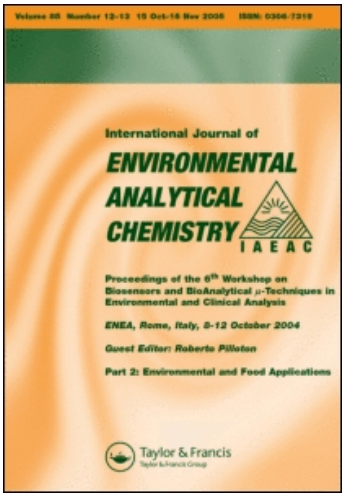

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A pilot study on the feasibility of the preparation of a new certified reference material based on the Antarctic Scallop *Adamussium Colbecki*

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A PILOT STUDY ON THE FEASIBILITY OF THE PREPARATION OF A NEW CERTIFIED REFERENCE MATERIAL BASED ON THE ANTARCTIC SCALLOP *ADAMUSSIUM COLBECKI*

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As foreseen by the Italian National Programme of Research in Antarctica, a preliminary investigation was performed to ascertain the feasibility of the production of a new Certified Reference Material for trace elements based on the bivalve *Adamussium colbecki*. The scallops sampled in Antarctica during the 1999–2000 Italian scientific expedition were analyzed by inductively coupled plasma atomic emission spectrometry and high-resolution inductively coupled plasma mass spectrometry for their content in selected trace elements (As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, V and Zn). The certification campaign will be undertaken on the basis of the findings of this feasibility study in close cooperation with the Institute for Reference Materials and Measurements, Joint Research Centre of the European Commission.

Keywords: Certified reference materials; Antarctica; Inductively coupled plasma mass spectrometry; *Adamussium colbecki*; Inductively coupled plasma atomic emission spectrometry

INTRODUCTION

This investigation was performed to ascertain the feasibility of the production of a new Certified Reference Material (CRM) for trace elements based on the bivalve *Adamussium colbecki*. The need of CRMs representative of Antarctic environmental matrices is dictated by the increasing quest for the validity and comparability of data produced and exploited by the international scientific community for the purpose of monitoring and assessing environmental contamination in Antarctica in the vicinity of research stations. The Italian National Programme of Research in Antarctica (Programma Nazionale di Ricerca in Antartide, PNRA) has in recent years undertaken

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a general project focused on the preparation and certification of such CRMs [1]. Two of these have already been accomplished and are commercially available, i.e., the MURST-ISS-A1 Antarctic Sediment, certified for the content of Al, As, Cd, Co, Cr, Fe, Mn, Ni, Pb and Zn, and the MURST-ISS-A2 Antarctic Krill, certified for the content of As, Cd, Co, Cu, Fe, Mn, Ni, Pb, Se and Zn [2–5]. Other candidate CRMs (e.g. Southern Ocean water for selected trace elements and krill for some PCB congeners) are in progress. In turn, the data obtained in this study are intended to pave the way to the actual certification project of As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, V and Zn in *Adamussium colbecki*.

EXPERIMENTAL

Sampling

The choice of *Adamussium colbecki* as a possible CRM for trace elements was suggested by its physiological and environmental characteristics, which make it an ideal bioindicator to reveal ongoing environmental contamination phenomena as caused by the presence and activities of research bases in the Antarctic continent. The selection of the elements to be certified has been dictated by the fact that they are contained as a rule in mineral oils, fuels and other materials of current use in an Antarctic research base and are therefore likely to locally contaminate the environment.

This benthic mollusc lives in shallow waters close to the coasts throughout Antarctica at a depth varying from 0 to 1500 m in depressions of the seafloor and is particularly abundant in the Terra Nova Bay with a density of 60–80 individuals per m^2 [6]. It is a filter-feeding organism, and its food includes phytoplankton, benthic diatoms, foraminifera and detritus. Its life expectancy may be up to 20 years. Because of its ability to bioaccumulate contaminants over a long period of time, this organism shows much promise as a valid bioindicator [7]. However, the Protocol on Environmental Protection to the Antarctic Treaty (the so-called Madrid Protocol) entered into force on January 1998. This must be implemented by all countries carrying out research in Antarctica, in particular, as regards the monitoring of the impact of the activities of their scientific bases on the surrounding environment [8–10].

