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Article

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Interlaboratory Comparison for the Characterization of a Brazilian Mussel Reference Material

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Bivalve mollusks have long been used in biomonitoring studies to assess seawater environmental status. The use of suitable certified reference materials is a straightforward means to attain quality assurance in such studies as they allow the traceability of measurement results, making it possible the comparison of data obtained at different places and periods of time. This paper describes the certification of element mass fractions for a *Perna perna* mussel reference material produced in Brazil. After homogeneity and stability studies, an interlaboratory comparison was performed for element characterization. Robust means were taken as assigned values and contributions due to element characterization and from homogeneity and stability studies were taken into account to yield expanded uncertainties. From the adopted certification criteria, informative values for 20 elements were obtained and it was possible to assign certified values for the following 16 elements: Al, As, Ca, Cd, Cl, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Se, Th and Zn.

Keywords: reference material, mussel, *Perna perna*, interlaboratory comparison, value assignment

Introduction

The use of bivalve mollusks such as mussels and oysters has long been established in the assessment of the environmental status of seawaters by means of biomonitoring studies.[1] However, the use of properly validated methods and the implementation of measurement traceability links are fundamental for proper evaluation of such studies and for the comparability of analytical results obtained at different places and periods of time.[2,3] The use of certified reference materials (CRM) with matrix similar to the matrix of the analyzed samples is a straightforward mean to attain quality assurance in analytical laboratories, i.e., to obtain analytical results with accuracy that is fit for its intended use.[4,5]

Reference materials (RMs) and certified reference materials (CRMs) play a fundamental role in quality assurance of analytical laboratories as they are used for calibration purposes, measurement procedure validation and in realization of the metrological traceability chain of measurement results. A CRM is defined as a reference material accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated measurement uncertainties and traceabilities, using valid procedures.[6] An RM is defined as a material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.[6] The production of CRMs and RMs for trace elements in biological matrix is a complex task which must assure that the material will be sufficiently homogeneous for the intended use and remain stable during its shelf life.

Interlaboratory comparisons are one of the recommended approaches used for property value assignment and are based on the premise that there is a population of laboratories or methods equally able to determine a property value with acceptable accuracy and that any differences observed among the measurement results are in principle random.[7]

This paper deals with the certification process for a *Perna perna* mussel reference material produced in Brazil by means of an interlaboratory comparison. The material was designed as a quality assurance tool for element biomonitoring studies along the Brazilian seashore. One important aspect of the certification process is that assigned values for most of the elements were obtained using instrumental neutron activation analysis (INAA),

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a method able to produce results with high metrological quality, as they are completely described and understood from the physical and chemical points of view, and hence, with similar metrological properties to the primary methods recognized by the Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM).[8]

For the estimation of the consensus values for the interlaboratory comparison, a robust mean approach was used. The Huber robust mean (H15) and its standard deviation (s_{H15}) were calculated[9,10] and H15 was taken as the estimate of the mass fraction while $s_{H15}/\sqrt{n'}$ was taken as the estimation of the standard uncertainty due to the characterization (u_{char}). The term n' is the number of result sets used, usually two *per* laboratory, which corresponds to the results obtained in one laboratory for two bottles of the mussel reference material.

Element mass fraction values were assigned to the mussel reference material taking in account not only u_{char} but also the standard uncertainty contributions due to the between-bottle homogeneity (u_{bb}); the long-term stability (u_{lts}) and short-term stability (u_{sts}) to the mass fraction combined standard uncertainties (u_{CRM}), which are combined to yield the expanded uncertainty associated to the mass fraction value (U_{CRM}), according to equation 1. This complies with ISO Guide 35 guidelines to the certification of reference materials:[7]

$$U_{CRM} = k \times \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2} \quad (1)$$

where, k is the coverage factor, usually taken as k = 2.

