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Expanded uncertainty assessment in fish samples analyzed by INAA and AAS

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ABSTRACT

Measurement uncertainty was estimated following the EURACHEM guide (*Quantifying Uncertainty in Analytical Measurement*) for the elements As, Br, Cl, Co, Cr, Cs, Fe, K, Mg, Mn, Na, Rb, Sb, Se and Zn determined by Instrumental Neutron Activation Analysis (INAA) and for the elements Cd, Hg and Pb determined by Atomic Absorption Spectrometry (AAS) in the fillet of the most consumed fish species at São Paulo city, Brazil. INAA expanded uncertainties ranged from 1.0 to 21% and the main contributions were due the counting statistics of sample and pipetted standards. For AAS, expanded uncertainties ranged from 6.5 to 13% and the main contributions were associated to the calibration curves. Uncertainty results were compared with the standard deviations of the mass fraction in the fishes (*n* = 10). Due to the wide variability in the mass fraction in the analyzed fish tissues, explained by factors such gender, age, fat content and fishing location of the specimens of each species, standard deviations were greater than expanded uncertainties, which means that the natural variability of the elements determined is greater than the data dispersion associated with the analytical techniques. Hence the used techniques were adequate for the fish fillet samples analyses.

Keywords: expanded uncertainty, fish samples, Neutron Activation Analysis, Atomic Absorption Spectrometry.

1. **INTRODUCTION**

Measurement uncertainty is defined such as a non-negative parameter that characterizes the dispersion of the quantity values being attributed to a measurand, based on the information used [1]. The uncertainty was calculated following the steps of EURACHEM guide: specify measurand, identify uncertainty sources, quantify uncertainty components, and calculate combined uncertainty [2].

1.1. **Specify measurand**

According to the EURACHEM guide, in many cases in chemical analysis, the measurand is the concentration of an analyte [2]. In this study, the measurand was the mass fraction of As, Br, Cd, Cl, Co, Cr, Cs, Fe, Hg, K, Mg, Mn, Na, Pb, Rb, Sb, Se and Zn determined by short and long term Instrumental Neutron Activation Analysis (INAA) [3], Cold Vapor Atomic Absorption Spectrometry (CV AAS) and Electrothermal Atomic Absorption Spectrometry (ET AAS) [4] in the fillet of two fish species: bluefish – *Pomatomus saltatrix* (Linnaeus, 1766) and Lebranche mullet – *Mugil liza* (Valenciennes, 1836). The former is known in Portuguese as *anchova* and the later as *tainha*.

The relationship among the measurand and the input quantities is given by a mathematical model. For AAS, according to the Lambert-Beer law, there are ranges of concentration in which the concentration varies linearly with the absorbance [4, 5]. Therefore, being a linear function, it is expressed by Equation 1:

$$A = mx + c \tag{1}$$

Where absorbance (A) is the dependent variable, concentration (x) is the independent variable, (m) and (c) are the angular and linear coefficients of the calibration curve, respectively. However, to obtain the mass fraction (mass/mass) from concentration (mass/volume), the treatment described in Equation 2 was necessary:

$$F = \frac{(A - c)}{m} \frac{V}{M} \tag{2}$$

In which (F) is the mass fraction and (V) the final volume of the digested mass sample (M).

For the comparative INAA, *F* is given by the Equation 3 [6]:

$$C_s = \frac{A_s m_c C_c}{A_c m_s} e^{-\lambda \Delta t}$$ (3)

In which the index "*s*" and "*c*" corresponding to sample and comparator, respectively, of activity (*A*), mass (*m*) and element mass fraction in the comparator (C_c). The decay constant (λ) is the ratio of ln(2) by half-life of the radionuclide, and the term *Δt* is the difference between the beginning to the counting of the comparator and sample, taken into account the time from the end of irradiation and the start of counting [$t_e(c) - t_e(s)$] [6]. In this study, the comparators used were pipetted standards, in which the term m_s has a value equal to 1.