For this pilot study, a small number of adult individuals of the mollusc were collected by divers along the coast of Terra Nova Bay, close to the Italian Base, during the 1999–2000 scientific expedition (see Fig. 1). After collection, samples were immediately sealed in decontaminated polyethylene bags, frozen at $-30^{\circ}C$ and kept at the same temperature until their delivery to the Istituto Superiore di Sanità (ISS, Rome, Italy).

Sample Treatment

To test the key steps of the future certification process, the collected material was subjected to a number of preliminary tests. Among these, priority was attached to the decision as to whether the entire organism or only selected tissues thereof should be included in the mass candidate to certification. The parts taken into account were the muscle, foot, mantle and internal organs, including gills, kidney, gonads

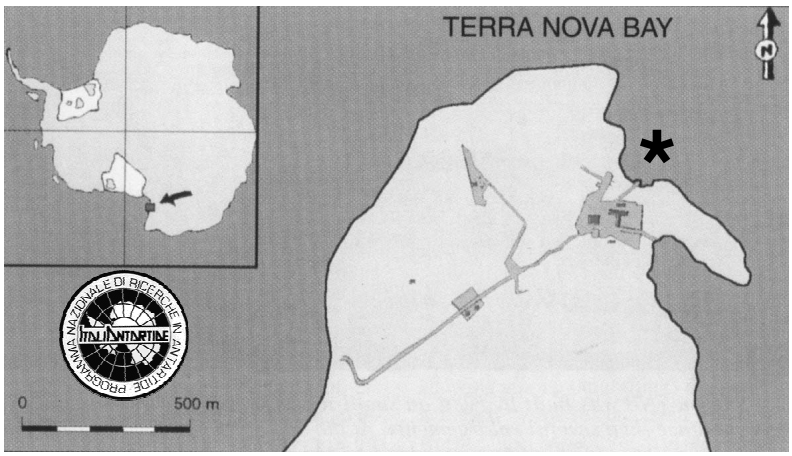

FIGURE 1 Sampling site of *Adamussium colbecki* (*) in the shallow waters facing the Italian Base at Terra Nova Bay, modified from PNRA [15].

and digestive gland. This last is of particular interest because of the bioaccumulation of Cd and Zn probably connected to the production of metallothioneines [11]. To this end, after removing the valves, a few individuals were dissected using tools made from Teflon®, polyethylene and tungsten carbide, thus minimizing any possible contamination of the samples by exogenous elements. Manipulation of samples was always performed in a Class-100 clean room (Tamco, Rome, Italy), thus further reducing the risk of exogenous contamination. The various organs thus obtained were pooled according to type and subsequently freeze-dried by means of a *Lyolab 3000* apparatus (Heto-Holten A/S, Allerød, Denmark). The freeze-drying procedure required a total of 72 h. Each freeze-dried mass was finely ground and homogenized for 5 h in an *Analysette* ball mill (Fritsch GmbH, Idar-Oberstein, Germany) equipped with an agate jar and ball. Approximately 500 mg each of the resulting powders were placed into Teflon® vessels, added to 5 mL of suprapur HNO_3 (Merck, Darmstadt, Germany) and then mineralized by acid-assisted microwave digestion in a MLS-1200 Mega oven (FKV Milestone, Sorisole, Italy; 10 min at 250 W, 10 min at 400 W, 10 min at 600 W).

Analysis

Determinations were performed by inductively coupled plasma atomic emission spectrometry (ICP-AES) for the content in some trace elements (As, Cd, Cr, Cu, Fe, Mn, Mo, Ni and Zn).

In the case of the whole organism, measurements were also performed by magnetic sector high-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS). In both instances, all determinations were performed in triplicate, and the resulting values were averaged.

Information on the spectrometers used and working parameters are listed in Tables I and II. The method of standard additions was applied to all determinations. Accuracy of measurements was checked by using the BCR 278R (Mussel Tissue) CRM.

