).

An important advantage of total digestion is the use of 'normalisation' procedures which allows discrimination between the background concentrations and the anthropogenic content, (other than by grain size). This is achieved by measuring the concentration of a metal such as aluminium or lithium, which is partly bound in the silica lattice. Another advantage of total digestion is the ability to use reference materials which are certified with a total metal content to verify analytical results.

A disadvantage of using hydrofluoric acid is its corrosive nature and its toxicity. It is therefore necessary to either remove the acid or render it less harmful for instrumental analysis. A further disadvantage of HF digestion is the increase in mass of material in solution which leads to problems with spectral and other interferences during analysis.

The following method has been adapted from Loring and Rantala (1990). A microwave oven is used with polytetrafluorethylene (PTFE) lined and sealed digestion vessels. This reduces the digestion time. Following digestion, boric acid is used to complex the hydrofluoric acid and decrease the toxic hazard. This also prevents aluminium fluoride precipitation. The digest is contained in a sealed vessel, preventing losses of volatile components, therefore mercury can be determined on the same digest.

15.2 Preparation of marine sediments

The marine sediments are coarse sieved through a 2 mm mesh and then freeze dried to constant weight and ground to a fine powder using a rotating agate mill.

15.3 Reagents

15.3.1 Ultra pure water

Ultra pure water as supplied by a Millipore ion exchange unit, of a resistivity of >18 megohm. cm.

15.3.2 Hydrofluoric acid

Ultra pure grade hydrofluoric acid (40%) is used. *SEE SAFETY NOTE ON HANDLING (Appendix)*

15.3.3 Nitric acid

Ultra pure grade nitric acid (sg 1.42) is used for the following solutions and for the preparation of the *aqua regia* reagent. A 0.1 M solution of ultra pure grade nitric acid is prepared by adding 6 ml of nitric acid to 500 ml of ultra pure water and making up the volume to 1 l with ultra pure water. A 5% solution is prepared by diluting 50 ml of nitric acid with ultra pure water to a total volume of 1 l. Both solutions are stored in high-density polythene bottles.

15.3.4 Aqua regia reagent

Aqua regia is prepared 1 h before use by combining ultra pure grade hydrochloric (sg 1.18) and nitric acids (sg 1.42) in the ratio 3:1 in a polytetrafluorethylene (PTFE) beaker.

15.3.5 Boric acid

Analytical grade boric acid is used to neutralise excess HF.

15.4 Apparatus

15.4.1 Microwave oven

A CEM CM81D microwave oven of 650 watt power rating and with additional pressure control is used.

15.4.2 Digestion vessels

Sealed digestion vessels with PTFE liners and fitted with safety pressure release valves 200 psi are used.

15.5 Method

15.5.1 Cleaning procedure

The PTFE liners of the digestion vessels are soaked in a bath of detergent (e.g. 5% Decon 90) for 8 h. They are then rinsed with deionised water and placed in a 5% v/v nitric acid bath for 12 h. After a further thorough rinsing with ultra pure water, the liners are air dried and wrapped in cling film until required to prevent contamination with airborne dust.

15.5.2 Digestion procedure

Samples are prepared in batches of 12, consisting of one blank, two certified reference materials and nine sediment samples.

Digestion vessels, outer and inner liners should be checked each time for signs of cracks or distortions, they should not be used if any are found.

The cleaned PTFE liners are tared using a 4 figure balance. 400-600 mg of finely ground material is accurately weighed into each PTFE liner.

The sample liners are then placed into their holders and transferred to a fume cupboard. 2 ml of *aqua regia* is then pipetted into each vessel and the reaction is allowed to proceed for at least five minutes. 6 ml of 40% hydrofluoric acid is then pipetted into each vessel (see safety note on HF). The digestion vessels are then sealed and placed in the microwave digestion unit. One of the 12 vessels used has a modified cap allowing a pressure line to be connected between it and a pres-

sure controller to monitor the pressure and control the microwave heating.