1.2. Identify uncertainty sources

The uncertainty sources of this study were identified and presented in a cause and effect diagram (also known as Ishikawa Diagram). The diagram allows: (i) visualization of the influence of uncertainties on measurand, (ii) grouping together of components whose combined effect can be evaluated and (iii) avoids double counting of sources [2]. The main sources of uncertainty associated with the input quantities of AAS and INAA are shown in Figure 1 and Figure 2, respectively.

Figure 1: *Ishikawa Diagram for AAS uncertainty sources.*
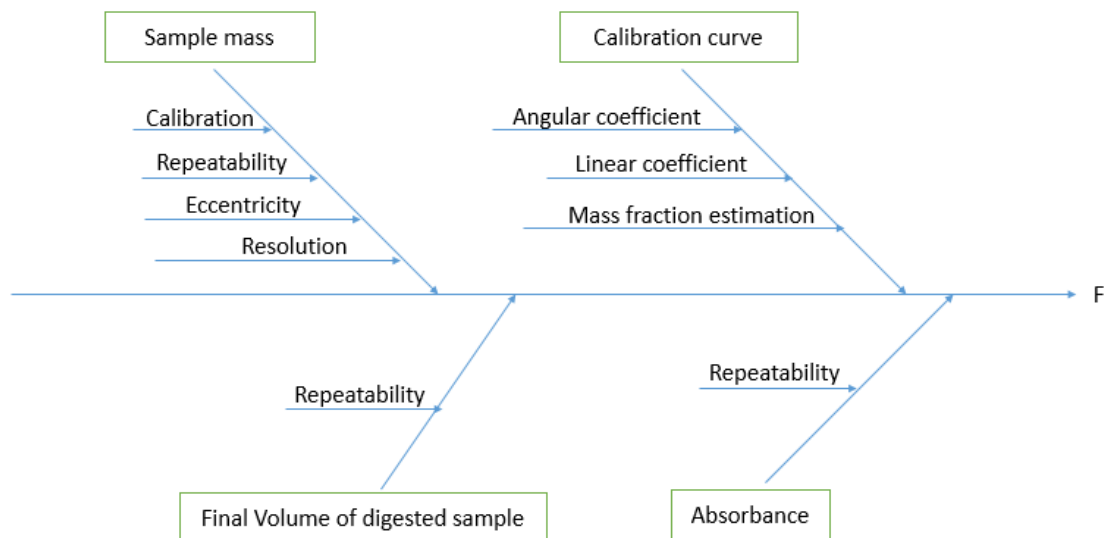
Figure 2: *Ishikawa Diagram for INAA sources – comparative method.*
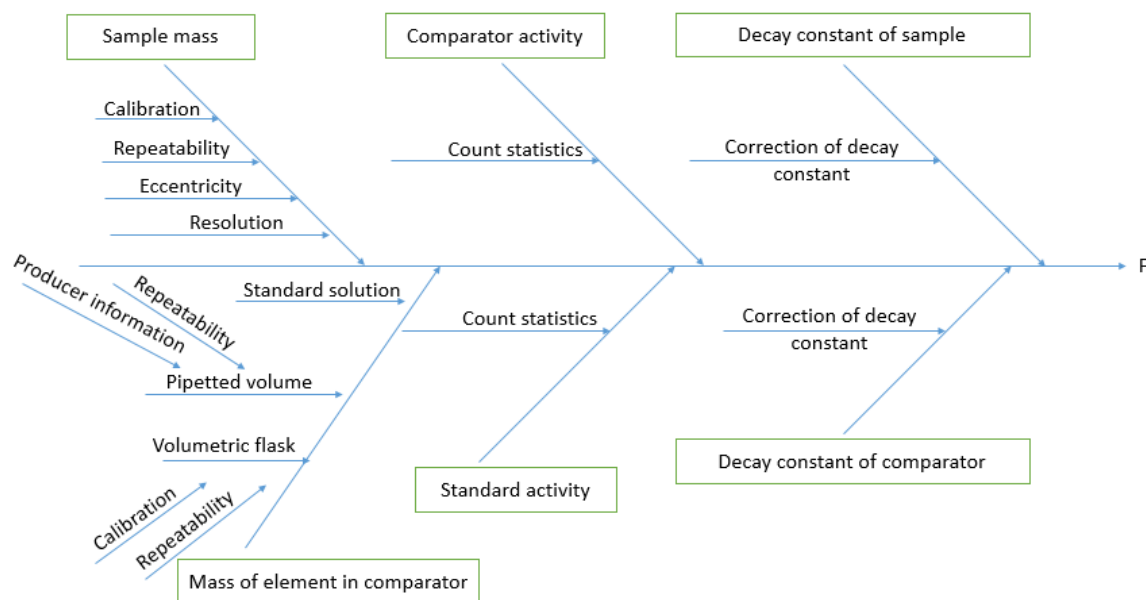
2. EXPERIMENTAL