TABLE I ICP-AES apparatus and settings

Spectrometer	Optima 3100 XL, axial view (Perkin-Elmer Co., Norwalk, CT, USA)
RF generator	Frequency, 40 MHz; power output, 1.3 kW
Polychromator	*Echelle* grating (ruling density, 79 lines mm^{-1}) combined with a Schmidt cross disperser; detection by a simultaneous solid-state segmented-array charged-coupled device detector; wavelength, range 165–403 nm; maximum resolution, 0.006 nm at 200 nm
Torch	PerkinElmer, quartz, four coils
Nebulizer	(i) Cross-flow with a Ryton Scott chamber; (ii) Ultrasonic, U-5000 AT^+ (CETAC Technologies Inc., Omaha, NE, USA)
Argon flows ($L\,min^{-1}$)	Plasma, 15; auxiliary, 0.5; aerosol, 0.6
Analytical wavelength (nm)	As, 193.6; Cd, 226.5; Cr, 284.3; Cu, 224.7; Fe, 238.2; Mn, 257.6; Mo, 202.1; Ni, 231.6; Zn, 213.8
Internal standard (nm)	Y, 360.0

TABLE II HR-ICP-MS apparatus and settings

Spectrometer	ELEMENT (Finnigan MAT, Bremen, Germany)
Geometry	Double focusing reverse Nier–Johnson
Resolution ($m/\Delta m$)	300–3000
RF generator power output	1.3 kW
Interface	Sampler and skimmer cones in Pt alloy
Data acquisition	Electric scan; five runs; five passes
Nebulizer	Ultrasonic, U-5000 AT^+ (CETAC Technologies Inc., Omaha, NE, USA)
Argon flows ($L\,min^{-1}$)	Plasma, 13; auxiliary, 1; aerosol, 1
Analytical masses (amu)	^{75}As, ^{114}Cd, ^{52}Cr, ^{65}Cu, $^{200,\,201,\,202}Hg$, ^{55}Mn, ^{96}Mo, ^{60}Ni, ^{51}V
Internal standard mass (amu)	^{115}In

Characterization of the Freeze-Dried Material

The production and certification of a CRM based on *Adamussium colbecki* at this stage of the project do not appear to pose any problems other than those generally encountered with similar matrices [12–14]. Preliminary experiments performed on the freeze-dried material of the mollusc under test do not raise particular concerns as regards the possibility of grinding it down to the necessary average particle size (ideally $< 125\,\mu m$). Also, homogeneity and stability, as based on experimental evidence gained with other mussel tissues, are expected to be fully compatible with the prerequisites of a CRM, in particular of its shelf-life. However, the thorough assessment of the homogeneity and stability of the ground freeze-dried *Adamussium* can be undertaken only once this candidate CRM has been produced and bottled. To this end, the criteria established by IRMM will be implemented.

RESULTS

As a rule, element concentrations were found to increase in the order muscle–foot–mantle–internal organs (Tables III and IV). The difference between the lowest and the highest value for a given element generally spanned only one order of magnitude. Table IV also lists the percentage distribution of the elements in organs and tissues.

TABLE III Concentrations as determined by ICP-AES on the whole freeze-dried organism of *Adamussium colbecki* (pool of two individuals)

Element	*Concentration* ($mg\,kg^{-1}$)		
	Organism A	*Organism B*	*Mean of A+B*
As	11.0 ± 1.2	12.9 ± 0.5	11.9 ± 1.4
Cd	30.7 ± 0.5	28.1 ± 1.2	29.4 ± 1.9
Cr	3.6 ± 0.2	2.2 ± 0.1	2.9 ± 0.9
Cu	3.55 ± 0.02	4.5 ± 0.1	4.0 ± 0.6
Fe	117.8 ± 1.9	76.6 ± 2.6	97.2 ± 29.1
Mn	2.68 ± 0.04	2.6 ± 0.1	2.65 ± 0.04
Mo	0.78 ± 0.03	0.61 ± 0.02	0.7 ± 0.1
Ni	5.5 ± 0.9	4.8 ± 0.2	5.1 ± 0.5
Zn	69.3 ± 0.9	67.8 ± 2.8	68.5 ± 1.0

TABLE IV Concentrations as determined by ICP-AES on freeze-dried organs of *Adamussium colbecki* and element percentage (shown in parentheses)

