

Mussel Reference Material Preparation Proposal as a Quality Assurance Tool for Brazilian Seashore Biomonitoring

E. G. MOREIRA,* M. B. A. VASCONCELLOS, V. A. MAIHARA, M. G. M. CATHARINO & M. SAIKI

Instituto de Pesquisas Energéticas e Nucleares – IPEN-CNEN/SP,
Av. Prof. Lineu Prestes, 2242, CEP 05346-000, Butantã, São Paulo, SP, Brasil

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ABSTRACT

Analytical measurement results may affect economical, political and environmental protection decisions. Therefore, there is a growing demand for quality assurance of such results. The use of certified reference materials, CRMs, is a simple and efficient way to validate analytical methods and to keep them under control. However, CRMs are expensive and usually obtained abroad, which makes their use prohibitive for a large number of Brazilian chemical laboratories. This paper presents a proposal for the preparation of a mussel reference material using the *Perna perna* species. This material will be used in biomonitoring studies of the Brazilian Seashore. The various aspects of the preparation such as sampling, processing, physical and chemical characterization are presented. Preliminary results for the concentration of elements such as As, Br, Co, Cr, Fe, K, Na, Se and Zn determined by INAA and Cd and Hg determined by AAS, are also presented.

Key words: reference material, mussel, *Perna perna*, INAA, AAS, metals.

RESUMO

Proposta de preparo de um material de referência de mexilhão como ferramenta de controle de qualidade para a biomonitoração do litoral brasileiro

Resultados de medições analíticas podem afetar decisões econômicas, políticas e de preservação ambiental. Assim, há crescente demanda para a garantia da qualidade de tais resultados. A utilização de materiais de referência certificados, MRCs, é uma maneira simples e eficiente de validar métodos analíticos e monitorá-los de forma a garantir que estejam sob controle. No entanto, MRCs são materiais de alto valor agregado, em geral obtidos no exterior, o que torna seu uso rotineiro proibitivo para grande parte dos laboratórios nacionais. Neste trabalho é apresentada proposta de preparo de material de referência de mexilhão da espécie *Perna perna* para ser utilizado em estudos de biomonitoração de elementos tóxicos realizados em pontos do litoral brasileiro. Os diversos aspectos do preparo, como amostragem, processamento, caracterização física e química do material, serão abordados. Além disso, são apresentados resultados preliminares para a concentração de elementos como As, Br, Co, Cr, Fe, K, Na, Se e Zn, determinados por INAA, e Cd e Hg, determinados por AAS.

Palavras-chave: material de referência, mexilhão, *Perna perna*, INAA, metais.

INTRODUCTION

Reference materials in the quality assurance of analytical measurements

Analytical measurement results may affect economical, political and environmental protection decisions regarding pollutant limits implementation in environment monitoring and in remediation planning (Zschunke, 2000). Therefore, there

has been a growing demand for quality assurance of such results, i.e., that obtained results are accurate through analytical techniques and that they are accompanied by their respective associated uncertainties in order to warrant the confiability for result comparison between different locations and different times. A straightforward and efficient method to achieve this goal is the comparison of the result obtained for a real sample with the value assigned to a known material, the reference

*Corresponding author: Edson Gonçalves Moreira, e-mail: emoreira@ipen.br.

material, RM, which has sufficiently homogeneous and well-established property values. RMs are also used in the validation of analytical methods, inter-laboratory studies and proficiency tests (Kramer *et al.*, 2001). When the property values of the RM are certified by a procedure that establishes the traceability of the values to an exact value of the unit of the International System of Units, SI, in which the property values are expressed, followed by an uncertainty for an established confidence level, the RM is called certified reference material, CRM. In the case of CRM for chemical composition, the property value is the element or compound concentration in the material (ISO, 1997).

Despite its importance in analytical result quality assurance, the use of CRMs is far from being widely disseminated. The complexity in the preparation and characterization of CRMs make their final price high, which may make their use economically unviable. Besides, there may be import constraints and restrictions for such materials. Last but not least, it is not always possible to obtain a CRM with a similar matrix to the sample under investigation and/or with element levels at the same range of concentration.