The assessment of the uncertainty components was performed on data of the homogeneity and stability studies which were previously carried out on the mussel candidate reference material.[11,12]

Experimental

Preparation of the material

For the preparation of the Brazilian mussel reference material, 164 kg of *Perna perna* (Linnaeus, 1758) mussels were purchased from a single producer, from Cocanha Beach in Caraguatatuba City, São Paulo State North Shore where mussels are cultivated by the longline system. Only individuals with commercial size were used (6.7 ± 0.5 cm long and 5.5 ± 0.4 cm wide). The mussels were frozen and taken to the laboratory for further processing. Portions of the mussels were allowed to thaw at room temperature and, after pre-cleaning, the shells were removed and the soft tissues were homogenized in a domestic blender adapted with titanium blades. This process yielded approximately 36 kg of material which was freeze-dried in a Thermo Savant Modulyo D freeze-dryer, resulting in 5.4 kg of freeze-dried material. The material was grinded in the blender and the portion with particle size above 105 µm was discarded. The resulting powder (2.4 kg) was freeze-dried once again and it was homogenized for 72 h in a Y-type Marconi MA 201 homogenizer with polytetrafluoroethylene coated bowl. Then the bulk material was bottled in 171 bottles with approximately 13 g each. Afterwards the material was irradiated with a gamma ray dose of 5 kGy to enhance its stability. The moisture content of the material was estimated as 3.17 ± 0.21%, by means of isothermal curves at 85 °C, using a Shimadzu TGA-50 thermogravimetric analyzer. A Gaussian particle size distribution was observed for the material, with mean particle size $\mu = 94.6 \pm 0.8$ µm, characterized by laser diffraction technique using a CILAS 1064L particle size analyzer.

Homogeneity and stability studies

In order to assess the homogeneity and stability properties of the material and for the estimation of the homogeneity and stability components of the combined uncertainty, two analytical techniques were used: INAA for the determination of Ag, As, Br, Co, Cr, Cs, Eu, Fe, La, Na, Rb, Sc, Se, Th, and Zn; and atomic absorption spectrometry (AAS), for the determination of Cd, Hg and Pb. For the other elements, which no homogeneity or stability studies were available, u_{bb} and u_{lts} were estimated as the mean relative uncertainty values obtained for the elements determined in the homogeneity and stability studies.

Instrumental neutron activation analysis

Subsamples of the mussel reference material, with approximately 150 mg, were weighed in properly cleaned polyethylene vials using a Shimadzu AEM-5200 analytical balance. Elemental standards with the same geometry of the samples were prepared by pipetting Spex standard element solutions onto Whatman paper filters, using variable volume pipettes (Eppendorf). Subsamples and elemental standards were irradiated simultaneously for 8 h at 10^{12} cm^{-2} s^{-1} thermal neutron flux of the IEA-R1 Nuclear Research Reactor at IPEN-CNEN/SP and the radionuclides ^{110m}Ag, ^{76}As, ^{82}Br, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{152}Eu, ^{59}Fe, ^{140}La, ^{24}Na, ^{233}Pa (for Th determination), ^{86}Rb, ^{46}Sc, ^{75}Se and ^{65}Zn were measured after appropriate decay periods, using suitable gamma ray decay photopeaks. Gamma ray measurements were performed using a GC2018 Canberra high purity germanium detector coupled to a Canberra DSA-1000 multichannel analyzer. Gamma ray spectra were collected and processed using a Canberra Genie 2000 version 3.1 spectroscopy software. Element mass fraction calculations were carried out using a Microsoft Excel spreadsheet.

Atomic absorption spectrometry

Subsamples of the mussel reference material, with approximately 300 mg, were weighed in perfluoralcoxi vessels (Savillex). Afterwards, 4 mL of concentrated HNO_3 (Merck) and 1 mL of 30% (v v^{-1}) H_2O_2 (Merck) were added to the vessels, which were closed and left overnight at room temperature. Then, samples were digested in a digestion block (Tecnal) for 3 h at 90 °C. Digests were allowed to cool to room temperature and were diluted with high purity water (Milli-Q) up to 25 mL. Reagent blanks were analyzed in the same manner as the subsamples.

