

TRACE ELEMENTS IN SEAFOOD – IPEN-CNEN/SP NEUTRON ACTIVATION ANALYSIS LABORATORY PARTICIPATION IN A SIM PILOT STUDY WITH UNCERTAINTY ASSESSMENT

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Abstract – This study presents the Neutron Activation Laboratory (LAN-IPEN) participation in the SIM 8.16P Pilot Study on Toxic Element in Seafood organized by the National Institute of Standards & Technology (NIST) and the “Comisión Chilena de Energía Nuclear” (CCHEN). LAN-IPEN presented results for As, Se and Zn obtained by Instrumental Neutron Activation Analysis and Cd obtained by Electrothermal Atomic Absorption Spectroscopy. Concentration results were submitted to the intercomparison providers with their combined and expanded standard uncertainties. For this SIM pilot study, thirteen laboratories from nine countries determined the toxic elements by five analytical techniques (ICP-MS, AAS, NAA, XFR, and ICP OES). The seafood sample was described as the Antarctic Krill Certified Reference Material (MURST ISS-A2) in the intercomparison report. The comparison of the LAN-IPEN results to the SIM 8.16P reference values leads to the following z-score values: As +0.34; Cd -0.73; Se +0.59 and Zn +0.20, demonstrating the suitability of the methods employed.

Key-words: Interlaboratory comparison, toxic elements, uncertainty assessment.

Introduction

Participation in proficiency testing programs is one of the internationally accepted quality assurance tools in order to a laboratory to produce consistently reliable data [1] being also one requirement for laboratory accreditation [2]. Other important quality assurance tools are validation of analytical methods, use of certified reference materials (CRMs) and the employment of routine internal quality control. In the framework of the Inter-American Metrology System (SIM), it is essential to ensure regional cooperation in metrology in order to obtain result comparability from measurement processes performed in laboratories within the system [3], and hence, SIM proposed the 8.16P Pilot Study on Toxic Element in Seafood, organized by the NIST and CCHEN, for the determination of As, Cd, Pb, Se and Zn.

This study presents the Neutron Activation Laboratory, LAN-IPEN, participation in the SIM 8.16P Pilot Study. LAN-IPEN presented results for As, Se and Zn

obtained by Instrumental Neutron Activation Analysis (INAA) and Cd obtained by Electrothermal Atomic Absorption Spectroscopy (ET AAS) [4]. Concentration results were submitted to the intercomparison providers with their combined and expanded standard uncertainties, whose sources are described in the following sections.

Uncertainty sources in Instrumental Neutron Activation Analysis

The measurand is the concentration C (mg kg^{-1}) of the elements As, Se and Zn in a seafood sample by the relative method of Instrumental Neutron Activation Analysis, INAA.

In the relative method of INAA, where the unknown sample is irradiated simultaneously with standards of the elements of interest, the concentration C is determined by means of the following equation:

$$C = \frac{m A_u e^{\lambda(t_u - t_s)}}{M A_s} \quad (1)$$

where:

m = mass of the element in the standard;

M = mass of the sample;

A_u = activity of the sample;

A_s = activity of the elemental standard;

t_u = sample decay time;

t_s = elemental standard decay time;

λ = decay constant, where $\lambda = \frac{\ln 2}{t_{1/2}}$ and $t_{1/2}$ is the element half life.

The uncertainty sources for the relative method of INAA are shown in the cause and effect diagram in Figure 1 [5-9].

Uncertainty sources in Electrothermal Atomic Absorption Spectroscopy

The measurand is the concentration C (mg kg^{-1}) of Cd in a seafood sample by Electrothermal Atomic Absorption Spectroscopy, ET AAS.

The concentration C is obtained by means of the following equation:

$$C = \frac{A - b}{a} \times \frac{V}{m} \quad (2)$$

where:

A = sample absorbance;

a = calibration curve angular coefficient;

b = calibration curve linear coefficient;

V = volume of the digested sample;

m = sample mass.

The uncertainty sources for the ETAAS method are shown in the cause and effect diagram in Figure 2 [5, 10].