Use of mussels as biomonitors

Mussels are sedentary organisms that feed by seawater filtration and are in close contact with seawater. As a consequence, they may bioaccumulate several pollutants in their tissues. Although it may be detrimental to aquaculture, this fact has been extensively used in water quality monitoring.

Generally speaking, the objective of environmental monitoring is not only to determine the natural concentration of chemical constituents but also the concentration above which chemicals become pollutants in the environment. Many human activities such as mining, fossil fuel burning, industrial processing, agriculture, domestic sewage discharges and nuclear activities contribute to the pollution of the marine environment. Many contaminants pose drastic effects due to their bioavailability and toxicity to biological processes and persistence in the environment (Kayasth & Swain, 2004; Krammer *et al.*, 2001).

National and international environmental monitoring programs have been launched worldwide to assess the quality of aquatic environments. Even though it can be done using seawater, the use of marine organisms permits pollution evaluation over longer periods of time including concentration peaks and biological absorption effects. In this kind of study, marine organisms act as "biological samplers".

Mussels, oysters and other benthic organisms are used in marine and fresh water biomonitoring in more than seventy

countries (Krammer *et al.*, 2001). It is of fundamental importance that biomonitoring data are produced with sufficient accuracy if results obtained in different laboratories over long periods of time are to be compared. For the assessment of pollutants and their byproducts in the environment, it is important not only to use accurate techniques but also pay careful attention to every step of the analytical process from meaningful sampling and appropriate sample preparation and storage to the use of quality assurance and quality control protocols for analytical procedures. The availability of CRMs with similar properties as those of the real environmental samples is of utmost importance for the quality assurance and quality control of the analytical methods used in biomonitoring.

Various studies have been done in Brazil using marine organisms as indicators of anthropic toxic compound contamination of the environment (Callil, 1998; Medeiros *et al.*, 1998).¹ Nowadays, there is a biomonitoring study under development at IPEN in collaboration with the Oceanographic Institute of the São Paulo University. This study, subject of a Ph. D. program, aims the biomonitoring at some regions of São Paulo State Seashore for the contamination of toxic elements such as arsenic, cadmium, lead and mercury, using the *Perna perna* mussel.

Mussel reference material preparation proposal

From the previous observations, the relevance of the preparation of a mussel CRM, to help in the biomonitoring of seawater pollution, is evident. The development of skills for the preparation and certification of a mussel RM, i.e., a biological matrix reference material to be used in environmental studies, is an important step in Brazilian metrological evolution. In this paper, the preparation of a *Perna perna* mussel RM is proposed. The material will be used in toxic element biomonitoring studies of the Brazilian Seashore.

MATERIAL AND METHODS

In this work, the steps for the production of a mussel reference material are shown in Figure 1. The various steps were defined from the expertise of our laboratory in handling and sampling of biological materials, from the evaluation of detailed international mussel CRMs production reports, and they attend to international guidelines for the preparation, characterization and certification of CRMs (ISO, 1992, 1997, 2000a, 2000b, 2000c, 2006).

1. CALLIL, C. T., 1998, Utilização de moluscos como indicadores de mercúrio: proposta de biomonitoração. In: Encontro Brasileiro de Ecotoxicologia, 5., 05-08 out., Itajaí, SC. *Anais...* Itajaí. CD-ROM.

MEDEIROS, I. D., ALMEIDA, E. A., VENTURA, E. C., MÜLLER, I. C., MEZZARI, M. P. & BAINY, A. C. D., 1998, Biomonitoramento da contaminação aquática na Baía Norte da Ilha de Santa Catarina utilizando mexilhões *Perna perna* como organismos sentinela. In: Encontro Brasileiro de Ecotoxicologia, 5., 05-08 out., Itajaí, SC. *Anais...* Itajaí. CD-ROM.