2.1. Quantify uncertainty components

To express the uncertainties associated with the input quantities as standard uncertainty, it was necessary to classify them in Type A, calculated from statistical distribution of the quantified values from series of measurements, that can be characterized by a standard deviation; or in Type B, evaluated from probability density functions based on experience or other information (e.g. producer catalog or the expanded uncertainty of standard solution) that can also be characterized by standard deviation [2]. Table 1 shows the conversion of input quantities of AAS into standard uncertainties.

Type B uncertainties associated with the sample mass: resolution, eccentricity and calibration were obtained from the balance calibration certificate. The repeatability was obtained from a series of weightings of a standard weight on the balance ($n = 10$). To the final volume, the uncertainty was

obtained by internal calibration of the volumetric flask ($n = 10$), which provided a standard deviation that was considered.

Table 1: Uncertainties sources of AAS and their expression into standard uncertainty.

Uncertainty sources in AAS				
Input quantity	Uncertainty associated	Distribution	Quantification of the standard deviation	
			Uncertainty factor	Type (A or B)
Sample mass	Repeatability	Normal	$1/\sqrt{n}$	A
	Resolution	Rectangular	$1/\sqrt{3}$	B
	Calibration	Normal	$1/k$	B
	Eccentricity	Rectangular	$1/\sqrt{3}$	B
Final Volume	Repeatability	Normal	$1/\sqrt{n}$	A
Absorbance	Repeatability	Normal	$1/\sqrt{n}$	A
Calibration curve	angular coefficient	Normal	$1/\sqrt{n}$	A
	linear coefficient	Normal	$1/\sqrt{n}$	A
	Mass fraction interpolation	Normal	$1/\sqrt{n}$	A

The uncertainty associated with the absorbance is the standard deviation ($n = 2$) between the measurements, performed by the software WinLab 32 for AAS, version 6.2.0079 of the spectrometer. To the uncertainties associated with the calibration curve, the standard deviations of the angular and linear coefficients were obtained by ordinary least squares regression, using the software Past 3.

To calculate the standard deviation associated to the mass fraction predicted by interpolation ($\hat{x}$), Equation 4 was used [7]:

$$DP(\hat{x}) = \frac{rsd}{m} \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{(\bar{y}_0 - \bar{y})^2}{m^2((\sum_{i=1}^{n}(x_i x_i) - (\sum_{i=1}^{n} xi)^2/n))}} \qquad (4)$$

In which (n) is the number of standard solutions in the calibration curve, (N) the number of replicates of the absorbance measurement, ($\bar{y}_0$) absorbance mean ($\bar{y}$) of the calibration curve and *rsd* defined by the Equation 5 [7]:

$$rsd = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_\iota)^2}{n-2}} \qquad (5)$$

Where (y_i) is the absorbance mean for standard solution *i* of the analytical curve and ($\hat{y}_i$) the predict absorbance for the standard solution *i* of the analytical curve.

Input uncertainties were converted into standard uncertainties through the product between input uncertainties by the factor of uncertainty, taking into account their probability density functions.

Regarding to the INAA – comparative method– the input uncertainties used in this study are showed in the Table 2.