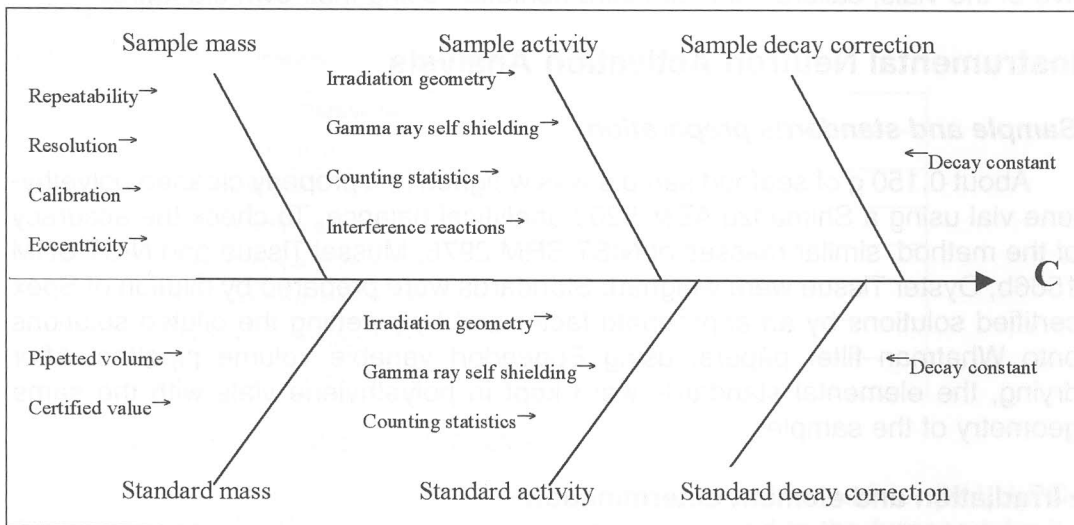


Figure 1 – Uncertainty sources in INAA.

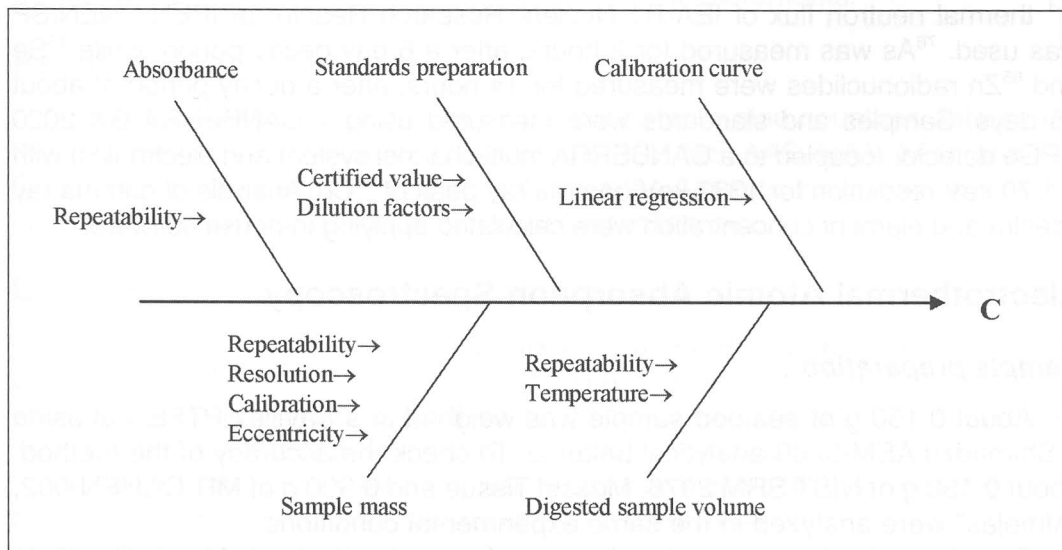


Figure 2 – Uncertainty sources in ET AAS.

