trivalent actinide activity levels at Windscale are most probably negligible, and its contribution to the $^{238}Pu$ inventory by subsequent decay through $^{242}Cm$ would appear unimportant.

## 5. Trace element studies

### 5.1 Sensitivity and accuracy of atomic absorption spectrophotometry for trace elements in marine biological samples

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During the course of 1974-75 atomic absorption spectrophotometry (AAS) has been used extensively in our laboratory for measuring various trace elements in marine biological materials in order to conduct homogeneity tests on the intercalibration samples for trace metal analysis as well as to obtain baseline data for trace elements in various kinds of marine organisms collected from different locations in the Mediterranean Sea. Several series of test experiments have been conducted on the current methodology in use in our laboratory to ensure satisfactory analytical performance in measuring a number of trace elements for which analytical problems have not completely been solved. Sensitivities of the techniques used were repeatedly checked for various elements and the accuracy of the analyses were always critically evaluated by analyzing standard reference materials. The results of these test experiments have uncovered critical points relevant to the application of the AAS to routine analysis.

Our instrumentation consists of a Perkin-Elmer atomic absorption spectrophotometer Model 403 equipped with a three slot burner head 303-0401 for flame analysis or a graphite furnace HGA 72 for flameless analysis. A Perkin-Elmer 56 XY recorder is connected directly to the spectrophotometer and all absorbance signals are measured by the peak height technique. The flame technique was applied to trace metals such as Cr, Mn, Fe, Co, Ni, Cu, Zn, Ag, Cd and Pb, while the graphite furnace technique was used to verify the results obtained by the flame technique for Cr, Co, Ag and Pb. A deuterium arc background corrector was used for the graphite furnace technique (Model 303-0295).

For mercury measurements, the digestion procedure and cold vapour technique described by Uthe *et al.*[100] was slightly modified and used in conjunction with a Beckmann DB-G spectrophotometer with the atomic absorption accessories. A 10cm quartz cuvette was used as the absorption cell.

In order to determine the sensitivity of measurements attainable by our system for various trace elements of interest, repeated

measurements were made using solutions of appropriate concentration. The average values of these measurements are presented in Table 30. The

Table 30. Sensitivities of the flame technique and of the graphite furnace technique in atomic absorption spectrophotometry for various trace elements

| Element | Flame technique sensitivity (ng/ml) | Graphite furnace technique sensitivity | |
|---|---|---|---|
| | | (ng) | (ng/ml) |
| Cr | 100 | 0.02 | 2 |
| Mn | 60 | 0.008 | 0.8 |
| Fe | 150 | 0.06 | 6 |
| Co | 150 | 0.08 | 8 |
| Ni | 150 | 0.09 | 9 |
| Cu | 90 | 0.01 | 1 |
| Zn | 20 | 0.002 | 0.2 |
| Ag | 100 | 0.006 | 0.6 |
| Cd | 40 | 0.008 | 0.8 |
| Pb | 500 | 0.04 | 4 |

sensitivity is expressed in terms of the concentrations of the elements giving a reading of 1% absorbance for the flame technique or of the absolute quantities of the elements giving a similar reading for the graphite furnace technique. Although the sensitivities between the two different techniques are not directly comparable, the sensitivities for the graphite furnace technique are also given in terms of specific concentrations for convenient comparison. For these conversions it was assumed that the same volume, 10 μℓ, was always used for each injection into the graphite furnace. By comparing the sensitivities between these two techniques, it can be seen that the graphite furnace technique increases the sensitivity by approximately two orders of magnitude for many of the trace elements listed. However, since the specific atomic absorption of solid samples in the graphite furnace is expected to be influenced by sample matrices, the sensitivity for some elements on real samples may be much lower than that given in the table.

Broad-band non-specific background absorption and/or scattering of the light caused by spattering and fuming of materials in the destruction of sample matrices or by evaporation of solvents pose serious interference in the atomic absorption spectrophotometry especially in solid source atomization, such as that using a graphite tube furnace. A series of measurements were performed using a graphite furnace technique with and

without the deuterium background corrector, to evaluate the magnitude of such effects for two heavy metals, Co and Pb. From 50 to 200mg of a freeze-dried oyster tissue was wet-ashed with 2ml of a mixture of nitric and perchloric acids (3:1 v/v) in a Pyrex glass tube under heating. The initial digestion was carried out at a temperature of approximately 100°C for 30-45 min and then the temperature was increased to 180°C to let the acids evaporate under steady boiling, until no visible liquid was left. The residue was dissolved with 10ml 0.1M HCl. Ten µℓ of the solution was introduced into a graphite tube furnace and the following steps were followed for the step-wise atomization:

Co: drying at 82°C (30 sec); programmed thermo-destruction at 170°C (10 sec), between 170°C and 1020°C (30 sec) and at 1020°C (60 sec); atomization at 2540°C (10 sec).

