REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

12 November 1984

Determination of total cadmium, zinc, lead and copper in selected marine organisms by flameless atomic absorption spectrophotometry

Reference Methods for Marine Pollution Studies No. 11 Rev. 1

Prepared in co-operation with

FAO IAEA IOC

UNEP 1984

Note: This document has been prepared in co-operation between the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), the Intergovernmental Oceanographic Commission (IOC) of UNESCO and the United Nations Environment Programme (UNEP) under projects FP/ME/0503-75-07, ME/5102-81-01, FP/5102-77-03 and FP/5101-84-01.

For bibliographic purposes this document may be cited as:

UNEP/FAO/IAEA/IOC: Determination of total cadmium, zinc, lead and copper in selected marine organisms by flameless atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 11 Rev. 1, UNEP 1984.

REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

12 November 1984

Determination of total cadmium, zinc, lead and copper in selected marine organisms by flameless atomic absorption spectrophotometry

Reference Methods for Marine Pollution Studies No. 11 Rev. 1

Prepared in co-operation with

FAO IAEA IOC

UNEP 1984

PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes ten regions and has over 120 coastal States participating in it. 1/ 2/

One of the basic components of the action plans sponsored by UNEP in the framework of Regional Seas Programme is the assessment of the state of marine environment and of its resources, of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of reference methods and guidelines for marine pollution studies are being developed and are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations system as well as other organizations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions the users are invited to convey their comments and suggestions to:

International Laboratory of Marine
Radioactivity
International Atomic Energy Agency
c/o Musée Océanographique
MC98000 MONACO

which is responsible for the technical co-ordination of the development, testing and intercalibration of reference methods.

1/ UNEP: Achievements and planned development of UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1 UNEP, 1982.

2/ P. HULM: A Strategy for the Seas. The Regional Seas Programme: Past and Future UNEP, 1983.

This issue (Rev.1) of the Reference Method for Marine Pollution Studies No. 11 was prepared in co-operation with the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO. It includes comments received from IOC's GIPME Group of Experts on Methods, Standards and Intercalibration (GEMSI), from the FAO/UNEP/IAEA Experts Consultation Meeting on Reference Methods for the Determination of Chemical Contaminants in Marine Organisms (Rome, 4-8 June 1984) and from a number of scientists who reviewed and tested the method. The assistance of all those who contributed to the preparation of Revision 1 of this reference method is gratefully acknowledged.

CONTENTS

1. SCOPE AND FIELD OF APPLICATION

This reference method describes the determination of total cadmium, total copper, total lead and total zinc in biological material by atomic absortion spectrophotometry after the organic matter has been decomposed by wet chemical digestion under pressure. Detection limits of the method will vary with the individual instruments used, typical ranges of the detection limits are as follows (in $mgkg^{-1}$):

- with flame atomization

Fresh weight	Cd	0.1-0.2
	Cu	0.5-1.0
	Pb	5-8
	Zn	2.5-5

- with electrothermal atomization

Fresh weight	Cd	0.0005-0.001
	Cu	0.01-0.03
	Pb	0.01-0.05
	Zn	Not normally used

2. REFERENCES

BERNHARD, M. (1976) Manual of methods in aquatic environment research. Part 3. Sampling and analyses of biological material. FAO Fish.Tech.Pap. No. 158 (FIRI/T158), pp. 124. FAO, Rome.

UNEP/FAO/IAEA (1984) Sampling of selected marine organisms and sample preparation for trace metal analysis. Reference Methods for Marine Pollution Studies No. 7 Rev. 2, UNEP, Geneva.

UNEP/FAO/IAEA (in preparation) Guidelines for monitoring chemical contaminants in marine organisms. Reference Methods for Marine Pollution Studies No. 6, UNEP, Geneva.

3. PRINCIPLES

An aliquot of the sample, prepared according to UNEP/FAO/IAEA (1984), is decomposed in a pressure container in the presence of nitric acid at 140°C. Then cadmium, copper, lead and zinc are determined by atomic absorption spectrophotometry with flame atomization or, after appropriate drying and charring, with electrothermal atomization at a wavelength of 228.8 nm (cadmium), 324.7 nm (copper), 283.3 nm (lead) and 213.9 nm (zinc).

4. REAGENTS

All reagents, including the distilled water, should be of recognized analytical quality, with as low as possible Cd, Cu, Pb and Zn concentration. All reagents must be checked for contamination with these elements by analysing blanks.

4.1 Demineralized distilled water or water of equivalent quality, with Cd, Cu, Pb and Zn content below detection limits of this method.

4.2 Nitric acid ($d_{20°C}$ = 1.4 gl^{-1}).

4.3 Hydrochloric acid ($d_{20°C}$ = 1.19 gl^{-1}).

4.4 Cadmium standard solutions.

4.4.1 Cadmium stock solution: 1 g Cd^{-1}. Transfer a commercially available stock solution (e.g. Titrisol, Merck) containing 1 $\pm$ 0.002 g Cd into a 1 litre volumetric flask (5.3) and bring to volume with distilled water (4.1).

