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REPORT No. 35

INTERCALIBRATION OF ANALYTICAL METHODS ON MARINE ENVIRONMENTAL SAMPLES

Results of MEDPOL II Exercise for the Intercomparison of Trace Element Measurements on Fish Tissue Homogenate MA(F)-MED 86/TM

April 1988

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Prepared in co-operation with UNEP

1. INTRODUCTION

In recent years the question has often been raised whether the Mediterranean Sea as an almost closed basin may be significantly more polluted than the oceans. Therefore, much work has been undertaken in Mediterranean States in the framework of UNEP's Action Plan for the Mediterranean in order to check this hypothesis, evaluate temporal tendencies and variations between different areas, etc. Pelagic organisms of the Mediterranean Sea, in particular, may be more contaminated by heavy metals and chlorinated hydrocarbons than those of the oceans and this can be very important from the health and economical point-of-view owing to the intensive use of fishing in the Mediterranean area.

Many scientists in Mediterranean States, therefore, are busy nowadays with the determination of heavy metals and other pollutants in marine biota samples, e.g., shellfish, plankton and pelagic fish. Such determinations at very low concentration levels are often very delicate and require sophisticated equipment as well as professional experience. It is, therefore, highly desirable for chemists who work in the field of trace element determination (and in particular for those who just entered this field) to check the validity of their analytical results. For this reason, the International Atomic Energy Agency in cooperation with the United Nations Environment Programme organized in past years laboratory intercomparisons in the framework of the MEDPOL Phase II activities. These intercomparison runs concerned the determination of trace elements in mussel and shrimp tissues as well as marine sediment (1,2). The present intercomparison is relative to the analysis of a fish tissue homogenate and the results of the participating laboratories are presented here.

2. SCOPE OF THE INTERCOMPARISON

Each participant received a sample of lyophilised fish tissue. This sample originated from a batch of homogenized material which was prepared as described below. Each sample was accompanied by an information sheet and a report form. Participants were requested to determine as many as they could from among the following 15 elements: Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, V and Zn.

In total, 17 laboratories from 10 countries participated in this intercomparison run: 101 laboratory means were reported for 14 elements but for three of them only isolated results (one or two laboratory means) were supplied. On the other hand, eight results were not accurately determined and concentrations simply reported as lying below a given detection limit.

3. DESCRIPTION OF THE MATERIAL

About 600 kg of garfish (Belone belone) were collected from the Western Baltic Sea in April 1984. Fillets were separated from other tissues and lyophilised.

Lyophilised tissues were first ground in a laboratory cutting mill and the resulting powder, which passed through a 1mm sieve, was further homogenized by mixing the totality of the powdered material in a stainless steel rotating drum for 150 hours. A portion of this material was taken and packed into small bottles for other intercomparison purposes such as the determination of radionuclides and organochlorine compounds. The remaining material, however, appeared to be rather fibrous and its homogeneity doubtful as far as trace element concentrations were concerned. It was decided, therefore, to further grind the material in a porcelain ball mill. The resulting powder was sieved through a clean stainless-steel sieve (300 µm) and the fraction of the material passing through the sieve was retained.

By proceeding in this way it was possible to prepare about 7.5 kg of powder having a particle size smaller than 300 µm. This powder was further homogenized by mixing in a stainless-steel rotating drum for about one week. Aliquots of about 30 g of this powder were put into acid-washed small glass bottles equipped with plastic screw caps. These bottles were labelled MA(F)-MED86/TM, denoting "Marine animal (fish) MEDPOL 1986/Trace metals". Each sample bottle was individually numbered.

The homogeneity of the material for trace elements was checked by determining the concentrations of *Cr*, *Cu* and *Zn* in ten 500 mg-samples taken randomly from the bulk of the powder. The samples were mineralized by wet-ashing with nitric and perchloric acids and zinc was determined by flame atomic absorption spectroscopy while the flameless technique was applied to the determination of chromium and copper. Each sample solution was analysed 10 times in the case of chromium and copper and 7 times in the case of zinc. For each element a one-way variance analysis was performed to compare the variability between samples and the variability within samples. In each case both variances did not differ significantly, i.e., the variance between samples could be explained by the analytical variance (at a significance level of 0.05). On the other hand, in no case did the relative standard deviation of the results of analysis of the different samples exceed 3%. This material was, therefore, considered as homogeneous for the purpose of the intercalibration (at least for a sample weight $\geqslant$ 500 mg).