Pb: drying at 100°C (30 sec); programmed thermo-destruction at 170°C (30 sec) and between 170°C and 420°C (120 sec); atomization at 2040°C (10 sec); cooling at 46°C (20 sec).

For determining Co and Pb concentrations in the sample, the standard addition procedure was adopted and appropriate blanks were run with adequate frequency. Similar measurements were repeated with or without a deuterium arc background corrector.

Absorbances measured without the deuterium background corrector (DBC) in the Co and Pb determinations on the oyster sample are compared schematically with those obtained with the DBC in Figure 18. In this figure, relative absorbances on the standard solutions of Co and Pb are also illustrated. As shown in Figure 18, in spite of the fact that there is no difference between the absorbance observed without the DBC and that with the DBC on the standard solution of either Co or Pb, substantial differences exist between the absorbances measured with and without the DBC on the oyster sample as well as between those on the sample spiked with 100ng of Co or Pb. These differences in the absorbance measured represent the matrix effect, which may lead to gross errors in calculating the concentrations of the elements in the sample. Figures 19 and 20 represent the relationships between the sample weight taken and the resulting absorbance measured as well as standard addition curves, respectively, for Co and Pb based on measurements made by using the DBC. Linear increases of the absorbance with increasing sample weight or with increasing standard addition are demonstrated in these figures for both Co and Pb. However, these linear relationships do not necessarily ensure satisfactory performance of the analytical technique used. From Figure 18, it can be

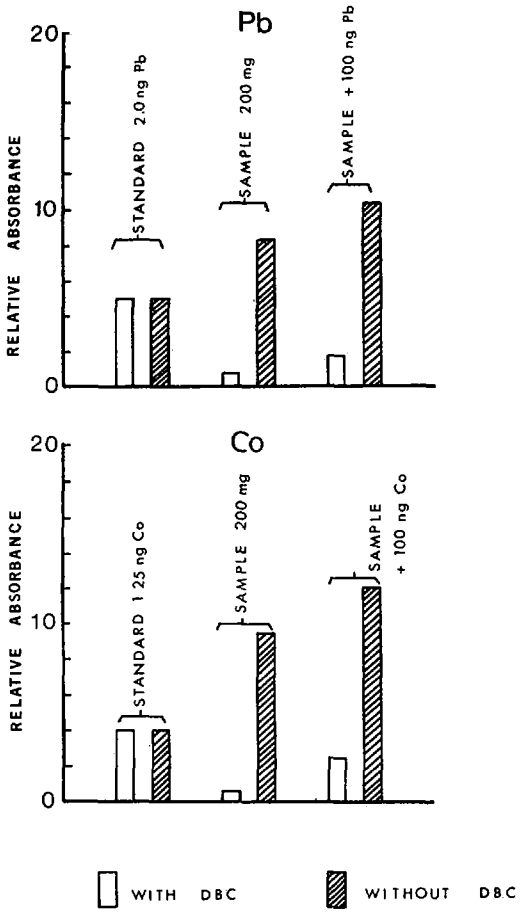


Figure 18. Comparisons between absorbances obtained with a deuterium background corrector (DBC) and without a DBC in the graphite furnace technique.

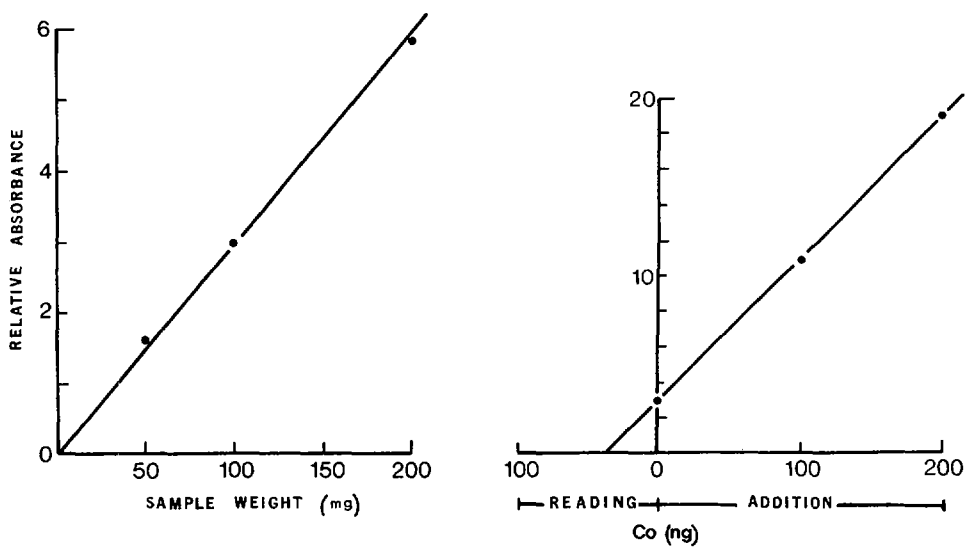


Figure 19. Relationship between the sample weight and absorbance measured, for the oyster sample (MA-M-1) and a standard addition curve for cobalt determination by the graphite furnace technique with a deuterium background corrector.