Alternatively: Dissolve 1 g metallic cadmium of analytical grade in 10 ml of HCl (4.3) diluted 1:1 with distilled water (4.1) in a 1 litre volumetric flask (5.3) and bring up to volume with distilled water (4.1).

Alternatively: Dissolve 1.833 g $CdCl_2$ prepared from $CdCl_2.2.5H_2O$ (previously dried at 105°C for 2 hours), in a 1 litre volumetric flask (5.3) with distilled water (4.1), stabilize with 10 ml HCl (4.3) diluted 1:1 with distilled water (4.1), and bring up to volume.

4.4.2 Cadmium standard solution: From the stock solution (4.4.1) prepare, by appropriate dilutions using micropipettes (5.4), a cadmium standard solution which in 0.1 ml contains the lowest amount of cadmium standard to be used for the standardization (10.1). Prepare this standard solution daily using distilled water (4.1) acidified with an appropriate amount (e.g. 0.2 ml HNO_3/100 ml of distilled water) of nitric acid (4.2) for the dilutions.

NOTE: The concentration of Cd in the standard solution should depend on the Cd levels anticipated in the samples to be analyzed.

4.5 Copper standard solutions

4.5.1 Copper stock solution: 1 g Cu^{-1}. Transfer a commercially available stock solution (e.g. Titrisol, Merck) containing 1 $\pm$ 0.002 g Cu into a 1 litre volumetric flask (5.3) and bring to volume with distilled water (4.1)

Alternatively: Dissolve 1.000 g of copper metal (strip or wire, purity 99.9%) in a minimum volume of 1:1 diluted nitric acid (4.2) and dilute to 1 litre (5.3) with distilled water (4.1).

4.5.2 Copper standard solution: From the stock solution (4.5.1) prepare, by appropriate dilutions using micropipettes (5.20), a copper standard solution which in 0.1 ml contains the lowest amount of copper standard to be used for the standardization (10.1). Prepare this standard solution daily using distilled water (4.1) acidified with an appropriate amount (e.g. 0.2 ml HNO_3/100 ml of distilled water) of nitric acid (4.2) for the dilutions.

NOTE: The concentration of the Cu in the standard solution should depend on the Cu levels anticipated in the samples to be analyzed.

4.6 Lead standard solutions

4.6.1 Lead stock solution: 1 g Pbl^{-1}. Transfer a commercially available stock solution (e.g. Titrisol, Merck) containing 1 $\pm$ 0.002 g Pb into a 1 litre volumetric flask (5.3) and bring to volume with distilled water (4.1).

Alternatively: Dissolve 1.000 g of lead metal (strip or wire, purity 99.9%) in 1:1 diluted nitric acid (4.2). Dilute to 1 litre (5.3) with distilled water (4.1).

4.6.2 Lead standard solution: From the stock solution (4.6.1) prepare, by appropriate dilutions using micropipettes (5.4), a lead standard solution which in 0.1 ml contains the lowest amount of lead standard to be used for the standardization (10.1). Prepare this standard solution daily using distilled water (4.1) acidified with an appropriate amount (e.g. 0.2 ml HNO_3/100 ml of distilled water) of nitric acid (4.2) for the dilutions.

NOTE: The concentration of the Pb in the standard solution should depend on the Pb levels anticipated in the samples to be analyzed.

4.7 Zinc standard solutions

4.7.1 Zinc stock solution: 1 g Znl^{-1}. Transfer a commercially available stock solution (e.g. Titrisol, Merck) containing 1 $\pm$ 0.002 g Zn into a 1 litre volumetric flask (5.3) and bring to volume with distilled water (4.1)

Alternatively: Dissolve 1.000 g of zinc (metal granules, purity 99.9%) in 40 ml 1:1 diluted hydrochloric acid (4.3) and dilute to 1 litre (5.3) with distilled water (4.1).

4.7.2 Zinc standard solution: From the stock solution (4.7.1) prepare, by appropriate dilutions using micropipettes (5.4), a zinc standard solution which in 0.1 ml contains the lowest amount of zinc standard to be used for the standardization (10.1). Prepare this standard solution daily using distilled water (4.1) acidified with an appropriate amount (e.g. 0.2 ml HNO_3/100 ml of distilled water) of nitric acid (4.2) for the dilutions.

NOTE: The concentration of the Zn in the standard solution should depend on the Zn levels anticipated in the samples to be analyzed.

4.8 Combined standard solution: From the respective stock solutions (4.4.1, 4.5.1, 4.6.1 and 4.7.1 respectively) prepare, by appropriate dilutions using

micropipettes (5.4), a standard solution which in 0.1 ml contains the lowest amount of each metal to be used for the standardization (10.2). Prepare this standard solution daily using distilled water (4.1) acidified with an appropriate amount (e.g. 0.2 ml HNO_3/100 ml of distilled water) of nitric acid (4.2) for the dilutions.