The water content of the lyophilised material as determined by drying to a constant weight at 85°C was found to be approximately 4.5%. At this temperature, the constant weight was reached after a drying time of 24-48 hours. As, however, the moisture content may vary with changes in the ambient humidity and temperature, it was recommended that the water content of this material be always determined in a separate sub-sample (not that taken for analysis) by drying for 48 hours at 85°C.

All results were to be reported on a dry-weight basis.

4. EVALUATION OF RESULTS

The data provided by laboratories participating in this intercomparison are presented in Tables 1-14 (no result was reported for the determination of the element Ag). All results relevant to a given element are grouped in the same table. The terms used in the tables are defined as follows:

Unit: Units in which the concentration of an element to be determined is expressed (in this intercomparison, all results are expressed in micrograms per gram of dry-weight).

Laboratory Code No.: Each laboratory was represented by a code number, which remains unchanged throughout the tables. The numbers, however, do not correspond to the sequency of laboratories in the list of participants given at the end of this report, so that anonymity is preserved.

Method: Participating laboratories were requested to give basic information on the analytical methods which they applied to the determination of trace elements. These methods are described in the tables by a code, namely:

I-NAA	:	Neutron activation analysis (instrumental)
R-NAA	:	" " " (with radiochemical separations)
AAS	:	Atomic absorption spectroscopy (without specifications)
Flame-AAS:	:	" " " (flame technique)
GF-AAS:	:	" " " (graphite-furnace technique)
Hydride-AAS:		" " " (hydride-generation technique)
CV-AAS	:	" " " (cold-vapour technique)
SP	:	Spectrophotometry, colorimetry
EM	:	Electrochemical methods

No. of determinations: The number of individual determinations of a given trace element, performed by a laboratory using the same analytical procedure.

Laboratory mean: The arithmetic mean computed from all individual results supplied by a laboratory for the determination of a given trace element. An asterisk next to a laboratory mean denotes that this mean was classified as an outlier and was not taken into account when computing the overall mean.

Coefficient of variation: The ratio (expressed in percent) of the standard deviation of the individual results of determination of a given trace element to the laboratory mean (the standard deviation is computed in the usual way). The coefficient of variation was not computed when less than 3 individual results were reported.

5. DISCUSSION

An average concentration value was computed for each element from the laboratory means given in Tables 1 - 14.

The data sets from which these overall means were computed were first scrutinized in order to eliminate doubtful results. This was done by assuming that these data sets are normally distributed and by applying Student's t-test at a one-sided significance level equal to 0.01 (3).

This procedure of elimination of outliers was necessary since it was obvious that some results in each data set were very different from those of the dominant group and their influence in the calculation of the overall mean had to be removed before computing this value.

The detected outliers are identified by an asterisk next to the corresponding values in Tables 1 - 14.

The mean values of the sets of results obtained after elimination of outliers and their confidence intervals were then computed in the usual way, i.e.:

$$\bar{x} - t.\ S/\sqrt{n} < \mu < x + t.\ S/\sqrt{n}$$

where:

u = theoretical mean of the distribution of results
x = arithmetical mean " " "
S = standard deviation of the "accepted" results
n = number of "accepted" results
t = Student's factor for (n-1) degrees of freedom and a significance level α = 0.05 (two-sided).

Results given in the form "less than" are reported in Tables 1-14 but were not taken into account when computing the overall means.

The test of outlier detection was applied when at least four results (other than those given in the form "less than") were available. Overall means of concentrations and their confidence intervals were computed from at least three "accepted" results. These overall means are given in Table 15.