Materials and methods

Due to the hygroscopic nature of the test material, each participant was provided with twelve vials of it (each containing approximately 0.5 g) for the determination of As, Cd, Pb, Se and Zn and each vial should be analyzed in its entirety as one sample. The method to be used would be the choice of the participating laboratory, and should reflect normal operating procedures. A complete description of the methods used as well as a description of how the method was calibra-

ted and how the concentrations were calculated should also be provided to the organizer. Participants should have determined the elements of interest in at least five of the vials, calibrating their instrumentation using their own standards.

Instrumental Neutron Activation Analysis

Sample and standards preparation

About 0.150 g of seafood sample was weighed in a properly cleaned polyethylene vial using a Shimadzu AEM-5200 analytical balance. To check the accuracy of the method, similar masses of NIST SRM 2976, Mussel Tissue and NIST SRM 1566b, Oyster Tissue were weighed. Standards were prepared by dilution of Spex certified solutions by an appropriate factor and by pipetting the diluted solutions onto Whatman filter papers, using Eppendorf variable volume pipettes. After drying, the elemental standards were kept in polyethylene vials with the same geometry of the samples.

• Irradiation and element determination

Sample aliquots and reference materials were simultaneously irradiated with elemental standards. For element determination, an 8 hour irradiation at 10^{12} n cm⁻² s⁻¹ thermal neutron flux of IEA-R1 Nuclear Research Reactor at IPEN-CNEN/SP was used. ⁷⁶As was measured for 2 hours, after a 6 day decay period, while ⁷⁵Se and ⁶⁵Zn radionuclides were measured for 14 hours, after a decay period of about 15 days. Samples and standards were measured using a CANBERRA GX 2020 HPGe detector (coupled to a CANBERRA multi-channel system and electronics) with a 1.70 keV resolution for 1332 keV gamma ray peak of ⁶⁰Co. Analysis of gamma ray spectra and element concentration were calculated applying in-house software.

Electrothermal Atomic Absorption Spectroscopy

Sample preparation

About 0.150 g of seafood sample was weighed in a Savillex PTFE vial using a Shimadzu AEM-5200 analytical balance. To check the accuracy of the method, about 0,150 g of NIST SRM 2976, Mussel Tissue and 0.300 g of MR-CCHEN-002, "Almejas" were analyzed in the same experimental conditions.

Sample and reference materials were digested with 4 mL Merck PA 65 % HNO₃ and 1mL Merck PA 30% H₂O₂ in Savillex PTFE vials. Vials were closed and left overnight at room temperature. In the following day, vials were heated at 90 °C for 3 hours in a digestion block. The digests were cooled to room temperature and diluted to a final volume of 25.3 mL with Milli-Q water.

• Standards preparation

A Spex certified standard solution of Cd (1000 ± 3 mg L⁻¹) was diluted accordingly to obtain a 7.0 ng mL⁻¹ stock solution. This solution was further diluted by the AS-800 autosampler for construction of a Cd calibration curve of: 1.4; 4.2 and 7.0 ng mL⁻¹.

• Instrument parameters

The used instrument parameters for the Perkin-Elmer AAnalyst 800 are described in Table 1.

Table 1 – Instrument parameters used in the Cd determination by ETAAS.

Parameter	Cd
Wavelength (nm)	228.8
Slit width (nm)	0.7
Drying temperature 1 (°C)	110
Drying temperature 2 (°C)	130
Pyrolysis temperature (°C)	500
Atomization temperature (°C)	1500
Cleaning temperature (°C)	2400

• Cadmium determination by ET AAS

A 20 μL aliquot of the sample solution and 10 μL of matrix modifier ($\text{NH}_4\text{H}_2\text{PO}_4$ 0.5 % (m/v) and $\text{Mg}(\text{NO}_3)_2$ 0.03 % (m/v)) were introduced to the furnace tube by the autosampler. After the atomization step, the Cd concentration was calculated by the spectrometer software after two replicate measurements.

Results

For this SIM pilot study, thirteen laboratories from nine countries determined the toxic elements by five analytical methods (ICP-MS, AAS, NAA, XFR, and ICP OES). The seafood sample was described as the Antarctic Krill Certified reference material (MURST ISS-A2) in the intercomparison report.