The microwave conditions are:-

Pressure set to a maximum 80 psi
100% power for 25 min
75% power for 60 min.

The sealed vessels are allowed to cool until the pressure drops to zero psi on the readout display. The pressure line is removed and the vessels transferred to a fume cupboard where they are opened and 5.6 g boric acid added to each one to neutralise excess HF. They are then resealed and returned to the microwave oven and the pressure line reconnected ready for further heating as follows:-

Pressure set to a maximum 20 psi
100% power for 10 min
50% power for 30 min.

The vessels are again allowed to cool and transferred as before to a fume cupboard. They are opened and the contents quantitatively transferred to 50 ml calibrated polypropylene containers and made up to 50 ml using ultra pure water.

Note: The microwave conditions are quoted for a set of 12 samples with weights and acid volumes as described above. If the number of samples, weight or volume are changed, different microwave settings may be required.

16. ANALYSIS OF HF DIGESTS FOR COPPER, ZINC, CHROMIUM, IRON, ALUMINIUM, CADMIUM, LEAD AND MERCURY

16.1 Reagents

16.1.1 Standards

Composite Cu, Zn, Fe and Al standards are prepared in the same reagent matrix as the digests.

Cadmium and lead Standards are made up in 5% nitric acid.

Mercury standards are prepared in the same reagent matrix in the digests.

16.1.2 Tin (II) chloride stock solution

Analytical grade tin (II) chloride (200 g) is dissolved in 500 ml of analytical grade hydrochloric acid and diluted with 500 ml of DDW to give a 20% w/v solution. This is stored in a glass stoppered bottle and will remain stable for at least two weeks.

16.1.3 Tin (II) chloride working solution

A working solution of 2% w/v tin (II) chloride is made by diluting the stock solution ten fold with DDW.

16.1.4 Wash solution

The wash solution is 0.1 M nitric acid in DDW.

16.2 Apparatus

A Unicam SP9 spectrophotometer, equipped with a deuterium continuous background correction and PU9090 data graphics system, using a air/acetylene flame is used for the analysis of copper, zinc and iron. A nitrous oxide/acetylene flame is used for aluminium and chromium. Details of use are given in Harper *et al*. (1989).

Cadmium and lead are determined using a Unicam SP9 spectrophotometer with a graphite furnace attachment, PU9090 data graphics and PU9050 video graphic display.

Mercury is analysed using an automated atomic fluorescence system (PS Analytical).

These instruments are used in conjunction with manufacturer's instructions. A suitable external calibration is made and samples are diluted to read within the standard calibration range.

16.3 Performance of the method

In Table 7, results of ten different samples analysed by both HF and *aqua regia* digestion methods are compared. The total digestion technique gives consistently higher concentrations results for all metals except mercury for which comparable results are obtained. Results for chromium by HF digestion are generally 50% higher than those of *aqua regia* digests due to chromium forming refractory oxides during the *aqua regia* digestion. The differences between the digestion techniques are as expected as *aqua regia* is unable to release the metals bound to the silica lattice.

Table 8 shows results from six replicate HF digests of a marine sediment. The relative standard deviations for these are better than six percent, showing that the method gives good reproducibility. The detection limits are relatively high due to the low sediment weight to high sample volume ratio, necessary to prevent a borosilicate gel forming in solution.

Table 9 shows results obtained for certified reference sediment samples using the HF digestion technique. In all cases, comparable results were achieved with relative standard deviations similar to those of the certified reference data.