4.9 Working matrix: Prepare according to (7) a large (e.g. 300 g of fresh weight) sample of the same tissue and species which will be analyzed. Test the homogeneity of the working matrix by analysing 5 subsamples for their Cd, Cu, Pb and Zn content (according to 9 and 10). If the coefficient of variation of five analyses is less than 10% the working matrix is ready for use. Otherwise, homogenize the working matrix again or prepare a new working matrix until the above coefficient of variation is obtained.

5. APPARATUS

5.1 Eight or more Teflon digestion vessels of at least 25 ml capacity, single or in a "digestion block".

NOTE: If smaller digestion vessels are used, the amounts digested (9) must be accordingly reduced to avoid explosions.

5.2 Oven or hot plate for the digestion vessels, working temperature 120 - 150 C complete with double independent temperature control.

NOTE: Oven or hot plate must have two independent temperature controls so that an overheating and consequent explosion can be avoided.

5.3 At least nine 25 ml, several 10 ml, 100 ml, 500 ml and 1 litre volumetric flasks (borosilicate glass).

5.4 Micropipettes to deliver accurately: 0.1 ml. Micropipettes or automatic sampler for injection into electrothermal device (see operator's manual).

5.5 Stainless steel, glass or Teflon tissue homogenizer.

5.6 Pipettes for 1, 2, 10 ml.

5.7 Laminar flow hood and/or "clean room".

5.8 Analytical balance (100-200 g) with a precision of 0.001 g for weighing reagents; preferably a 'top-load' balance.

5.9 Atomic absorption spectrophotometer (AAS) with hollow cathode lamps for Cd, Cu, Pb and Zn and with background correction for use with acethylene/air flame or alternatively complete with programmable graphite furnace atomizer.

NOTE: Lower detection limits can be reached using an electrodeless discharge lamp.

5.10 Signal indicator, recorder or similar.

5.11 Air compressor or high-pressure cylinder with compressed air (for flame atomization only).

NOTE: A source of clean, compressed air may be required for some spectrophotometers in the furnace mode to purge ozone which is generated by the deuterium lamp used for background correction from the optics.

5.12 High-pressure cylinder with acetylene of analytical grade purity (for flame atomization only).

NOTE: If acetylene intended for welding is utilized it should be checked against a pure supply. Nitrogen of adequate purity or any other gas or gas mixture specified by the AAS manufacturer may be used.

5.13 Weighing bottles with ground stoppers.

5.14 Drying oven (105°C).

5.15 Stainless steel tweezers.

5.16 Desiccator.

5.17 Freeze-dryer.

NOTE: All glassware that is used for the first time in the analytical procedure (9 and 10) must be thoroughly cleaned by washing with tap water followed by rinsing first with distilled water (4.1) and then with diluted 10% nitric acid (4.2).

6. SAMPLING

For a sampling plan follow UNEP/FAO/IAEA (in preparation) and for sampling of marine organisms follow UNEP/FAO/IAEA (1984).

7. SAMPLE PREPARATION

For sample preparation follow UNEP/FAO/IAEA (1984).

8. DETERMINATION OF DRY WEIGHT

NOTE: Results expressed on the basis of the dry weight (DW) of the analyzed tissues are more reliable than results expressed on the basis of its

fresh weight (FW) because the continuous loss of water from the fresh biological samples does not allow an accurate determination of FW. However, results expressed on the basis of FW are easier to interpret as most transfer models of pollutants are based on FW.

8.1 Oven-drying

A clean weighing bottle (5.13) with its ground stopper removed, is placed into a drying oven (5.14) set at 105°C. It is important to use the tweezers (5.15) every time the glass is touched, to avoid leaving fingerprints and particles of dirt on the weighing bottle. After 2 hours at 105°C place the stopper and the bottle separately into a desiccator (5.16) to cool.

Weigh the empty bottle with its stopper in place on the analytical balance (5.8). Note the weight. Place 1-2 g of the subsample of the specimen sample (7) in the weighing bottle and close with the stopper. Weigh it again and note the result.

Place the weighing bottle containing the subsample in the drying oven set at 105°C, remove the stopper with tweezers (5.15) and place the stopper also in the oven.

After 24 hours replace the stopper on the bottle, remove the bottle with stopper from the drying oven, remove the stopper and place bottle and stopper in a desiccator to cool.

Weigh the bottle with stopper in place and note the weight.

Repeat the drying cycle until the difference between subsequent weighing is less than 0.5% of the total weight; calculate the dry weight (DW) and DW/FW ratio.

NOTE: Biological materials containing large amounts of lipids cannot be oven-dried to constant weight and must, therefore, be freeze-dried.

8.2 Freeze-drying

Place a 1-2 g exactly weighed subsample of the specimen sample (7) in the clean sample container suitable for freeze-drying and freeze-dry (5.17) for 24 hours. Weigh the subsample exactly and freeze-dry for another 24 hours. Determine again the weight of the subsample. If the difference between the 2 weighings is less than 0.5% determine the DW and DW/FW ratio. Otherwise repeat the drying cycle until the difference between successive weighings is less than 0.5%.