LAN results

For validation of the methods, CRMs were analyzed and except for Zn in the oyster tissue reference material, all the z scores calculated for the obtained results and certified values were lower than 1, indicating that the used methods were appropriate for element determination in seafood samples.

In Table 2 are presented the obtained concentrations for the analyzed vials, the mean, and the combined and expanded uncertainties.

Table 2 – As, Cd, Se and Zn concentrations in seafood samples (mg kg^{-1}).

Element	Vial number					mean	u_c	U^1
	140	773	1258	1442	1449			
As	4.67	4.66	4.12	4.72	4.38	4.51	0.24	0.47
Cd	0.698	0.687	0.680	0.681	0.686	0.686	0.035	0.071
Se	8.08	8.42	7.89	8.13	7.70	8.04	0.24	0.48
Zn	64.3	67.4	69.7	65.5	65.3	66.4	1.1	2.2

¹Expanded uncertainties were calculated using a coverage factor of 2, which gives a level of confidence of approximately 95%;

Quantification of uncertainty components

• Instrumental Neutron Activation Analysis

Contributions of the mass of sample and elemental standards, the decay constants, the irradiation process and the gamma ray spectrometry measurement to the uncertainty in INAA were evaluated. Detailed description of uncertainty evaluation results are presented elsewhere [11].

The standard uncertainties from the relevant sources of uncertainty were combined, using the relative method, yielding the combined standard uncertainty, u_c , for the concentration of As, Se and Zn in seafood. The expanded uncertainty, U , was determined from the combined standard uncertainties, using a coverage factor $k = 2$, which gives a level of confidence of approximately 95%. In Table 3 the contributions to the combined standard uncertainties and expanded uncertainties are summarized. It may be noticed that sample and elemental standards activities uncertainties are the larger contributions to the expanded uncertainty in element determination by INAA.

Table 3 – Combined standard uncertainty, u_c , and expanded uncertainty, U , for the determination of of As, Se and Zn in seafood by INAA (mg kg^{-1}).

Element	Concentration	Standard uncertainty ¹					u_c	U
		u_M	u_m	U_λ	u_{Au}	U_{As}		
As	4.51	6.47×10^{-4}	2.14×10^{-2}	6.26×10^{-4}	0.231	0.038	0.24	0.47
Se	8.04	1.12×10^{-3}	6.40×10^{-2}	2.78×10^{-6}	0.049	0.228	0.24	0.48
Zn	66.4	8.91×10^{-3}	2.39×10^{-1}	3.46×10^{-4}	0.341	1.02	1.1	2.2

¹ u_M , u_m , U_λ , u_{Au} , U_{As} – standard uncertainties for sample mass, mass elemental standard, decay constant, sample activity and standard activity, respectively.

• Electrothermal Atomic Absorption Spectroscopy

The contribution of the sample mass, the volume of digested sample, the absorbance, the working standards preparation and the regression fit of the calibration curve to the uncertainty in ET AAS was evaluated. Detailed description of uncertainty evaluation results are not presented for brevity.

The standard uncertainties from the relevant sources of uncertainty were combined, using the relative method, yielding the combined standard uncertainty, u_c , for the concentration of Cd in seafood. The expanded uncertainty, U , was determined from the combined standard uncertainties, using a coverage factor $k = 2$, which gives a level of confidence of approximately 95%. In Table 4 the contributions to the combined standard uncertainties and expanded uncertainties are summarized. As expected, the calibration curve regression fit was the major contribution to the expanded uncertainty in Cd determination by ET AAS.

Table 4 – Combined standard uncertainty, u_c , and expanded uncertainty, U , for the determination of Cd in seafood by ETAAS.