Input uncertainties associated with pipette volumes were calculated through internal calibration (analyst) and from information obtained from the producer catalog. To elements where dilution was required (e.g. Cs that has a target nuclide with high thermal neutron cross section), both uncertainties – in the volumes used to pipet the solutions into the volumetric flask and the volume used to pipet the volumetric flask solution in the filter paper were taken into account.. Besides, uncertainties obtained from the calibration certificate and by internal calibrations of volumetric flasks were also considered.

Combined standard uncertainties of standard solutions were obtained by the ratio of their expanded uncertainties with the respective coverage factors (k). Uncertainties associated with sample mass were obtained in an analogous form to those obtained for the AAS technique. Counting statistics uncertainties were taken as the square root of the measured activity, as it follows the Poison Distribution [8]. Radionuclide half-life uncertainties were obtained from a half-life database [9].

Table 2: Uncertainties sources of INAA and their expression into standard uncertainty.

Uncertainties sources in INAA				
			Quantification of the standard deviation	
Input quantity	**Uncertainty associated**	**Distribution**	**Uncertainty factor**	**Type (A or B)**
Element mass in comparator	SD pipettor (analyst)	Normal	$1/\sqrt{n}$	A
	SD pipettor (producer)	Rectangular	$1/\sqrt{3}$	B
	Volumetric flask (catalog)	Normal	$1/k$	B
	Expanded uncertainty of standard solution	Normal	$1/k$	B
Comparator activity	Count statistics	Poisson distribution	-	A
Decay constant	Correction of decay constant	Rectangular	$1/\sqrt{3}$	B
Sample Mass	Repeatability	Normal	$1/\sqrt{n}$	A
	Resolution	Rectangular	$1/\sqrt{3}$	B
	Calibration	Normal	$1/\sqrt{n}$	A
	Eccentricity	Rectangular	$1/\sqrt{3}$	B
Sample activity	Count statistics	Poisson	-	A

distribution

2.2. Calculate combined uncertainties

The calculation of the combined uncertainty was performed using the Kragten spreadsheet method, as indicated by the EURACHEM Guide. Expanded uncertainties were obtained by means of the combined uncertainties with the coverage factor (k) = 2, which provides approximate level of confidence of 95%. Also, expanded uncertainties were presented as percentage of the mass fractions [2].

3. RESULTS AND DISCUSSION

Estimated expanded uncertainties (U, %) and experimental relative standard deviations (RSD, %) of macro, micro and toxic elements in the fillet of some of the most consumed fish species at São Paulo city are presented in Table 3.

As a general trend for the expanded uncertainties obtained, the smaller the mass fraction of the element (e.g. Sb – µg kg^{-1}) the higher the expanded uncertainty, and the inverse is also true (e.g. Na – g kg^{-1}), therefore, the order of magnitude of the element is roughly inversely proportional to the expanded uncertainty. Figure 3 shows that while uncertainties were < 5% at g kg^{-1} mass fraction level, they were < 10% for most elements at mg kg^{-1} level and < 15% for most elements at µg kg^{-1}. When expanded uncertainties are compared to relative standard deviations, RSD is remarkably higher, especially for inorganic contaminants. This is due to the nature of the matrix analyzed is this study, since the mass fraction of contaminants of each specimen depend of the element level in the environment, fat content, size, age, contaminant type and exposure time [10, 11].