9. MINERALIZATION OF THE BIOLOGICAL MATRIX

NOTE: Explosion hazard!! If too high amounts of organic material are placed in closed digestion vessels, (e.g. instead of the amount in fresh weight

the same amount in dry weight is used) the vessels may burst with great energy (explode). An explosion may also occur if the vessels are overheated. Therefore, the entire digestion procedure must be carried out with the appropriate precautions required for working with acids under pressure. For example, the digestion operation must be carried out under a closed fume hood. Defective teflon bottles must be discarded and bottles which have been used for a certain length of time must be replaced before there is any risk of bursting. To avoid overheating the oven or the hot plate (5.2) must be equipped with a second thermostat which intervenes above the digestion temperature (e.g. 160°C).

NOTE: Contamination hazard!! Due to virtual omnipresence of trace metals great precautions have to be taken to avoid contamination of the sample to be analyzed and of the test solutions. Whenever possible all operations should be carried out in "clean rooms" and/or under laminar flow hoods (5.7).

9.1 Cleaning of the digestion vessels before and between digestions

Clean the digestion vessels (5.1) with detergent, if necessary, and rinse with distilled water (4.1), then proceed with the digestion procedure (9) without adding the sample (blank run). If the blank value is greater than detection limit repeat the blank run.

9.2 Predigestion experiments

Determine, by digestion procedure (9.3), for every new matrix the minimum amount of concentrated nitric acid (4.2) necessary to destroy completely the organic matter by adding to about 1 g FW sample (7) increasing amounts of nitric acid (from 1 ml to not more than 6 ml).

9.3 Digestion (mineralization) procedure

Place an exactly weighed sample (7) of about 1 g FW in each of the digestion vessels (5.1), one vessel being charged with an exactly weighed amount of the working matrix (4.9) to check the efficiency (11) of each digestion.

Add concentrated HNO_3 in amount needed for digestion (9.2), cover the vessels and close them tightly.

Let the samples in the vessels predigest at room temperature for at least one hour (preferably overnight).

Place the vessels in a preheated oven or on a hot plate (5.2) at 140 $\pm$ 2°C for three hours.

Remove the vessels from the oven (hot plate), let them cool to room temperature and then open them. If the solution is not clear or has a yellow-brownish colour, the digestion is not complete. In which case, take a new sample (7) and repeat the experiment as described in 9.3 ensuring that the digestion conditions are followed exactly. If the solution is clear, transfer the contents of each vessel into clean 25 ml volumetric flasks (5.3) and bring up to volume with distilled water (4.1).

The contents of the volumetric flasks represent the test solutions. Analyse the samples as soon as possible.

10. ANALYTICAL DETERMINATION

10.1 Standardization of cadmium, copper, lead and zinc determination by addition of individual standard solutions

Before a new matrix is analyzed, and at periodic intervals as specified in the quality control procedure (12.3), carry out a digestion procedure (9.3) with 8 digestion vessels (5.1), all but one charged with the working matrix (4.9) in order to standardize (calibrate) the method and the apparatus used.

Prepare the appropriate standard solution for each metal separately (4.4.2, 4.5.2, 4.6.2 or 4.7.2) so that 0.1 ml of each individual standard added to 25 ml of the test solution (9.3) will result in a final metal concentration in the test solution approximately double that of the lowest concentration anticipated in the samples to be analyzed for that metal. Before starting a set of analyses it is important to check the calibration of the instrument using standard solutions.

Prepare a series of 8 clean digestion vessels (9.1) for each metal of interest. Place 1.00 g FW or equivalent to not more than 0.2 g DRY WEIGHT of the working matrix (4.9) to five vessels (5.1). No working matrix is added to the vessels 6-8, these vessels will be the blanks. In the case that high blank values are found, check for cleanliness of reagents and apparatus, but also calculate the blank value by standard additions. The first vessel should contain the working matrix (4.9) only, without any additions. With a micropipette (5.4) add 0.1 ml of standard solution (4.4.2, 4.5.2, 4.6.2 or 4.7.2) to the second vessel, 0.2 ml to the third vessel, 0.3 ml to the fourth vessel and 0.4 ml to the fifth vessel. Then add the predetermined amount of (9.2) of concentrated nitric acid to all eight vessels and carry out the digestion procedure (9.3) and the metal determinations according to the appropriate paragraph of (10).

Determine the metal concentration in the eight digestion vessels and construct a standardization (calibration) curve for each metal separately. Verify that the concentrations to be analyzed are in the straight part of the curve. If they are not, change the amounts to be analyzed by appropriate dilutions of the test solution (9.3) and of the corresponding standard solution (4.4.2, 4.5.2, 4.6.2 or 4.7.2).

10.2 Standardization of cadmium, copper, lead and zinc by addition of a combined standard solution

Before a new matrix is analysed, and at periodic intervals as specified in the quality control procedure (12.3), carry out a digestion procedure (9.3) with 8 digestion vessels (5.1) all but one charged with the working matrix (4.9), in order to standardize (calibrate) the method and the apparatus used.

Prepare the appropriate combined standard solution for Cd, Cu, Pb and Zn (4.8) so that 0.1 ml of combined standard added to 25 ml of the test solution (9.3) will result in a final metal concentration in the test solution approximately double than the lowest concentration anticipated in the samples to be analyzed for each metal.