Table 7. A comparison of 10 different sediment samples digested by the 'Total' method and aqua regia method (All results are in mg kg^{-1})

Cr		Cu		Pb		Zn		Hg	
HF	AR	HF	AR	HF	AR	HF	AR	HF	AR
63	34	13	6.6	33	25	67	57	0.07	0.08
68	36	13	7.7	42	27	74	63	0.11	0.09
66	35	11	7.7	43	27	69	60	0.06	0.09
66	35	14	7.7	44	27	68	59	0.04	0.06
62	33	12	6.8	37	26	63	58	0.06	0.07
54	28	11	5.9	48	23	58	50	0.09	0.09
60	30	9.4	6.4	33	24	59	53	0.05	0.10
51	27	7.9	5.5	32	20	55	47	0.07	0.05
42	20	3.2	3.5	23	16	44	36	0.10	0.10
39	20	5	3.6	21	16	40	37	0.11	0.12

HF = Total digestions
AR = Aqua regia digestion

Table 8. Results achieved by the analysis of six replicate 'Total' digests of marine sediment

No.	Cd (mg kg$^{-1)}$	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Hg (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Al (%)	Fe (%)
1	4.72	98	384	7.83	390	97	3.97	2.6
2	4.64	95	410	7.4	401	97	3.76	2.6
3	4.25	100	399	8.27	403	99	3.59	2.63
4	4.37	107	366	7.61	379	95	3.94	2.48
5	4.57	101	364	7.43	437	95	3.94	2.67
6	4.26	103	390	8.35	431	97	4.00	2.48
Mean	4.47	101	386	7.82	407	97	3.87	2.58
Standard deviation	0.20	4.13	18.2	0.41	22.8	1.51	0.16	0.08
% RSD	4.47	4.09	4.72	5.24	5.60	1.56	4.13	3.10
Detection limits	0.02	5.0	3.0	0.01	3.0	0.6	0.02	0.1

RSD = relative standard deviation

Table 9. Comparison of three certified reference materials analysed by the 'Total' method with their certified reference data

	MESS-1 (mg kg^{-1})	BCSS-1 (mg kg^{-1})	PACS-1 (mg kg^{-1})
Cadmium			
Certified value	0.59 ± 0.1	0.28 ± 0.04	2.38 ± 0.2
rsd %	17.0	142	8.4
Mean measured value	0.55 ± 0.14	0.22 ± 0.02	2.00 ± 0.22
rsd %	25	9.0	11.6
n	7	8	14
Chromium			
Certified value	71 ± 11	123 ± 14	113 ± 8
rsd %	15.5	11.4	7.1
Mean measured value	60 ± 3	111 ± 4.5	100 ± 5.3
rsd %	5.0	4.1	5.3
n	19	9	19
Lead			
Certified value	34 ± 6.1	27.7 ± 3.4	404 ± 20
rsd %	17.9	12.3	5.0
Mean measured value	36.1 ± 2.8	23.7 ± 2.3	438 ± 29
rsd %	1.7	9.7	6.7
n	7	9	14
Zinc			
Certified value	191 ± 17	119 ± 12	824 ± 22
rsd %	8.9	10.0	2.7
Mean measure value	169 ± 14	106 ± 4.9	890 ± 31
rsd %	8.3	4.6	3.5
n	19	9	19
	MESS-1 (%)	BCSS-1 (%)	PACS-1 (%)
Aluminium			
Certified value	5.84 ± 0.02	6.26 ± 0.02	6.47 ± 0.11
rsd %	0.3	0.3	1.7
Mean measured value	6.0 ± 0.1	6.5 ± 0.2	6.6 ± 0.14
rsd %	1.7	3.1	2.1
n	7	9	13

rsd= relative standard deviation

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APPENDIX. Safety note — handling of hydrofluoric acid in the laboratory

Hydrofluoric acid is a clear liquid, corrosive and toxic. It should not be used with glassware as it attacks silica.

At all times of handling HF the operator should wear protective clothing consisting of:- laboratory coat, gloves, sleeve protectors, rubber apron and a face visor.

If spilt onto the skin, HF may not produce a burning sensation immediately as other acids do, but may be delayed for up to 24 hours. All spillages affecting the operator's person, however small, must be reported and immediately treated by a suitably qualified first aider.

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