Figure 3: *Expanded uncertainty behavior by concentration range.*
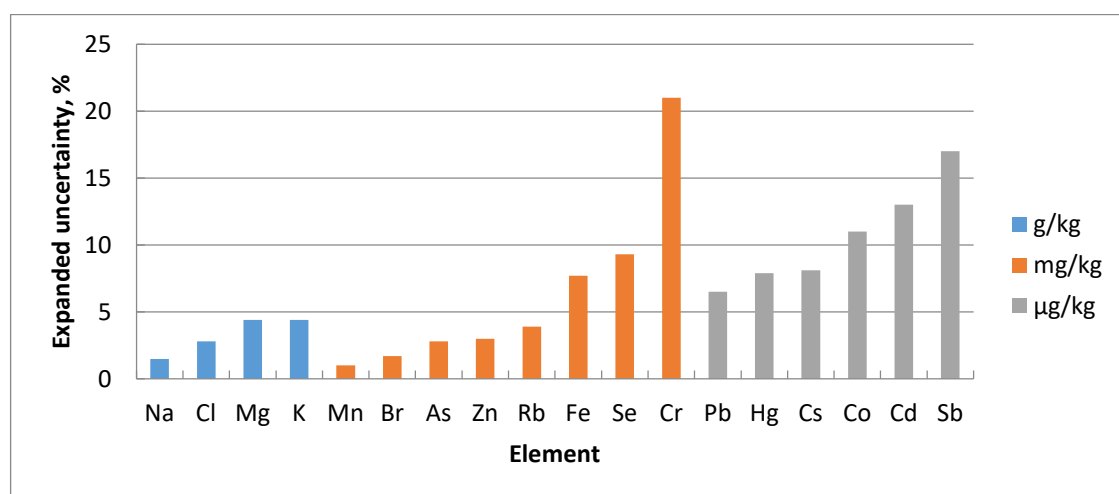
Table 3: Expanded uncertainty obtained by INAA and AAS (dry weight, $k = 2$, $n = 10$).

Mass Fraction	Element	U, %	RSD, %		Technique
			Bluefish	**Lebranche Mullet**	
g kg^{-1}	Cl	2.8	14	34	Short-term INAA
	K	4.4	13	18	Short-term INAA
	Mg	4.4	10	11	Short-term INAA
	Na	1.5	18	28	Short-term INAA
mg kg^{-1}	As	2.8	21	42	Long-term INAA
	Br	1.7	12	37	Long-term INAA
	Cr	21	< 0.11	50	Long-term INAA
	Fe	7.7	21	40	Long-term INAA
	Mn	1.0	30	50	Short-term INAA
	Rb	3.9	17	33	Long-term INAA
	Se	9.3	5.1	21	Long-term INAA

|				
	Zn	3.0	16	22	Long-term INAA
	Cd	13	< 2.6	61	ET AAS
	Co	11	40	26	Long-term INAA
$\mu g\ kg^{-1}$	Hg	7.9	26	28	CV AAS
	Cs	8.1	17	12	Long-term INAA
	Pb	6.5	< 86	< 86	ET AAS
	Sb	17	43	< 0.92	Long-term INAA

< less than quantification limit.

The Kragten spreadsheet method used allowed to evaluate how each standard uncertainty component contributed to the overall expanded uncertainty. Figure 4 shows typical expanded uncertainty weights to the AAS technique used in this study. The input quantities that most contribute to the expanded uncertainty are derived from calibration curve, while uncertainties associated with the balance and dilution were practically negligible. Figure 5 presents typical expanded uncertainty weights to the INAA technique, which had as main contribution to the expanded uncertainty the count statistics of sample and comparators (pipetted standards). Uncertainties associated to element mass in comparators and sample weighting had a minor contribution to expanded uncertainty as expected.

Assessment of the expanded uncertainty is indispensable, especially in studies in which levels of contaminants must be within specific limits, as some elements reported in this study such as, Cd, Pb or Hg. It is crucial to calculate the expanded uncertainty to verify if any mean value that is below legal limits may become higher when the uncertainty is taken into account [2].