Prepare a series of 8 clean digestion vessels (9.1). Add an exactly weighed aliquot of about 1 g FW of the working matrix (4.9) to five vessels (5.1). No working matrix is added to the 6-8 vessels, these vessels will be the blanks. The first vessel should contain the working matrix (4.9) only, without any additions. With a micropipette (5.4) add 0.1 ml of combined standard solution (4.8) to the second vessel, 0.2 ml to the third vessel, 0.3 ml to the fourth vessel and 0.4 ml to the fifth vessel. Then add the predetermined amount of (9.2) of concentrated nitric acid to all six vessels and carry out the digestion procedure (9.3) and the metal determinations according to 10.3, 10.4, 10.5, 10.6 or 10.7.

Determine the metal concentration in the six digestion vessels and construct a standardization (calibration) curve for each metal separately. Verify that the concentrations to be analyzed are in the straight part of the curve. If they are not, change the amounts to be analyzed by appropriate dilutions of the test solution (9.3) and of the combined standard solution (4.8).

10.3 Determination of cadmium with flame atomization

Set up the AAS (5.9 and 5.10) for Cd determination by selecting the Cd lamp, wavelength 228.9 nm and adjusting the acetylene/air flow (5.11 and 5.12) according to the manufactor's instruction. Introduce the test solution (9.3) into the atomizer and record the signal obtained.

10.4 Determination of copper with flame atomization

Set up the AAS (5.9 and 5.10) for Cu determination by selecting the Cu lamp, wavelength 324.7 nm and adjusting the acetylene/air flow (5.11 and 5.12) according to the manufacturer's instructions. Introduce the test solution (9.3) into the atomizer and record the signal obtained.

NOTE: No interferences have been reported for copper in the air-acetylene flame but some depression has been noted at high Zn/Cu ratios. This can be minimized by the use of a lean air-acetylene flame or a nitrous oxide-acetylene flame.

10.5 Determination of lead with flame atomization

Set up the AAS (5.9 and 5.10) for Pb determination by selecting the Pb lamp, wavelength 283.3 nm and adjusting the acetylene/air flow (5.11 and 5.12) according to the manufacturer's instructions. Introduce the test solution (9.3) into the atomizer and record the signal obtained.

NOTE: Due to the frequently high ambient Pb levels, Pb contamination of samples is highly probable and consequently samples have to be treated with great precaution to avoid sample contamination.

NOTE: No cationic interferences have been reported for the air-acetylene flame. However, phosphate, carbonate, iodide, fluoride and acetate suppress lead absorbance significantly. These interferences can be overcome by adding EDTA so that the final test solution is 0.1 molar with respect to EDTA.

10.6 Determination of zinc with flame atomization

Set up the AAS (5.9 and 5.10) for Zn determination by selecting the Zn lamp, wavelength 213.9 nm and adjusting the acetylene/air flow (5.11 and 5.12) according to the manufacturer's instruction. Introduce the test solution (9.3) into the atomizer and record the signal obtained.

NOTE: No chemical interferences have been observed in an air-acetylene flame.

10.7 Determination of Cd, Cu, Pb and Zn with electrothermal atomization

10.7.1 Determination of optimal conditions for the atomization programme: for each matrix determine the optimal parameters of the atomization programme (10.7.2) by changing the time intervals and temperatures of the four steps (drying, charring, atomizing and cleaning). This should be done by reference to the manufacturer's manual, followed by a careful and critical assessment of the recommended parameters in relation to those determined experimentally using suitable matrix materials.

10.7.2 Electrothermal atomization: Set up the AAS (5.9 and 5.10) and the graphite furnace for determination and select the appropriate lamp, wave length, optimal atomizing programme (10.7.1), etc.

Inject the test solution (9.3) with the appropriate micropipette or preferably, if available, with an automatic sample injector (5.4) and start the atomizing programme. Record the signal (e.g. peak height).

After 10 determinations check the correct functioning of the graphite furnace by injecting 20 µl of the test solution from the first digestion vessel used in the standardization procedure (10.1 or 10.2). Compare this signal with the signal obtained during standardization (10.1 or 10.2). If the signal is reduced below its mean less standard deviation determine the cause of this reduction. Most probably the matrix has reacted with the graphite tube and the graphite tube has to be replaced.

NOTE: The amount (5 µl to 50 µl) injected into the graphite furnace depends on its size and on the concentration of the metal in the test solution. When using electrothermal devices, the use of an automatic (or manual) background compensation system is recommended.

11. EXPRESSION OF RESULTS

From the height of the peak obtained on the test solution, determine, by reference to the standardization (calibration) curve (10.1 or 10.2) and making allowance for the blank determination, the concentration of analyzed metals in the test solution. When using electrothermal atomizers, the presence of the biological matrix often causes sensitivity decreases; then the blank evaluations must be made by an addition to the blank matrix.

In the case of an apparatus with digital read-out or a maximum response indicator, prepare a graph of instrument read-out against the corresponding mass of analyzed metals.