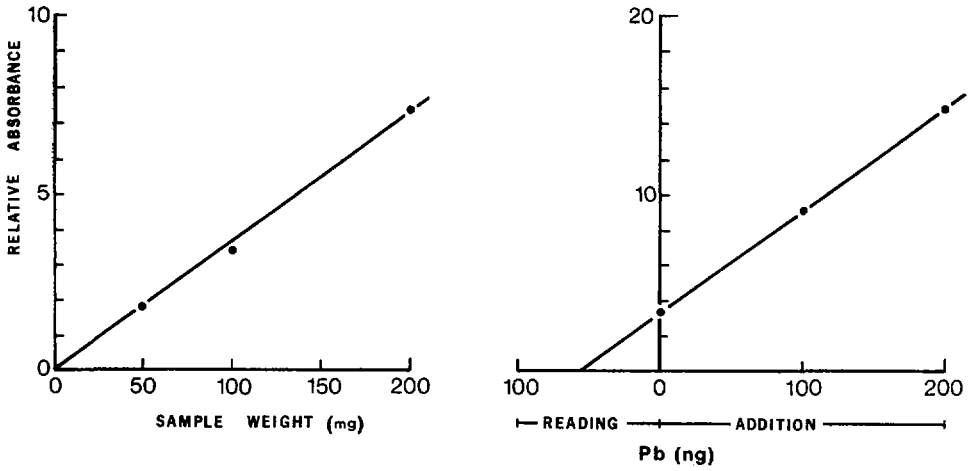


Figure 20. Relationship between the sample weight and absorbance measured for the oyster sample (MA-M-1) and a standard addition curve for lead determination by the graphite furance technique with a deuterium background corrector.

presumed that the absorbances measured without the DBC may also give the linear relationships, when the background absorption is much greater than the specific atomic absorption caused by the atomization of the element of interest. In Table 31, examples of the results obtained by different techniques on the oyster sample and the NBS's Orchard Leave sample are given. As can be seen, the results obtained by the graphite furnace technique without the DBC are consistently higher than those with the DBC for both Co and Pb, and the difference may reach more than one order of magnitude in many cases. It seems that the flame technique produces comparable results

Table 31. Determinations of cobalt and lead in the IAEA oyster sample, MA-M-1, and the NBS orchard leaf sample by various atomic absorption techniques

| Sample | Technique used | Co found (μg/g-dry) | Pb found (μg/g-dry) |
|---|---|---|---|
| Oyster homogenate MA-M-1 | Flame | - | 5.8 ± 0.8 |
| | Graphite furnace without DBC* | 7 ± 2 | 8.1 ± 0.6 |
| | Graphite furnace with DBC* | 0.38 ± 0.03 | 0.61 ± 0.04 |
| NBS Orchard Leave | Flame | 0.25 | 44 |
| | Graphite furnace without DBC* | 5.2 ± 0.2 | 67 ± 6 |
| | Graphite furance with DBC* | 0.21 ± 0.01 | 44.3 ± 0.7 |
| | Certified value by NBC | 0.2 | 45 ± 3 |

* Deuterium arc background corrector

to those obtained with the graphite furnace technique using the DBC, when the Pb content of the samples are sufficiently high or the matrix of the samples allows it to attain higher sensitivity for Co.

Based on these observations, it should be emphasized that for some trace elements the use of a deuterium background corrector is essential to obtain reliable results by atomic absorption spectrophotometry using the graphite furnace technique.

Although the cold vapour atomic absorption technique is extensively utilized for the determination of total and organically-bound mercury in marine biological samples[100-104], controversial observations have been made concerning the possible loss of mercury during the drying step of samples before the analysis, as well as during the wet-ashing step[105-108]. In order to verify the satisfactory recovery of mercury using the technique of Uthe *et al.*[100], a step-by-step examination of the analytical procedure is in progress. As a first step, it was necessary to determine possible loss of mercury in freeze-drying or low-temperature drying. Approximately 200mg freeze-dried or oven-dried (at 50°C) biological material were placed in a 50ml round borosilicate flask, fitted with a 15cm Liebig condenser and digested with 5ml mixture of $H_2SO_4$-$HNO_3$ (4:1 v/v) under heating at 60°C on a hot plate. The digestion was continued until a clear solution was obtained. After cooling, 10ml $KMnO_4$ solution (5% w/v) were slowly added to the flask and the flask was allowed to stand overnight at room temperature. In case manganese dioxide was precipitated during this time, it was dissolved by adding 1ml $NH_2OH \cdot HCl$ solution (10% w/v). Total volume was adjusted to 25ml with 0.1M $HNO_3$. The total volume of the flask was transferred to the reaction cell, which was prewashed with 5ml dil. $HNO_3$. To the solution 3ml $SnCl_2$ solution (10% w/v) were added, the stop cocks of the cell were closed and the contents of the cell were stirred for 2 min. with a magnetic stirrer. Then the stop cocks were opened and the reduced mercury was driven from the solution with $N_2$-gas at a flow-rate of 1 liter/min through an optical cuvette to the exit. Absorbance measurements were made using a wave-length of 253.7µm.