Four results only were reported for the determination of arsenic (Table 1). The consistency of these results shows that this element could be determined in a satisfactory way by different techniques such as spectrophotometry, atomic absorption (hydride generation) and neutron activation. The graphite-furnace technique (Laboratory No. 8) does not seem to have a sufficient sensitivity but the reported detection limit agrees with the other determinations of this element.

As far as the results reported for cadmium are concerned (Table 2), it is obvious that they are very scattered, in spite of the fact that 75% of them were obtained by the same basic technique (flameless atomic absorption). Five outliers out of 16 results were detected by the t test and are all significantly higher than a dominant cluster of results around 0.04 $ug.g^{-1}$, thereby indicating contamination problems in the determination of this element.

Five results of determination of chromium (out of a total of 6 results) were obtained by graphite-furnace AAS (Table 4). The t test detected two outliers which are significantly higher than the other results and are probably caused by contaminations. One result was obtained by instrumental neutron activation analysis and is similar to the dominant group of the results of flameless AAS.

With the exception of three outliers (one on the lower side and two on the higher side) results reported for the determination of copper are well grouped around 3 $\mu g.g^{-1}$ (Table 5). Most results were obtained by atomic absorption spectroscopy (either by the flame or by the flameless technique). One result was obtained by neutron activation analysis and an other one by polarography. These results are similar to the results of atomic absorption.

Four results only were reported for the determination of iron (Table 6). One result is obviously too low and was detected as outlier by the t-test. The three other results are in good agreement in spite of the fact that they were obtained by three different techniques.

The results reported for the determination of mercury are well grouped around a central value of about 0.5 $ug.g^{-1}$ (Table 7). One outlier only was detected by the t-test. One laboratory determined mercury by neutron activation analysis and the result agrees with the other results which were all obtained by atomic absorption spectroscopy (mainly by the cold-vapour technique).

Four results only were reported for the determination of manganese (Table 8). With the exception of one result which is obviously too low and may be due to a calculation error, the results are in good agreement in spite of the fact that they were obtained by three different techniques.

As far as the results reported for the determination of lead are concerned (Table 10) one can see that they are very scattered. A dominant group, however, exists around a central value of about 4 $\mu g.g^{-1}$. One result was detected as outlier by the t-test and is maybe due to a contamination error. Most results were obtained by graphite-furnace atomic absorption spectroscopy. One determination was done by polarography and agrees with the dominant group of results obtained by atomic absorption.

Results reported for zinc are given in Table 14. Most of them are grouped around a central value of about 110 $\mu g.g^{-1}$ but three outliers (out of 14 results) were detected by the t-test. Most results were obtained by atomic absorption spectroscopy (flame technique). Two results given respectively by instrumental neutron activation analysis and polarography are in good agreement with the dominant group of results of atomic absorption.

An insufficient number of data was available for the determination of cobalt, nickel, antimony, selenium and vanadium. A thorough evaluation of these data is not possible under such circumstances.

7. CONCLUSIONS

On the whole one can state from the results given in Tables 1-14 that the quality of analyses does not show any significant improvement in comparison with previous intercomparison runs (1,2).

Strong analytical problems still seem to exist in the case of cadmium, chromium and lead since very scattered results were reported for these elements. It is surprising to notice that elements normally easy to determine like copper and zinc caused problems in some cases.

The number of analytical methods which were used in this intercomparison was rather limited. The method of analysis which was predominantly used was atomic absorption spectroscopy in its various forms (about 83% of all results), followed by neutron activation analysis (12%), polarography (4%) and spectrophotometry (1%). No determinations by other analytical techniques such as X-ray fluorescence or plasma atomic emission spectroscopy were reported.

The within-laboratory precision is satisfactory. About 74% of the reported coefficients of variation lie between 0 and 10%, 12% are between 10 and 20%, 11% between 20 and 30% and 3% only are higher than 30%.

The total number of outliers detected by the t-test is moderate (about 16% of all results). This figure, however, does not show a significant improvement when compared with that of previous intercomparisons (1). About 69% of the outlier's results are too high while 31% are too low. This probably reflects contamination problems during the analysis of some elements.