Check if the digestion vessel with the working matrix of the digestion series (9.3) yielded a result within 10% of the result obtained in the homogeneity test (4.9). If it did not, check the digestion procedure (9.3) for errors and repeat it with the same samples until a satisfactory result is obtained.

Using the result obtained with the known concentration of test solution calculate the metal concentration of sample, taking into account the exact weight of the sample placed in each digestion vessel (9.3). Express this concentration both in $mgkg^{-1}$ FW and in $mgkg^{-1}$ DW utilizing for the latter the results of (8).

12. ESTIMATION OF PRECISION AND ACCURACY

12.1 Precision

Estimate the precision of the entire analytical procedure (9 to 10) by digesting 5 subsamples from one original sample by calculating standard deviation (S) and coefficient of variation (CV) (CV=S.100/mean). If the coefficient of variation is greater than 20%, check the procedure for possible errors and contamination.

NOTE: The working matrix test (10.1) can be used for estimation of precision.

12.2 Accuracy

Analyze a certified standard with a matrix similar to the material under study together with your own working matrix (4.9) using this reference method. Calculate the mean and the standard deviation for the certified standard and the working matrix. If the value given for the certified standard is within the interval of your mean $\pm$ standard deviation, your method has the required accuracy and the working matrix can be used as standard for checking the accuracy of your procedure. If not, check procedure for errors.

NOTE: Standards are distributed as dried material so reduce the aliquot for digestion (9.3).

NOTE: In addition, by participating in intercalibration exercises involving several analytical laboratories, the accuracy of the method as used by the analyst can be checked and compared with the accuracy obtained by other participants in the exercise.

12.3 Quality control

Analyse periodically, at least once a week, or whenever the routine has been interrupted for more than a week, the working matrix (4.9) standardizing the method according to (10.1 or 10.2) in order to guarantee the precision and accuracy of your results.

If the quality control checks reveal a fluctuation in the standard deviation or the accuracy of the results by more than 5%, check the following factors: stability of stock solutions (prepare new solutions); instrumental drift or inadvertant changes in operational parameters, contamination of the working matrix (select alternative reference material for analysis); contamination of equipment, e.g. glassware; operator error(s).

13. TEST REPORT

Fill in the test report (table 1) giving full details in every column. Attach the corresponding sampling and sample preparation protocol (UNEP/FAO/IAEA (1984)).

Table 1 : Test Report on Total Metal Concentration in Biological Material:

___ Cadmium, ___ Copper, ___ Lead, ___ Zinc

1. Sample code: _____

2. Determination of dry weight by freeze-drying ___ or in oven ____

   2.1 Duration of drying: _____ hours

   2.2 Date of drying (day, month, year): _____

   2.3 DW/FW ratio: _____

3. Mineralization (digestion)

   3.1 Duration of mineralization: _____ hours

   3.2 Temperature used for mineralization: _____°C

   3.3 Date of mineralization (day, month, year) _____

   3.4 Anomalies observed which may influence test results:

   _____

   _____

   _____

4. Standardization (calibration) using working matrix

   4.1 Standardization

   4.2 Date (day, month, year): _____

4.3 Result obtained by _____ flame atomization or _____ electrothermal atomization:

digestion vessel		1	2	3	4	5	6	7 (blank)	8
added FW (g)	for Cd						-	-	-
	for Cu						-	-	-
	for Pb						-	-	-
	for Zn						-	-	-
added stand. sol. (ml)		-	0.1	0.2	0.3	0.4	-	-	-
units of recorded signal	for Cd								
	for Cu								
	for Pb								
	for Zn								
$mgkg^{-1}$ FW	for Cd								
	for Cu								
	for Pb								
	for Zn								

4.4 Atomizing programme (when electrothermal atomization was used):

metal	Cd	Cu	Pb	Zn	
wavelength					(nm)
drying time at 95°C					(seconds)
charring temp.					(°C)
atomizing temp.					(°C)
atomizing time					(seconds)

4.5 Anomalies observed at standardization and observations relevant to the interpretation of the results: _____

_____

_____

5. Test result and estimation of precision using subsamples of same sample

5.1 Date (day, month, year): _____

5.2 Result obtained by _____ flame atomization or _____ electrothermal atomization:

digestion vessel		1	2	3	4	5
added FW (g)	for Cd for Cu for Pb for Zn					
units of recorded signal	for Cd for Cu for Pb for Zn					
$mgkg^{-1}$ FW	for Cd for Cu for Pb for Zn					

5.3 Atomizing programme (when electrothermal atomization was used:

metal	Cd	Cu	Pb	Zn	
wavelength					(nm)
drying time at 95°C					(seconds)
charring temp.					(°C)
atomizing temp.					(°C)
atomizing time					(seconds)
cleaning the graphite furnace					(seconds)