Mussels were obtained from a single population, from a single producer, from Cocanha Beach in Caraguatatuba City, São Paulo State North Shore, where mussels are cultivated by the longline system. Using cultivated mussels poses the advantage of using animals with the same size (age), at the same place and the same time of origin (same pollution burden history), which enhances the homogeneity of the product. Furthermore, sampling is easy and usually at a reasonably low cost.

Tissue masses shown in Figure 1 are approximate and were obtained from preliminary tests. An 80% mass loss in the freeze drying process and in the disposal of individuals with size outside the range of the mean size of the population used was considered. A 20% dry material loss was also foreseen due to storage, drying, vessel transfers and inappropriate particle size fractions disposal after cryomilling in a ball mill.

After the bulk preparation, the candidate RM will be homogenized in an appropriate “Y shape” mixer and bottled in glass bottles with about 20 g of RM each. The moisture content

of the material will be tested in a moisture content determination balance and, if necessary, the RM will be subjected to the freeze-drying process until the moisture content level be lower than 3-4% (w/w). The bottles will be gamma ray irradiated at the ^{60}Co irradiation facility at IPEN in order to enhance its stability. The homogeneity will be evaluated with respect to the within-bottle homogeneity and between-bottles homogeneity by means of a two-way analysis of variance test, ANOVA (ISO, 2006). The stability of the material will be checked at different temperatures using a previously established experimental design to define the most favorable transport and storage conditions (ISO, 2006). The content of the elements of interest in the candidate RM will be determined by national and international laboratories in an interlaboratory program by means of various validated analytical methodologies. Element content certification will be accomplished by applying statistical treatments to the results reported by the interlaboratory program participants in order to assign the certified values and their associated uncertainties.

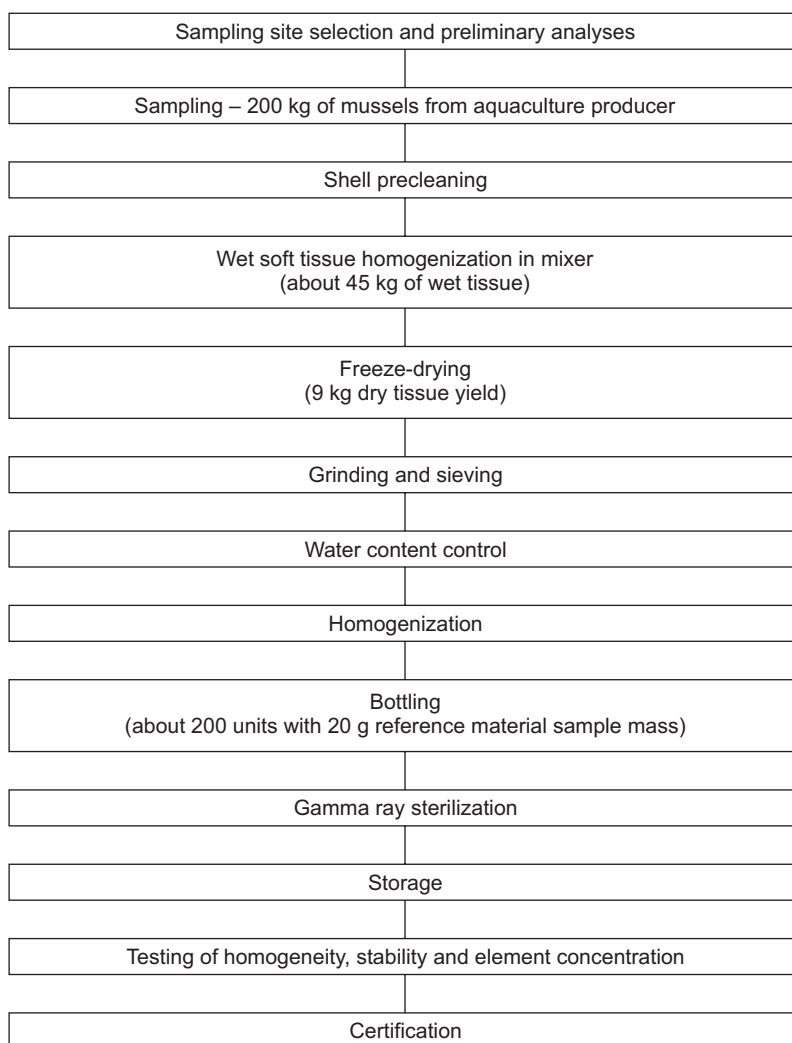


Figure 1 – Preparation steps for the mussel reference material.