The number of outlying results by participating laboratory varied between 0 and 4. Three laboratories produced 1 outlier, three laboratories reported 2 outliers and one laboratory produced 3 outliers. One laboratory reported 4 outliers (for 5 reported results). This laboratory should carefully revise its analytical procedures for trace element analysis.

REFERENCES

(1) International Atomic Energy Agency, International Laboratory of Marine Radioactivity: Intercalibration of Analytical Methods on Marine Environmental Samples, Results of MEDPOL II Exercise for the Intercomparison of Trace Element Measurements on Mussel Tissue Homogenate and Marine Sediment (MA-M-2/TM and SD-N-1/2/TM), Report IAEA/RL/137 (MONACO/31), December 1986.

(2) International Atomic Energy Agency, International Laboratory of Marine Radioactivity: Intercalibration of Analytical Methods on Marine Environmental Samples, Trace Element Measurements on Shrimp Homogenate, Results of the Worldwide Intercomparison Run MA-A-3/TM and of the MEDPOL Exercise MA(S)MED86/TM, Progress Report No. 34, June 1987.

(3) Commissariat a l'Energie Atomique, Methodes Statistiques en Chimie Analytique, Volume III, Calcul d'erreurs, fascicule 4, Recherche des Valeurs Aberrantes, DUNOD, Paris, 1969.

Table 1
Results of intercomparison for arsenic

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
5	SP	6	2.00	6.3%
8	GF-AAS	-	<4.00	-
9	Hydride-AAS	6	1.11	6.8%
11	R-NAA	6	1.88	7.7%

Table 2
Results of intercomparison for cadmium

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No of Determinations	Laboratory Mean	Coefficient of Variation
1	GF-AAS	6	0.015	9.7%
2	GF-AAS	6	0.16*	24.1%
3	GF-AAS	6	0.31*	11.1%
4	GF-AAS	3	0.047	24.7%
5	GF-AAS	6	0.10	35.8%
6	GF-AAS	3	0.63*	9.1%
7	Flame-AAS	-	<0.002	-
8	GF-AAS	4	0.051	9.1%
9	GF-AAS	6	0.034	32.1%
10	GF-AAS	2	0.82*	-
11	R-NAA	6	0.049	6.4%
12	GF-AAS	3	0.017	21.1%
13	EM	3	0.022	3.0%
14	Flame-AAS	6	0.36*	5.0%
15	GF-AAS	6	0.10	9.3%
17	GF-AAS	6	0.018	10.1%

Table 3
Results of intercomparison for cobalt

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
7	Flame-AAS	-	< 0.005	-
8	GF-AAS	-	< 0.2	-
11	I-NAA	4	0.065	7.9%

Table 4
Results of intercomparison for chromium

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
2	GF-AAS	6	2.30*	29.9%
3	GF-AAS	6	0.57	8.5%
5	GF-AAS	6	1.37*	6.1%
8	GF-AAS	4	0.38	10.0%
9	GF-AAS	6	0.58	21.3%
11	I-NAA	4	0.70	3.4%

Table 5
Results of intercomparison for copper

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
1	GF-AAS	6	3.02	6.9%
3	GF-AAS	6	4.30	5.8%
4	Flame-AAS	3	3.43	4.4%
5	GF-AAS	6	4.00	9.5%
6	GF-AAS	3	5.67*	7.3%
7	Flame-AAS	2	0.0086*	-
8	GF-AAS	4	3.12	6.1%
9	GF-AAS	6	3.05	6.0%
10	GF-AAS	2	9.44*	-
11	R-NAA	6	2.40	7.4%
12	GF-AAS	3	1.92	8.1%
13	EM	4	3.15	3.7%
14	Flame-AAS	6	3.36	4.4%
15	GF-AAS	6	3.05	7.6%
16	Flame-AAS	3	1.68	3.6%

Table 6
Results of intercomparison for iron

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
7	Flame-AAS	2	0.074*	-
8	GF-AAS	4	99.2	7.0%
9	Flame-AAS	6	111.0	3.5%
11	I-NAA	4	98.5	13.5%

