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NIES Certified Reference Material, Mussel

The National Institute for Environmental Studies (NIES) announces the availability of NIES Certified Reference Material No.6, Mussel.

Mussels (*Mytilus edulis* and similar species) have been extensively used as sentinel organisms to monitor coastal water pollution by heavy metals and organic compounds. Since the beginning of the International Mussel Watch Programme, the need for certified reference materials of matrix similar to samples under investigation has been stressed in order to verify the accuracy of analytical data provided by participating laboratories. The National Bureau of Standards (U.S.A.) issued Oyster Tissue Standard Reference Material in 1979. It is known, however, that the concentrations of certain elements, *i.e.*, Zn, Cu, Cd, Ag, in mussel (typically *Mytilus edulis*) are significantly different from those in oyster (typically *Crassostrea gigas*), therefore, the development of a mussel reference material has been undertaken.

The material was prepared from whole soft parts of reared common mussels (*Mytilus edulis*) obtained from Matoya Bay, Mie Prefecture, Japan. The mussel tissue was cryogenically ground, freeze-dried, sieved to pass a 80-mesh (177 μm) screen, blended, bottled and finally sterilized by Co-60 radiation. The bottles contain about 10 grams of material.

Certified values are provided for Na, K, Mg, Ca, Fe, Zn, Mn, As, Cu, Ni, Pb, Cd, Cr, Ag, while reference values are reported for P, Al, Sr, Se, Co and Hg. The elemental composition of this reference material is similar to those of mussels collected from unpolluted waters.

For further information on the availability of environmental reference materials write to Dr.M.Morita at the National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki, 305, Japan.

Preparation of Material

The mussels used for this reference material were the reared common mussels (*Mytilus edulis*) obtained from Matoya Bay, Mie Prefecture, Japan. The mussels (shell length 6-8 cm, average weight 22g) were collected in early July, 1981, and were shucked at the collection site. The whole soft parts (about 100kg) were iced and transported to the laboratory.

A batch of the mussel tissues (about 1kg) was washed with distilled water and immediately frozen in a liquid nitrogen bath. The frozen tissue was cryogenically ground for 1 hr in an alumina ball-mill, which had been precooled with liquid nitrogen. After repeating this procedure for the remaining batches, the mussel tissue was freeze-dried in one lot. At this stage, the dry mussel tissue powder was ground again for 1 hr in the ball-mill at room temperature.

The pulverized powder (about 13kg) was sieved through a set of nylon screens (upper, 50-mesh; middle, 80-mesh; bottom, reservoir) for 15 min, and then the powder which passed through a 80-mesh (177 μm) screen was collected for further processing.

The mussel powder (below 80-mesh, total amount 9.5kg) was mixed in a V-blender (150 L) for 2 hrs and packaged into acid-washed glass bottles (900 bottles, 10g each). Eight hundred bottles were sterilized by Co-60 radiation (2 M rad) at the Japan Atomic Energy Research Institute (Takasaki) and the remaining 100 bottles have been stored frozen at -20 ℃ without γ-ray radiation.

Homogeneity Assessment

In order to estimate homogeneity of the material, the variation of elemental content in several bottles was examined by acid-digestion followed by ICP-AES and AAS analysis. Six bottles were randomly selected from the lot of 800 bottles and 5 aliquots (about 250 mg dry wt) were taken from each bottle (total 30 samples).

The homogeneity of the mussel reference material was estimated using the analysis of variance. For the elements Mg, Ca, Zn, Sr, Mn, Cu and Cd, variations due to sample variability were estimated to be less than 1% (as relative standard deviation), indicating that the prepared Mussel satisfies the homogeneity criteria for a reference material.

Certified Values

The certified values are based on results of determinations by at least three independent analytical techniques. The uncertainties of the certified values were estimated based on consideration of 2 times the standard deviation of the mean of the acceptable values, and of the 95% confidence intervals for the mean of individual methods.

Instruction for Drying

The material should be dried in an air-oven at 85 ℃ for 4 hrs before use. The mean moisture loss was about 3.5%. For the determination of volatile elements such as Hg and Se, drying should be done on samples separate to those for analysis.

Sample Size

A minimum sample weight of 250 mg of the dry material should be used. A homogeneity test varying a sample size from 50 mg to 250 mg showed the best homogeneity for the 250 mg sample.

Storage

The material should be kept tightly closed in its original bottle and stored in a desiccator at room temperature.

Additional Information

This reference material contains siliceous material, probably the gut contents of the mussels. The certified and reference values are based on analyses performed on the entire sample. Therefore, decomposition procedures should be designed to achieve complete dissolution of the material such as through the use of a mixture of nitric/perchloric/hydrofluoric acids.

Certificate For
NIES Certified Reference Material,
NO. 6 "MUSSEL"

Certified Values

Element	Content*
Major and Minor Constituents	(Wt. Percent)
Sodium[a,b,c,e]	1.00 ± 0.03
Potassium[a,b,c,e]	0.54 ± 0.02
Magnesium[a,c,e]	0.21 ± 0.01
Calcium[a,c,e,f]	0.13 ± 0.01
Trace Constituents	(μg/g)
Iron[a,c,e]	158 ± 8
Zinc[a,c,e]	106 ± 6
Manganese[a,c,e,]	16.3 ± 1.2
Arsenic[a,c,e,g]	9.2 ± 0.5
Copper[a,c,d]	4.9 ± 0.3
Nickel[a,c,d,e]	0.93 ± 0.06
Lead[a,d,g]	0.91 ± 0.04
Cadmium[a,c,d,e]	0.82 ± 0.03
Chromium[a,c,e]	0.63 ± 0.07
Silver[a,d,e]	0.027 ± 0.003

a : atomic absorption spectrometry, b: flame emission spectrometry,
c : inductively coupled plasma emission spectrometry,
d : isotope dilution mass spectrometry, thermal ionization,
e : neutron activation analysis, f : spectrophotometry,
g : coulometry,

Reference Values

	(Wt. Percent)
Phosphorus	0.77
	(μg/g)
Aluminum	220
Strontium	17
Selenium	1.5
Cobalt	0.37
Mercury	0.05

*Based on dry weight: The material should be dried in an air-oven at 85°C for 4 hrs before use (mean moisture content, approximately 3.5%). A minimum sample size of 250 mg of the dry material should be used.

16-2 Onogawa, Tsukuba,
Ibaraki, 305, Japan
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for Environmental Studies
Environment Agency of Japan