For Cd and Pb determination by means of electrothermal atomic absorption spectrometry (ET AAS), a PerkinElmer AAnalyst 800 atomic absorption spectrometer equipped with Zeeman background correction and electrodeless discharge lamps (EDL) was used. For analysis, 20 µL of the diluted digests were taken by the AS-800 autosampler. As matrix modifier, 10 µL of $NH_4H_2PO_4$ 0.5% (m v^{-1}) and $Mg(NO_3)_2$ 0.03% (m v^{-1}) (PerkinElmer) were also introduced to the furnace tube by the autosampler for both elements. For each determination, analyses were performed in duplicate by the spectrometer.

For Hg determination by means of cold vapor atomic absorption spectrometry (CV AAS), a PerkinElmer flow injection mercury system (FIMS) was used. A 3% (m v^{-1}) HCl (Merck) solution was used as sample carrier while a 3% (m v^{-1}) $SnCl_2$ (Merck) solution was used as reductant reagent.

Certified standard solutions (Spex) were diluted in order to obtain suitable calibration curves for Cd, Pb and Hg. Cd and Pb standards were diluted by the autosampler, while Hg standards were diluted using pipettes (Eppendorf). Hg standards were submitted to the same digestion procedure as samples. Calibration curve coefficients were obtained by a linear regression fit with least-squares method performed by the spectrometer software.

Standard uncertainty due to between-bottle homogeneity

A random stratified scheme was applied for the selection of six bottles of the mussel candidate reference material for the between-bottle homogeneity study.[11] Eight subsamples from each bottle were weighed and randomized prior to element determination by INAA and AAS, depending on the element. This procedure was necessary in order to avoid interferences from any possible trends that might arise in the results during the measurement campaign.

The u_{bb} was estimated from the output of the one-way analysis of variance (ANOVA) tests performed in the homogeneity study according to equation 2.[7]

$$u_{bb} = \sqrt{\frac{MS_{between} - MS_{within}}{n_0}} \qquad (2)$$

where, $MS_{between}$ is the between-bottle mean square estimate from the homogeneity ANOVA test; MS_{within} is the within-bottle mean square estimate from the homogeneity ANOVA test; n_0 is the corrected number of results of the ANOVA test in the case of missing values.

For the elements that u_{bb} were less than the estimated repeatability standard deviation of the method (s_r), equation 3 was used for u_{bb} estimation.[13]

$$u_{bb} = \sqrt{\frac{MS_{within}}{n}} \times \sqrt[4]{\frac{2}{\nu_{MSwithin}}} \qquad (3)$$

where, n is the number of results of the ANOVA test; $\nu_{MSwithin}$ is the number of degrees of freedom for MS_{within}.

Standard uncertainty due to long-term stability

In order to implement the long-term stability study using an isochronous design, six bottles of the mussel candidate reference material were randomly selected and kept at 20 °C for various time periods up to 12 months.[12] After the test periods, element mass fractions were determined in four subsamples of each bottle by INAA and AAS, depending on the element, using a randomized order of analysis. Obtained results were compared to the ones obtained for a bottle kept at the control temperature (–20 °C). This temperature was chosen as the control temperature for the test, since biological reference materials are usually stable for element content at this temperature. According to ISO Guide 35 recommendations, u_{lts} was estimated from the uncertainty in the angular coefficient of the linear regression curve of mass fraction as a function of time from the long-term stability study, considering a two-year shelf life period.[7] The uncertainty in the angular coefficient is obtained from the output of the ANOVA test performed for the linear regression. In this study u_{sts} was considered negligible as the material proved to be stable for normal transport conditions in the short-term stability study performed.