Table 7
Results of intercomparison for mercury

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
1	CV-AAS	6	0.56	2.9%
2	CV-AAS	6	0.35	26.1%
3	CV-AAS	6	0.64	1.8%
4	CV-AAS	3	0.57	3.0%
5	CV-AAS	6	0.58	9.4%
8	AAS	4	0.32	23.4%
9	CV-AAS	6	0.46	3.2%
10	CV-AAS	2	2.30*	-
11	R-NAA	6	0.53	2.0%
12	GF-AAS	3	0.38	45.3%
13	CV-AAS	6	0.67	1.0%
14	CV-AAS	6	0.73	1.8%
15	CV-AAS	6	0.68	3.7%
17	CV-AAS	6	0.59	10.0%

Table 8
Results of intercomparison for manganese

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
7	Flame-AAS	-	< 0.003	-
8	GF-AAS	4	3.72	23.2%
9	Flame-AAS	6	3.00	8.6%
11	R-NAA	6	3.18	2.2%

Table 9
Results of intercomparison for nickel

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
7	Flame-AAS	-	< 0.005	-
8	GF-AAS	-	< 0.8	-

Table 10
Results of intercomparison for lead

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
1	GF-AAS	6	4.32	6.0%
2	GF-AAS	6	7.05	16.2%
3	GF-AAS	6	4.99	13.2%
4	GF-AAS	3	7.63	13.1%
5	GF-AAS	6	4.08	7.7%
6	GF-AAS	3	8.00	6.3%
7	Flame-AAS	2	0.021	-
8	GF-AAS	4	0.41	16.8%
9	GF-AAS	6	6.87	11.5%
10	GF-AAS	4	0.071	26.8%
12	GF-AAS	3	32.3*	14.0%
13	EM	3	3.96	3.7%
14	Flame-AAS	6	4.63	4.2%

Table 11
Results of intercomparison for antimony

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
11	R-NAA	6	0.023	8.6%

Table 12
Results of intercomparison for selenium

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
8	GF-AAS	-	< 15.0	-
9	Hydride-AAS	6	0.80	9.7%
11	T NAA	4	1.56	7.8%

Table 13
Results of intercomparison for vanadium

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
8	GF-AAS	4	0.92	2.8%
11	R-NAA	6	0.063	12.0%

Table 14
Results of intercomparison for zinc

Unit: Microgram/gram (dry-weight)

Laboratory Code No.	Method	No. of Determinations	Laboratory Mean	Coefficient of Variation
1	Flame-AAS	6	114.4	1.9%
3	Flame-AAS	6	117.3	8.6%
4	Flame-AAS	3	138.7	4.4%
5	Flame-AAS	6	127.6	1.3%
6	Flame-AAS	3	84.5	4.8%
7	Flame-AAS	2	0.14*	-
8	Flame-AAS	4	110.6	2.6%
9	Flame-AAS	6	104.3	3.0%
10	Flame-AAS	2	60.2*	-
11	I-NAA	4	113.0	2.6%
12	GF-AAS	4	8.5*	5.0%
13	RM	6	102.6	3.8%
14	Flame-AAS	3	106.8	1.2%
16	Flame-AAS	3	93.9	1.0%

Table 15

Intercomparison MA(F)-MED86/TM
Overall means of the determinations
of trace element concentrations in fish tissue homogenate

Element	Number of Accepted Results	Concentration* + Confidence Interval
As	3	1.66 ± 1.20
Cd	10	0.045 ± 0.023
Cr	4	0.56 ± 0.21
Cu	12	3.04 ± 0.48
Fe	3	102.9 ± 17.5
Hg	13	0.54 ± 0.08
Mn	3	3.30 ± 0.93
Pb	12	4.34 ± 1.83
Zn	11	110.3 ± 10.1

* All values are expressed in $\mu g.g^{-1}$ of dry-weight.

Confidence intervals are given for a significance level $\alpha = 0.05$

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