5.4 Summary of test results:

mean $mgkg^{-1}$ Cd FW: _____ standard deviation: _____

coeff. of variation (%): _____ DW/FW ratio: _____

mean $mgkg^{-1}$ Cd DW: _____

mean $mgkg^{-1}$ Cu FW: _____ standard deviation: _____

coeff. of variation (%): _____ DW/FW ratio: _____

mean $mgkg^{-1}$ Cu DW: _____

mean $mgkg^{-1}$ Pb FW: _____ standard deviation: _____

coeff. of variation (%): _____ DW/FW ratio: _____

mean mgkg Pb DW: _____

mean $mgkg^{-1}$ Zn FW: _____ standard deviation: _____

coeff. of variation (%): _____ DW/FW ratio: _____

mean $mgkg^{-1}$ Zn DW: _____

5.5 Anomalies observed at test and observations relevant to the interpretation of the results: _____

_____

_____

_____

6. Estimation of accuracy

6.1 Date (day, month, year): _____

6.2 Type (code) of certified standard used for Cd: _____

for Cu:_____, for Pb: _____, for Zn: _____

6.3 Declared mg of metal/kg of certified standard for Cd: _____

for Cu: _____, for Pb: _____, for Zn: _____

6.4 Result obtained by _____ flame atomization or _____ electrothermal atomization:

digestion vessel		1	2	3	4	5	6	7	8
added certified standard (g)	for Cd					-	-	-	-
	for Cu					-	-	-	-
	for Pb					-	-	-	-
	for Zn								
added matrix working matrix (g)	for Cd	-	-	-	-				
	for Cu	-	-	-	-				
	for Pb	-	-	-	-				
	for Zn								
units of recorded signal	for Cd								
	for Cu								
	for Pb								
	for Zn								
$mgkg^{-1}$ FW	for Cd								
	for Cu								
	for Pb								
	for Zn								

6.5 Summary of estimation of accuracy:

		certified standard	test sample
Cd	mean $mgkg^{-1}$ FW		
	stand. deviation		
	coeff. of variation (%)		
	mean $mgkg^{-1}$ DW		
Cu	mean $mgkg^{-1}$ FW		
	stand. deviation		
	coeff. of variation (%)		
	mean mgkg-1 DW		
Pb	mean $mgkg^{-1}$ FW		
	stand. deviation		
	coeff. of variation (%)		
	mean $mgkg^{-1}$ DW		
Zn	mean $mgkg^{-1}$ FW		
	stand. deviation		
	coeff. of variation (%)		
	mean $mgkg_{-1}$ DW		

7. Anomalies observed at estimation of accuracy and observations relevant to the interpretation of results: _____

_____

_____

_____

8. Intercalibration exercise (give details): _____

_____

_____

_____

_____

9. Full address of the institution which carried out the test:

_____

_____

_____

_____

10. Name(s) and signature(s) of the person(s) who carried out the test:

_____

_____

Date: _____

Attachment: Sampling and sample preparation protocol relevant to the analyzed sample.

LIST OF REFERENCE METHODS FOR MARINE POLLUTION STUDIES

LISTE DES METHODES DE REFERENCE POUR LES ETUDES DE POLLUTION MARINE

UNEP/WHO : Guidelines for monitoring the quality of coastal recreational waters. (Draft) Reference Methods for Marine Pollution Studies No. 1. UNEP 1982.

UNEP/WHO : Determination of total coliforms in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 2 Rev. 1, UNEP 1983.

PNUE/OMS : Détermination des coliformes totaux dans l'eau de mer par la méthode de culture sur membranes filtrantes. Méthodes de références pour les études de pollution marine No 2 rév. 1, PNUE 1983.

UNEP/WHO : Determination of faecal coliforms in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 3 Rev. 1, UNEP 1983.

PNUE/OMS : Détermination des coliformes fécaux dans l'eau de mer par la méthode de culture sur membranes filtrantes. Méthodes de références pour les études de pollution marine No 3 rév. 1, PNUE 1983.

UNEP/WHO : Determination of faecal streptococci in sea-water by the membrane filtration culture method. Reference Methods for Marine Pollution Studies No. 4 Rev. 1, UNEP 1983.

PNUE/OMS : Détermination des streptocoques fécaux dans l'eau de mer par la méthode de culture sur membranes filtrantes. Méthodes de références pour les études de pollution marine No 4 rév. 1, PNUE 1983.

UNEP/WHO : Determination of faecal coliforms in bivalves by multiple test tube method. Reference Methods for Marine Pollution Studies No. 5 Rev. 1, UNEP 1983.

PNUE/OMS : Détermination des coliformes fécaux dans les bivalves par le test des tubes multiples. Méthodes de références pour les études de pollution marine No 5 rév.1, PNUE 1983.

UNEP/FAO/IAEA : Guidelines for monitoring chemical contaminants in marine organisms. Reference Methods for Marine Pollution Studies No. 6, UNEP. (in preparation)

UNEP/FAO/IAEA/ IOC: Sampling of selected marine organisms and sample preparation for trace metal analysis. Reference Methods for Marine Pollution Studies No. 7 Rev. 2, UNEP 1984.

UNEP/FAO/IAEA/ IOC: Determination of total mercury in selected marine organisms by cold vapour atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 8 Rev. 1, UNEP 1984.