Interlaboratory comparison layout

The interlaboratory comparison for the characterization of the candidate mussel reference material was organized following international recommendations for the organization and control of interlaboratory programs.[7,14,15] A formal invitation letter was sent to laboratories with large experience in trace element determination in biological matrix materials. Seventeen laboratories from eight countries (Argentina, Brazil, Belgium, Chile, Hungary, Peru, Portugal and Malaysia) accepted the invitation. Two of them were from universities whereas the others belonged to state run research institutes. The laboratories were requested to present independent element mass fraction results in dry mass basis for six subsamples taken from each of two mussel reference material bottles and on one quality assurance sample of their choice. The laboratories were also allowed to select which elements to determine. Details on the measurement procedures and on dry mass determination on a separate subsample were also requested. Measurement uncertainty estimation was not mandatory but was considered very welcome to the program.

Results and Discussion

Homogeneity and stability studies

From the ANOVA tests performed on the mean values obtained by INAA and AAS for the elements in each bottle at the between-bottle homogeneity study, there was no evidence to reject the hypothesis that the bottles of the mussel reference material are homogeneous for the analyzed elements, within the 95% confidence interval. From the within bottle-homogeneity study, the minimum sample intake was estimated as 50 mg for most of the elements.[11]

With the application of the INAA and AAS methods to the isochronous long-term stability study, it was possible to confirm, on statistical grounds, that there were no systematic changes in mass fraction for 18 elements during the time period of this study. Thus it was concluded that the candidate mussel reference material is stable enough to be stored at room temperature, without any significant changes in mass fraction for the determined elements.[11] From the isochronous short-time stability test it was possible to confirm that there was no systematic changes in concentration for 18 elements during the time period of the study. It was concluded that the candidate mussel reference material is stable enough to be transported under normal transport conditions without any significant changes in composition for the determined elements.[12]

Interlaboratory comparison results

The interlaboratory comparison received information on 36 elements. Twelve laboratories used solid sampling techniques, such as neutron activation analysis (NAA), X-ray fluorescence (XRF) and alpha spectrometry (α-ESP); five laboratories used atomic absorption spectrometry (AAS); while seven laboratories used inductively coupled plasma optical emission or mass spectrometry (ICP OES or ICP-MS). Some of the laboratories did not report all the six subsample results for the two bottles as requested, but their results were considered. The identities of the participating laboratories are not informed due to the confidentiality criteria adopted.

In this study, for the estimation of consensus values for the interlaboratory comparison, recommendations from the International Harmonized Protocol for Proficiency Testing of Analytical Chemistry Laboratories were followed.[9] The Huber robust mean (H15) and its standard deviation (s_{H15}) were calculated. H15 was taken as the estimate of the mass fraction and the standard uncertainty due to the characterization (u_{char}) was estimated from s_{H15}.

Prior to robust mean estimation of the mass fractions, some results of the interlaboratory comparison were identified as invalid and were excluded from the data set, following Recommendation 1 of the IUPAC protocol, i.e., results outside the range of ± 50% of the median, were excluded.[9] It was concluded that the methods used by these laboratories must be better validated before participation on characterization programs. It should be emphasized that only obviously suspicious results, with large deviation from the rest of the data sets, were eliminated as robust statistics was used in the assignment of the consensus values.

For selected elements, Figure 1 presents the mean results and confidence intervals for data provided by the interlaboratory comparison participants in graphical form as well as the robust mean values of H15 and expanded standard uncertainty associated with the characterization, with a coverage factor $k = 2$. Robust estimates of the consensus values for mass fractions are in good agreement with the distribution of the interlaboratory comparison data, even though extreme laboratory values were observed for many elements, such as the observed for Se and Zn, suggesting that the robust mean method used was adequate for value assignment. For elements with reduced number of reported results, such as Cd, there is less confidence in the consensus value estimates.