Figure 4: *Contribution of standard uncertainties to expanded uncertainty in AAS.*
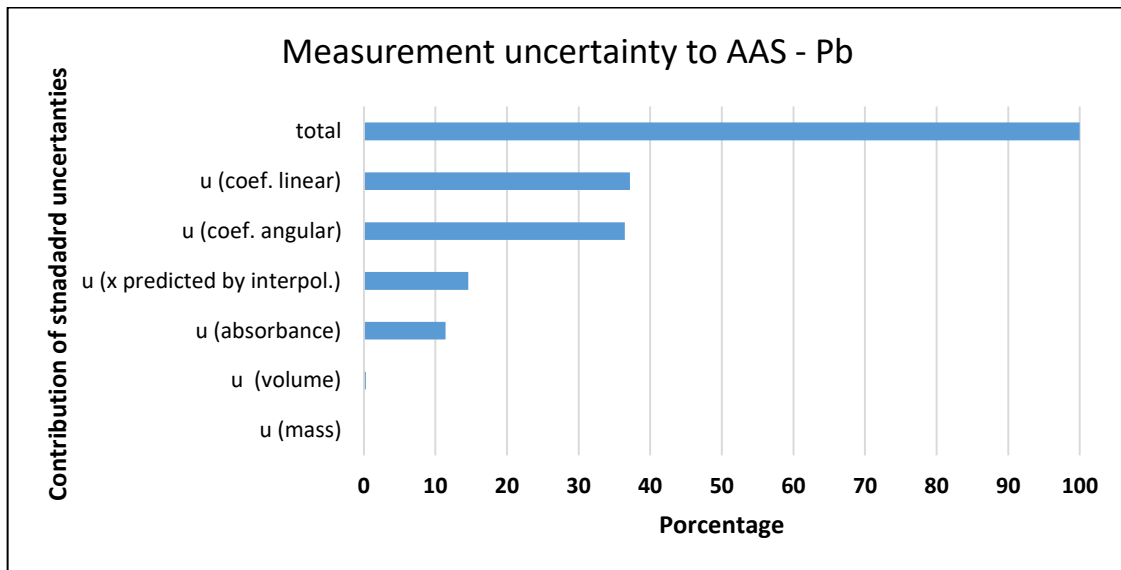
Figure 5: *Contribution of standard uncertainties to expanded uncertainty in INAA.*
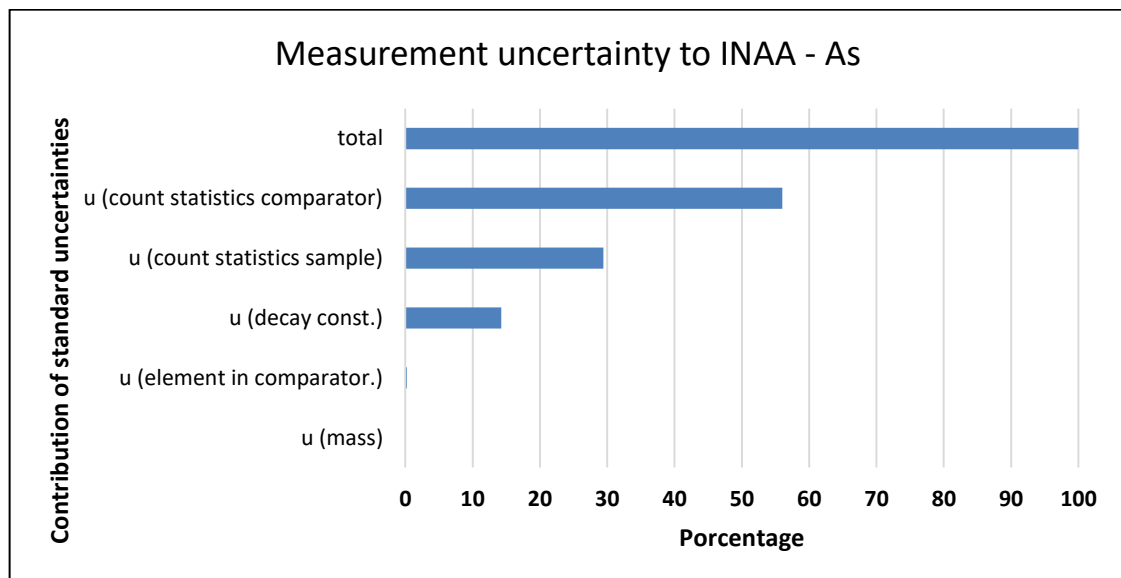
4. **CONCLUSION**

In this study, the expanded uncertainty of measurement calculated following the EURACHEM guide, to micro, macro and toxic elements in fish fillet determined by two analytical techniques – AAS and INAA was evaluated, being below 15% for most elements. In the future, food safety assessment will be carried out for the elements that had the uncertainty calculated in this study, and the uncertainty will be fundamental in assessing compliance with legal limits.

ACKNOWLEDGMENT

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