UNEP/FAO/IAEA : Determination of total arsenic in selected marine organisms by flameless atomic absorption spectrophotometry. (Draft) Reference Methods for Marine Pollution Studies No. 9, UNEP 1984.

UNEP/FAO/IAEA : Determination of total selenium in selected marine organisms by hydride generation atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 10, UNEP 1984.

UNEP/FAO/IAEA/ IOC: Determination of total cadmium, zinc, lead and copper in selected marine organisms by flameless atomic absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 11 Rev. 1, UNEP 1984.

UNEP/FAO/IAEA : Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons. Reference Methods for Marine Pollution Studies No. 12 Rev. 1, UNEP 1984.

UNEP/FAO/IAEA : Determination of methylmercury in selected marine organisms. Reference Methods for Marine Pollution Studies No. 13, UNEP UNEP 1984.

UNEP/FAO/IAEA : Determination of DDTs and PCBs in selected marine organisms. Reference Methods for Marine Pollution Studies No. 14. UNEP 1982.

UNEP/IOC/IAEA : Monitoring of tar on marine beaches. Reference Methods for Marine Pollution Studies No. 15, UNEP. (in preparation)

UNEP/IAEA : Determination of DDTs, PCBs, PCCs and other hydrocarbons in sea-water by gas chromatography. (Draft) Reference Methods for Marine Pollution Studies No. 16, UNEP 1982.

UNEP/IAEA : Determination of DDTs, PCBs and other hydrocarbons in marine sediments by gas liquid chromatography. (Draft) Reference Methods for Marine Pollution Studies No. 17, UNEP 1982.

UNEP/IOC : Determination of total dissolved cadmium in sea-water by differential pulse anodic stripping voltammetry. (Draft) Reference Methods for Marine Pollution Studies No. 18, UNEP 1983.

UNEP/IOC : Determination of total mercury in estuarine waters and suspended matter by cold vapour atomic absorption spectrophotometry. Reference Methods for Marine Pollution Pollution Studies No. 19, UNEP 1983. (in preparation)

UNEP/IOC : Monitoring of petroleum hydrocarbons in sediments. Reference Methods for Marine Pollution Studies No. 20, UNEP. (in preparation)

UNEP/WHO : Determination of total coliforms in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 21, UNEP 1983. (in preparation)

UNEP/WHO : Determination of faecal coliforms in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 22, UNEP 1983. (in preparation)

UNEP/WHO : Determination of faecal streptococci in sea-water by multiple test tube method. Reference Methods for Marine Pollution Studies No. 23, UNEP 1983. (in preparation)

UNEP/WMO : Sampling of aerosols and wet precipitation for analysis of chemical pollutants. Reference Methods for Marine Pollution Studies No. 24, UNEP (in preparation)

SPC/UNEP : Coral Reef Monitoring Handbook. Reference Methods for Marine Pollution Studies No. 25, UNEP 1984.

UNEP/IAEA : Determination of total mercury in marine sediments and suspended solids by cold vapour absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 26, UNEP 1984. (in preparation)

UNEP/IAEA : Determination of total cadmium in marine sediments by flameless absorption spectrophotometry. Reference Methods for Marine Pollution Studies No. 27, UNEP 1984. (in preparation)

UNEP/IOC : Monitoring of petroleum hydrocarbons in sea-water. (in preparation)

UNEP/IAEA : Guidelines for monitoring of estuarine waters and suspended matter. (in preparation)

UNEP/WHO : Determination of faecal cnliforms in estuarine waters, suspended matter and sediments. (in preparation)

UNEP/WHO : Determination of phosphorus in suspended matter and sediments. (in preparation)

UNEP/WHO : Determination of nitrogen in suspended matter and sediments. (in preparation)

UNEP/WHO : Determination of BOD_5 and COD in estuarine waters. (in preparation)

UNEP/FAO : Acute toxicity tests. (in preparation)

UNEP/UNESCO : Determination of total cadmium in estuarine waters and suspended matter. (in preparation)

UNEP : Biological non-acute toxicity tests. (in preparation)

UNEP/IOC : Determination of basic oceanographic and meteorological conditions. (in preparation)

UNEP/IOC : Determination of standard physical and chemical parameters. (in preparation)

UNEP/WHO : Statistical methods for the evaluation of results from monitoring the quality of coastal recreational and shellfish-growing waters. (in preparation)

UNEP/IAEA : Determination of selected trace metals in aerosols and in wet precipitation. (in preparation)

UNEP/IAEA : Determination of halogenated hydrocarbons in aerosols and in wet precipitation. (in preparation)

UNEP/WMO : Sampling of dry deposition. (in preparation)

Issued and printed by:

Regional Seas Programme Activity Centre
United Nations Environment Programme

Additional copies of this and other publications issued by the Regional Seas Programme Activity Centre of UNEP can be obtained from:

Regional Seas Programme Activity Centre
United Nations Environment Programme
Palais des Nations
GENEVA
Switzerland

Printed at U.N. Geneva
GE.84-03841—December 1984—1,000