Mass fraction values assignment

Table 1 presents the robust mass fraction estimates obtained in the interlaboratory comparison for the characterization of the mussel reference material.

Robust means were estimated from the individual results presented by the laboratories. Table 1 also presents the standard uncertainty contributions due to the

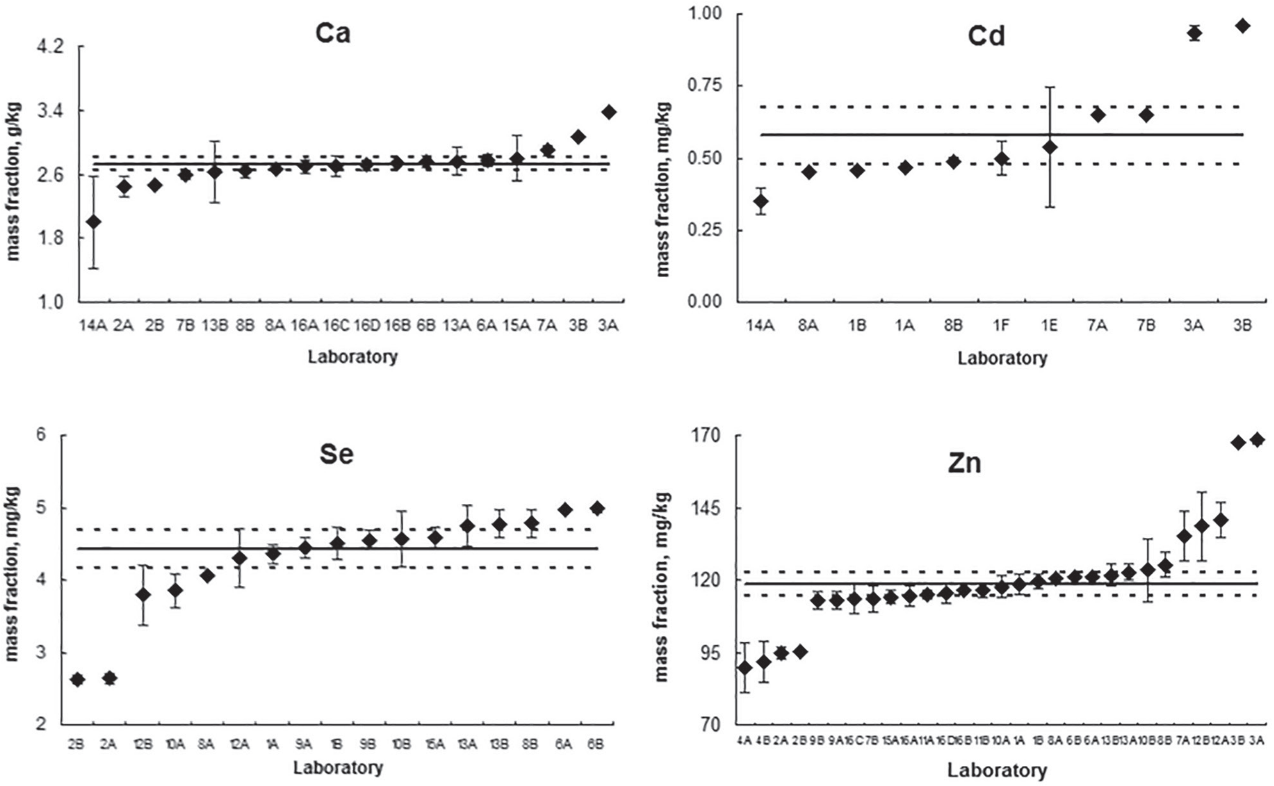

Figure 1. Interlaboratory comparison reported mass fractions (mean result and confidence interval at 95% confidence level) for two bottles (A and B) of the mussel candidate reference material (dry mass basis). Robust mean H15 (——); expanded uncertainty due to characterization, k = 2 (- - - -).