Contribution	Uncertainty, mg kg^{-1}
Sample mass	1.1×10^{-4}
Volume of digested sample	9.2×10^{-4}
Absorbance	2.68×10^{-3}
Working standards	4.54×10^{-3}
Calibration curve	3.49×10^{-2}
u_c	3.5×10^{-2}
U	7.1×10^{-2}

Interlaboratorial program assessment

For the assessment of the participating laboratories in the SIM 8.16P Pilot Study, the certified values for Cd, Pb, Se and Zn were used as reference values, as presented in Table 5 [12]. In the case of As, the organizers decided to use the mean of four laboratories that successfully participated in a previous CCQM study, including LAN-IPEN, as potential problems were detected in the original certified value for this element, possibly due to a loss over time, even though the starting material was collected in 1995 and the reference material was certified in 2001. The original certified value for As was $(5.02 \pm 0.40) \text{ mg kg}^{-1}$ [13].

Table 5 – Reference values for SIM 8.16P Pilot Study.

Element	Reference Value, mg kg^{-1}	Source
As	4.39 ± 0.35	Mean of four laboratories
Cd	0.73 ± 0.06	Certificate
Pb	1.11 ± 0.09	Certificate
Se	7.73 ± 1.13	Certificate
Zn	66.0 ± 2.0	Certificate

The comparison of the LAN-IPEN results to the reference values leads to the following z-score values: As +0.34; Cd -0.73; Se +0.59 and Zn +0.20, demonstrating the suitability of the methods employed.

The uncertainty values assessed for the INAA and ET AAS methods used in this study were in conformity to those obtained by other participating institutions. The overall participation of LAN-IPEN in the SIM 8.16P Pilot Study may be considered very positive as an estimation of its element determination skills, as presented in the Pilot Study summary report [12].

It is also worth mentioning the interesting quality indicators highlighted by the Pilot Study organizer, that is, laboratories that reported use of matrix CRMs as quality assurance checks and reported all requested information such as the complete description of the analytical methodology, including all relevant equations, a complete uncertainty budget with all potential sources of uncertainty assessed and the expanded uncertainty with its k value, were consistently more successful in reporting appropriate results.

Conclusion

This paper describes the well succeeded LAN-IPEN participation in the SIM 8.16P Pilot Study which was an important contribution to the continuous improvement of LAN-IPEN quality system. The SIM 8.16P Pilot Study was able to identify potential and actual problems in toxic element determination in seafood samples, such as the possible As loss after the certification campaign. The participation in such interlaboratorial programs is essential for assessment and maintenance of quality assurance systems for analytical laboratories.

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References

- [1] THOMPSON, M.; ELLISON, S. L. R.; WOOD, R. (2006) *Pure Appl. Chem.* 78 (1): 145-196.
- [2] ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories.
- [3] <http://www.sim-metrologia.org.br/>
- [4] MOREIRA, E. G.; MAIHARA, V. A.; CATHARINO, M. G. M.; VASCONCELLOS, M. B. A. (2005) Report on NIST-CCHEN SIM 8.16P Pilot Study.
- [5] EURACHEM/CITAC Guide (2000) Quantifying uncertainty in analytical Measurement.
- [6] IAEA, TECDOC-1401 (2004) Quantifying uncertainty in nuclear analytical measurements.
- [7] WEIZHI, T.; BANGFA, N.; PINGSHENG, W.; LEI, C.; YANGMEI, Z. (2001) *Accred. Qual. Assur.* (2001) 6: 488-492.
- [8] GREENBERG, R. R.; LINDSTROM, R. M.; SIMONS, D. S. (2000) *J. Radioal. Nucl. Chem.*, 245 (1): 57-63.
- [9] <http://www.nndc. bnl.gov/wallet/>
- [10] FARRANT, T. J. (1997) *Practical statistics for the analytical scientist – a bench guide.*
- [11] MOREIRA, E. G.; VASCONCELLOS, M. B. A.; SAIKI, M. (2005) *J. Radioal. Nucl. Chem.* 264 (1): 45-50.
- [12] GREENBERG, R. R. SIM 8.16P – Toxic metals in seafood summary report (2005).
- [13] CAROLI, S.; SENOFONTE, O.; CAIMI, S.; PAUWELS, J.; KRAMER, G. N.; ROBOUCH, P. (2001) *J. At. Spectrom.* 16: 1142-1146.