Element	*Concentration* ($mg\,kg^{-1}$)			
	Muscle	*Foot*	*Mantle*	*Internal organs*
As	n.d.	n.d.	7.5 ± 0.6 (n.d.)	7.8 ± 0.5 (n.d.)
Cd	1.49 ± 0.01 (1.3)	4.1 ± 0.3 (3.6)	11.2 ± 0.7 (9.7)	98.4 ± 1.0 (85.4)
Cr	0.4 ± 0.2 (4.6)	0.9 ± 0.1 (10.3)	4.4 ± 0.4 (50.5)	3.02 ± 0.04 (34.6)
Cu	0.889 ± 0.003 (6.1)	3.1 ± 0.6 (21.1)	2.9 ± 0.1 (19.7)	7.8 ± 0.1 (53.1)
Fe	37.9 ± 0.5 (8.0)	86.8 ± 1.7 (18.3)	84.9 ± 5.6 (17.9)	264.5 ± 3.0 (55.8)
Mn	1.10 ± 0.02 (9.1)	2.9 ± 0.1 (24.0)	4.5 ± 0.2 (37.2)	3.59 ± 0.03 (29.7)
Mo	0.24 ± 0.01 (7.2)	0.646 ± 0.001 (19.5)	0.66 ± 0.04 (19.9)	1.77 ± 0.02 (53.4)
Ni	1.4 ± 0.1 (8.1)	3.1 ± 0.3 (17.9)	5.0 ± 0.4 (28.9)	7.8 ± 0.1 (45.1)
Zn	40.8 ± 0.4 (11.7)	76.3 ± 2.0 (21.6)	156.8 ± 8.7 (44.5)	78.7 ± 1.3 (22.3)

n.d.: not determined.

From this viewpoint, therefore, it would seem unnecessary to resort only to one specific organ to produce the planned CRM. Rather, the whole organism is equally well representative of the average concentration expected for the elements of interest. This offers a definitive advantage in that the entire organism can be processed more easily, as no preliminary dissection needs to be carried out.

The concentrations measured in the entire mollusc agree rather well with those calculated by summing the individual amounts for each organ, the only exception being As. In this case, it was not possible to verify whether this was also true because no analytical data for muscle and foot were available due to an instrumental failure during the determination.

Data obtained by HR-ICP-MS confirmed those measured by ICP-AES and provided information also for Hg and V (Table V). In particular, also the concentration of As coincides with that measured by ICP-AES for the entire organism. As regards the possible mass interferences in HR-ICP-MS, those of the double ions $^{40}Ar^{35}Cl^{+}$, $^{40}Ar^{12}C^{+}$ and $^{40}Ar^{23}Na^{+}$ on $^{75}As^{+}$, $^{52}Cr^{+}$ and $^{63}Cu^{+}$, respectively, were taken into account. In consideration of the good agreement of the experimental data with results obtained for the above analytes by ICP-AES in the same samples as well as of the satisfactory results obtained in the analysis of the CRM (Table VI), the

TABLE V Element levels in the whole organism of *Adamussium colbecki* as obtained by HR-ICP-MS

Element	*Concentration* ($mg\,kg^{-1}$)
As	12.6 ± 0.7
Cd	32.4 ± 3.0
Cr	2.3 ± 0.8
Cu	4.8 ± 0.5
Fe	n.d.
Hg	0.67 ± 0.06
Mn	2.60 ± 0.55
Mo	1.04 ± 0.12
Ni	4.4 ± 0.6
V	0.61 ± 0.08
Zn	n.d.

n.d.: not determined.

TABLE VI Accuracy as determined through the analysis of the CRM BCR 278R ($mg\,kg^{-1}$)

Element	*Certified values*	*Found values*
As	6.07 ± 0.13	5.43 ± 0.31
Cd	0.348 ± 0.007	0.37 ± 0.03
Cr	0.78 ± 0.06	0.74 ± 0.02
Cu	9.45 ± 0.13	11.9 ± 0.34
Hg	0.196 ± 0.009	0.20 ± 0.03
Fe	n.c.	n.d.
Mn	7.69 ± 0.23	6.75 ± 0.38
Mo	n.c.	0.38 ± 0.03
Ni	n.c.	$1.93 \pm \pm 0.13$
V	n.c	0.26 ± 0.03
Zn	83.1 ± 1.7	n.d.

n.c.: not certified; n.d., not determined.

interferences mentioned turned out to be of little importance and were, therefore, disregarded.

CONCLUSIONS

On the basis of the results obtained, the material candidate to the certification will be prepared using the entire organism with the only exclusion of the valves. This solution avoids the separation of organs, a tedious procedure prone to the risk of possible contamination of the samples.

The certification campaign will be undertaken in close co-operation with the IRMM, in the frame of the Antarctic reference materials preparation programme, complying with the well-established BCR guidelines. Also, the expert laboratories will be selected according to the current rules of IRMM.

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