element characterization by means of the interlaboratory comparison (u_{char}), due to the between-bottle homogeneity study (u_{bb}), and due to the long-term stability study (u_{lts}) to the standard uncertainty of the assigned value (u_{CRM}), which was calculated according to equation 1. It was observed that u_{bb} was the lowest contribution to u_{CRM} while u_{lts} was the highest for most elements, even for the relatively short shelf life period that was chosen (2 years). Possibly the ISO Guide 35 approach used provided overestimated u_{lts} results, considering that freeze-dried biological materials of marine origin are usually certified for longer shelf life periods. Concerning the contribution of the interlaboratory comparison to u_{CRM}, approximately 70% of the elements presented relative u_{char} less than 5% while four elements presented u_{char} greater than 10% (Pb, S, U and Yb).

The metrological confidence in the interlaboratory comparison results varied from element to element as important factors such as number of participating laboratories, number of independent analytical techniques employed and contributions from the homogeneity and stability studies to the overall combined uncertainty must be taken into account.

For the purpose of certification, assigned values were classified according to the metrological confidence on the measurement results as:

(*i*) certified values: values obtained by 4 or more laboratories, using measurement methods with at least 2 measurement principles and with relative expanded uncertainties up to 20%;

(*ii*) informative values: values that did not fulfill the criteria to be considered certified values.

In relation to the relative expanded uncertainty associated with the assigned values, the criteria adopted for element certification may be regarded as restrictive, as compared to those adopted by the certification of an IAEA reference material of fish homogenate.[16] In that study, the limit of 20% for the relative uncertainty is set for elements with mass fractions above 100 mg kg^{-1}. The limit is 30% for elements with mass fractions in the range 10-100 mg kg^{-1}, and 50-60% for elements with mass fractions in the range of 0.1 to 10 mg kg^{-1}. In this study, the more restrictive criteria were set as the number of participating laboratories was considerably smaller and to assure greater confidence in the use of the certification information. The defined criterion was considered fit for the intended use of this mussel reference material which is to support environmental studies in Brazil using mussels as biomonitors.

Table 2 presents the certified mass fraction values and associated expanded uncertainties obtained for Al, As, Ca, Cd, Cl, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Se, Th and Zn at

Table 1. Mass fraction and uncertainty contributions at dry mass basis obtained in the characterization of the *Perna perna* mussel reference material