Table 1 – Element concentration determined in this study by INAA, CV-AAS (Hg) and ETAAS (Cd) for a test sample of the mussel candidate CRM and certified values for mussel CRMs.^a

Element	Candidate CRM	IRMM CE 278	NIST SRM 2976
As, mg kg ⁻¹	15.8 ± 0.5	6.07 ± 0.13	13.3 ± 1.8
Br, mg kg ⁻¹	338 ± 5	NC	(329 ± 15)
Cd, mg kg ⁻¹	0.654 ± 0.013	0.348 ± 0.007	0.82 ± 0.16
Co, mg kg ⁻¹	0.86 ± 0.01	NC	(0.61 ± 0.02)
Cr, mg kg ⁻¹	0.90 ± 0.06	0.78 ± 0.06	NC
Fe, mg kg ⁻¹	373 ± 6	NC	171.0 ± 4.9
Hg, mg kg ⁻¹	0.188 ± 0.020	0.196 ± 0.009	0.0610 ± 0.0036
K, %	1.3 ± 0.2	NC	(0.76 ± 0.03)
Na, %	2.79 ± 0.01	NC	(3.5 ± 0.1)
Se, mg kg ⁻¹	3.6 ± 0.2	1.84 ± 0.10	1.80 ± 0.15
Zn, mg kg ⁻¹	103 ± 1	83.1 ± 1.7	137 ± 13

NC – not certified; concentrations in parentheses are informative values (not certified); ^auncertainties are: the standard deviation for three determinations (candidate CRM); 95% confidence intervals (IRMM CE 278); expanded uncertainties (NIST SRM 2976).

RESULTS

At the time being, the material is in the freeze-drying step. Mean water content is about 84%. Preliminary element concentration results in test samples of the mussel candidate reference material are shown in Table 1. As, Br, Co, Cr, Fe, K, Na, Se and Zn were analyzed by Instrumental Neutron Activation Analysis, INAA; Hg by Cold Vapor Atomic Absorption Spectrometry, CVAAS and Cd by Electrothermal Atomic Absorption Spectrometry, ETAAS. For comparison purposes, the certified values for two CRMs are also presented: IRMM CE 278 “mussel tissue” and NIST SRM 2976 “mussel tissue (trace elements and methylmercury)” (IRMM, 2004; NIST, 1998).

Most element concentration values in the candidate RM are at the same order of magnitude of the certified values for IRMM CE 278, which was produced from the tissue of the *Mytilus edulis* mussel collected in the Dutch Wadden Sea and for NIST SRM 2976, which was produced from the tissue of the *Mytilus galloprovincialis* mussel obtained from French commercial producers close to Marseille. Important exceptions are As and Hg. As is present in higher amounts in the candidate RM than in IRMM CE 278. In contrast, Hg is present in the same order of magnitude in IRMM CE 278 but in considerably lower levels in NIST SRM 2976. Such differences are observable in matrix reference materials and reflect differences in water quality, species used in the CRM preparation and the degree of development of the used animals.

DISCUSSION

The preliminary results presented in this paper show the viability of the production of a Brazilian mussel certified reference material, which is contribution to Brazilian Metrology as it may be used in the quality assurance of analytical results and hence, they improve the confidence in the data used in biomonitoring as well as in ecotoxicological studies concerned with toxic element determination. It is also important to stress that the certification of the candidate reference material will be possible with the collaboration of other laboratories, as concentration values are usually assessed as the consensus mean among result values obtained in interlaboratory trials.

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