Figure 21 shows the relationship between the absorbance measured and the sample weights used as well as the standard addition curve obtained by using the above-mentioned procedure for the oyster sample. These relationships suggest that the analytical procedure adopted works satisfactorily. However, if the loss of mercury occurred during the drying step or if the loss was linear with respect to the sample weight, linearity would still have been observed.

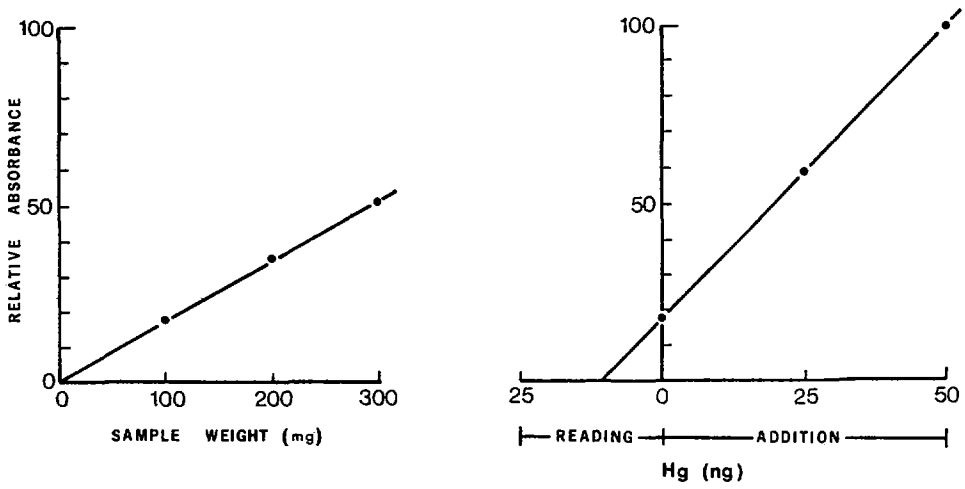


Figure 21. Relationship between sample weight and absorbance measured for the oyster sample (MA-M-1) and a standard addition curve for mercury determination by the cold vapour technique.

Table 32 gives the results obtained using the described procedure in the analysis of the NBS Orchard Leave standard and the oyster sample. Although the results show good agreement with the known values of mercury in these samples, they tend to be low.

Table 32. The results of mercury measurements on the IAEA oyster sample, MA-M-1 and the NBS Orchard Leave sample by the cold vapour atomic absorption spectrophotometry

| Sample | Oyster homogenate | NBS Orchard Leave |
|---|---|---|
| Hg found (µg/g-dry) | 0.130 ± 0.002 | 0.141 ± 0.003 |
| Probable or certified value | (0.16) | 0.155 ± 0.015 |

To investigate possible loss of mercury during drying, a mussel sample was collected from the Monaco coast and analyzed for mercury after freeze-drying as well as after oven-drying at 50°C. The results of these measurements are given in Table 33. There appears to be no difference between the results obtained by the two different drying methods. Since it has already been observed that freeze-drying causes only negligible loss of mercury [108], these results indicate that the oven-drying procedure at 50°C is also acceptable for mercury analysis for marine biological samples. This implies that if there is a systematic loss of mercury in the above-mentioned procedure, it has to be during the wet-ashing procedure. The re-examination of the wet-ashing procedure is now in progress.

Table 33. Comparison of the results of mercury determinations between oven-dried (50°C) and freeze-dried mussels

| Drying method | Sample weight taken* (mg) | Hg found (ng) | Hg concentration (ng/g-dry) |
|---|---|---|---|
| Freeze-drying | 192.7 | 43.8 | 227 |
| | 207.9 | 48.6 | 234 |
| | 172.1 | 43.3 | 251 |
| | 183.6 | 48.1 | 276 |
| | 180.7 | 44.9 | 248 |
| | | | Av. 247 ± 8 |
| Oven drying at 50°C | 205.2 | 46.7 | 228 |
| | 204.1 | 51.2 | 251 |
| | 227.9 | 53.3 | 234 |
| | 179.4 | 50.0 | 279 |
| | | | Av. 248 ± 12 |

* The weight was normalized to that dried at 50°C