Element	Analytical technique (number of results)	N	Mass fraction / (mg kg^{-1})	u_{char} / (mg kg^{-1})	u_{bb} / (mg kg^{-1})	u_{lts} / (mg kg^{-1})	u_{CRM} / (mg kg^{-1})
Ag	k_0-INAA (18); INAA (29)	5	2.45	0.11	0.081	0.15	0.21
Al / (g kg^{-1})	k_0-INAA (6); INAA (13); ICP OES (12); WD XRF (12)	5	1.19	0.062	0.013	0.083	0.10
As	k_0-INAA (50); INAA (35); ICP OES (24); ICP-MS (18)	11	13.59	0.27	0.12	0.82	0.87
Br	k_0-INAA (62); INAA (53)	8	248	8.6	2.9	19	21
Ca / (g kg^{-1})	k_0-INAA (26); INAA (1); ICP OES (36); ICP-MS (12); WD XRF (12); F AAS (12); PGNAA (1)	9	2.72	0.043	0.030	0.19	0.20
Cd	ICP OES (24); ICP-MS (6); F AAS (12); ET AAS (15); PGNAA (1)	5	0.578	0.050	0.0047	0.011	0.051
Ce	k_0-INAA (38); INAA (1)	4	1.82	0.049	0.020	0.13	0.14
Cl / (g kg^{-1})	k_0-INAA (30); INAA (25); WD XRF (12); PGNAA (1)	7	36.9	0.79	0.92	2.6	2.9
Co	k_0-INAA (50); INAA (38); ICP OES (12)	9	0.829	0.016	0.0031	0.035	0.038
Cr	k_0-INAA (44); INAA (28); ICP OES (36); ICP-MS (17)	10	1.24	0.055	0.0087	0.13	0.14
Cs	k_0-INAA (38); INAA (17)	5	0.106	0.0025	0.0011	0.013	0.013
Cu	ICP OES (48); ICP-MS (18)	6	11.27	0.27	0.12	0.79	0.84
Eu	k_0-INAA (32); INAA (16)	4	0.0291	0.0024	0.00021	0.0035	0.0042
Fe	k_0-INAA (38); INAA (38); ICP OES (60); ICP-MS (18); WD XRF (12)	14	593	12	4.6	23	27
Hf	k_0-INAA (20); INAA (1)	2	0.0411	0.0013	0.00045	0.0029	0.0032
Hg	INAA (1); CV AAS (27); SS AAS (12)	3	0.165	0.011	0.0018	0.018	0.021
I	k_0-INAA (18); INAA (1)	3	12.9	0.52	0.14	0.90	1.0
K / (g kg^{-1})	k_0-INAA (38); INAA (36); ICP OES (36); ICP-MS (12); WD XRF (12); F AAS (12)	11	8.18	0.25	0.14	0.57	0.64
La	k_0-INAA (39); INAA (30)	6	0.817	0.030	0.0063	0.062	0.069
Mg / (g kg^{-1})	k_0-INAA (18); INAA (25); ICP OES (36); ICP-MS (12); WD XRF (12); F AAS (12); PGNAA (1)	8	3.73	0.087	0.034	0.26	0.28
Mn	k_0-INAA (6); INAA (24); ICP OES (48); ICP-MS (6)	9	22.7	0.84	0.47	1.6	1.9
Na / (g kg^{-1})	k_0-INAA (62); INAA (29); ICP OES (36); ICP-MS (12); WD XRF (12); F AAS (12); PGNAA (1)	14	22.8	0.53	0.22	1.7	1.8
Ni	INAA (1); ICP OES (36); ICP-MS (6)	5	7.01	0.46	0.077	0.49	0.67
P / (g kg^{-1})	WD XRF (12); PGNAA (1)	2	15.9	0.53	0.18	1.1	1.2
Pb	k_0-INAA (3); ICP-MS (6); ET AAS (14)	2	0.559	0.060	0.011	0.040	0.073
Rb	k_0-INAA (50); INAA (17)	6	4.93	0.14	0.072	0.43	0.46
S / (g kg^{-1})	ICP OES (12); WD XRF (12); PGNAA (1)	3	21.9	2.2	0.24	1.5	2.7
Sc	k_0-INAA (50); INAA (39)	8	0.199	0.0021	0.0024	0.011	0.011
Se	k_0-INAA (38); INAA (34); ICP OES (12); WD XRF (12)	9	4.42	0.13	0.030	0.18	0.22
Sm	k_0-INAA (20); INAA (1)	2	0.152	0.0032	0.0017	0.011	0.011
Sr	k_0-INAA (6); INAA (1); ICP-MS (6)	3	34.0	2.4	0.37	2.4	3.4
Th	k_0-INAA (50); INAA (17); α-ESP (4)	7	0.280	0.0069	0.0017	0.019	0.020
U	ICP-MS (6); α-ESP (4)	2	0.143	0.017	0.0016	0.010	0.020
V	k_0-INAA (30); INAA (12); ICP OES (12); ICP-MS (6)	4	2.89	0.26	0.066	0.20	0.34
Yb	k_0-INAA (12)	1	0.273	0.040	0.0030	0.019	0.044
Zn	k_0-INAA (50); INAA (39); ICP OES (60); ICP-MS (12); WD XRF (12)	15	118.5	2.1	0.83	4.2	4.7

N: number of laboratories; CV AAS: cold vapor atomic absorption spectrometry; ET AAS: electrothermal atomic absorption spectrometry; FAAS: flame atomic absorption spectrometry; ICP OES: inductively coupled plasma optical emission spectrometry; ICP-MS: inductively coupled plasma mass spectrometry; INAA: instrumental neutron activation analysis; k_0-INAA: instrumental neutron activation analysis with k_0 standardization; PGNAA: prompt gamma neutron activation analysis; SS AAS: solid sample atomic absorption spectrometry; WD XRF: wavelength dispersive X-ray fluorescence; α-ESP: alpha spectrometry; u_{char}: standard uncertainty due to characterization; u_{bb}: standard uncertainty due to between-bottle homogeneity; u_{lts}: standard uncertainty due to long-term stability; u_{CRM}: standard uncertainty of the assigned value.

the certification campaign. Table 3 shows the mass fraction values for Ag, Br, Ce, Cr, Cs, Eu, Hf, Hg, I, La, P, Pb, Rb, S, Sc, Sm, Sr, U, V and Yb, elements whose results were considered informative in this certification campaign.

Table 2. Certified values obtained for the *Perna perna* mussel reference material. Mass fractions are robust means with expanded uncertainties, k = 2, at dry mass basis

Element	Mass fraction / (mg kg^{-1})	U / %
Al / (g kg^{-1})	1.19 ± 0.21	18
As	13.6 ± 1.7	13
Ca / (g kg^{-1})	2.72 ± 0.40	15
Cd	0.58 ± 0.10	18
Cl / (g kg^{-1})	36.9 ± 5.7	15
Co	0.829 ± 0.077	9.3
Cu	11.3 ± 1.7	15
Fe	593 ± 53	9.0
K / (g kg^{-1})	8.2 ± 1.3	16
Mg / (g kg^{-1})	3.73 ± 0.56	15
Mn	22.7 ± 3.7	16
Na / (g kg^{-1})	22.8 ± 3.6	16
Ni	7.0 ± 1.3	19
Se	4.42 ± 0.45	10
Th	0.280 ± 0.041	15
Zn	118.5 ± 9.5	8.0

U: relative expanded uncertainty.

Table 3. Informative values obtained for the *Perna perna* mussel reference material. Mass fractions are robust means with expanded uncertainties, k = 2, at dry mass basis

Element	Mass fraction / (mg kg^{-1})	U / %
Ag	2.45 ± 0.41	17
Br	248 ± 42	17
Ce	1.82 ± 0.28	15
Cr	1.24 ± 0.28	22
Cs	0.106 ± 0.026	25
Eu	0.0291 ± 0.0084	29
Hf	0.0411 ± 0.0064	16
Hg	0.165 ± 0.041	25
I	12.9 ± 2.1	16
La	0.817 ± 0.14	17
P / (g kg^{-1})	15.9 ± 2.5	16
Pb	0.56 ± 0.15	26
Rb	4.93 ± 0.92	19
S / (g kg^{-1})	21.9 ± 5.4	25
Sc	0.199 ± 0.023	11
Sm	0.152 ± 0.022	15
Sr	34.0 ± 6.9	20
U	0.143 ± 0.039	28
V	2.89 ± 0.67	23
Yb	0.273 ± 0.088	32

U: relative expanded uncertainty.

Conclusions

This paper describes the production of a *Perna perna* mussel reference material produced in Brazil to be used as a quality assurance tool in biomonitoring programs. Robust means from an interlaboratory comparison were taken as element mass fractions and contributions from the element characterization and from the homogeneity and stability studies were taken into account to yield combined standard uncertainties. For most of the elements, the stability component was the major uncertainty contribution. From the adopted certification criteria, informative values for 20 elements were presented an it was possible to assign certified values for the following 16 elements: Al, As, Ca, Cd, Cl, Co, Cu, Fe, K, Mg, Mn, Na, Ni, Se, Th and Zn.

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