Developing a new certified reference material of brown algae for trace metal analysis

A. Santoro, M. Ricci, M. F. Tumba-Tshilumba & A. Held
European Commission- Joint Research Centre,
Institute for Reference Material and Measurements, Belgium

Abstract

A certified reference material (CRM) of brown algae is being developed at the Institute for Reference Materials and Measurements (IRMM), European Commission, Joint Research Centre, in order to supply academic and industrial analysts with a new tool useful in the environmental analysis of trace metals.

Sixty kg of raw brown algae (species *Fucus vesiculosus*) was collected from two different sites in Galway (Ireland) and processed according to ISO 34 and ISO 35. A large amount of milled and sieved (at <125 μm) algae material was bottled and subjected to detailed homogeneity and stability tests with regard to the parameters to be certified.

The between bottle homogeneity of the total element content of As, Cd, Cu, Hg, Pb, Se and Zn was assessed via a dedicated test, which confirmed the suitable homogeneity of the candidate CRM. The short term stability of the material when stored at high temperature (60°C) for up to 4 weeks was also considered, confirming no degradation under the conditions applied.

Milled and sieved algae was also checked for water content by using Karl-Fischer titration and several oven temperatures (from 85°C to 110°C) in order to establish the most reliable water determination method for the material.

Along with these studies, a long term stability isochronous study and the characterisation of the total element content for the above-mentioned metals by using an inter-laboratory comparison approach are in progress, so as to finalise production and release onto the market the brown algae CRM for trace metal analysis.

Keywords: brown algae, trace metals, certified reference material, fucus vesiculosus, environmental analysis.

WIT Transactions on Ecology and the Environment, Vol 154, © 2011 WIT Press
www.witpress.com, ISSN 1743-3541 (on-line)
doi:10.2495/CHEM110161

1 Introduction

In recent decades, marine pollution has been evaluated and monitored not only by analysing water and sediment, but also by using aquatic bioindicator organisms. Aquatic organisms, such as mussels and algae, have displayed the capacity to accumulate organic and inorganic pollutants; and algae, in particular, showed a significant uptake capacity for trace metals.

Among the three main groups of algae (brown, red and green), brown algae seem to have the highest biosorbent capacity, especially for Cd, Cu, Zn, Pb, Cr and Hg in water and wastewater [1, 2]. Specifically, *Fucus vesiculosus* and *Ascophillum nodosus* have been increasingly used in water quality monitoring [3].

In this context, the Water Framework Directive [4], although not providing environmental quality standards for trace metals in brown algae, suggests the use of macroalgae as an indicator of water quality. Thus, a number of European countries (mainly Finland, Sweden, Germany and Great Britain) have used macroalgae (and in particular *Fucus vesiculosus*) as a marine bioindicator [5, 6].

Furthermore, *Fucus vesiculosus* is also used in food, cosmetic and clinical fields [7, 8] for its nutritional and therapeutic properties; increasing in this way the interest of the scientific community in monitoring the level of trace metals.

However, in trace metal monitoring, either in the environmental field or food market, the possibility of performing the analyses in a reliable and accurate way is of crucial importance. To this end, certified reference materials (CRMs) are developed with the specific scope of providing a common reference point useful for ensuring and improving the traceability of measurement results.

As specifically stated in ISO Guide 34 [9] and ISO Guide 35 [10], in order to be recognised as a reference material, special requirements have to be met. Furthermore, specific tests to prove its homogeneity and stability over a long period of time need to be performed.

By following these guidelines, raw Bladderwrack (species *Fucus vesiculosus*) was processed and tested at the Institute for Reference Material and Measurements (IRMM, Belgium), with the aim of producing a new CRM of brown algae for use in trace metals analysis.

2 Processing

Sixty kg of raw brown algae (Bladderwrack, *Fucus vesiculosus*) was collected at two sites in November 2009; Silver Strand beach and Spiddal in Galway (IRL). Plants were cut above the holdfast by using a sharp knife, immediately rinsed with seawater to remove debris and sand and then collected in bags. In the laboratory, plants were checked for large epiphytes and other animal material, quickly rinsed in freshwater and immediately frozen at - 20°C. The collection was performed taking into account the seasonal variation of element content in algae [11]. Several authors have reported an increase in the content of some trace elements between January and February; whereas a significant decrease was observed during the summer period (July, August) [12].

Although the temporal variation of metals in algae has been differently interpreted in literature [13, 14], in our case, the algae were collected in November, so as to reduce the risk of collecting raw material during a period of potential seasonal instability.

The frozen raw material was transported to the IRMM (Belgium) in order to be processed under controlled humidity and temperature conditions.

Algae were washed with deionised water to eliminate sand excesses, dried in a drying cabinet (Elbanton, NL) at 25 ± 5°C and milled by using a cryo-grinding vibrating mill (Palla mill, KHD, Humboldt-Wedag, Köln, DE) cooled at about -190°C. One of the main parameters which can seriously affect the quality of a reference material is the homogeneity; potential sources of heterogeneity were reduced by accurate milling and sieving steps. The material was sieved at 125 µm and finally mixed for several hours by using a DynaMix CM200 (WAB, Basel, CH).

Particle size distribution was checked by using a Sympatec Helos (Clausthal-Zellerfeld, DE) from the beginning to the end of the processing and as presented in Figure 1, the resulting material showed an average particle size of ca. 100 µm.

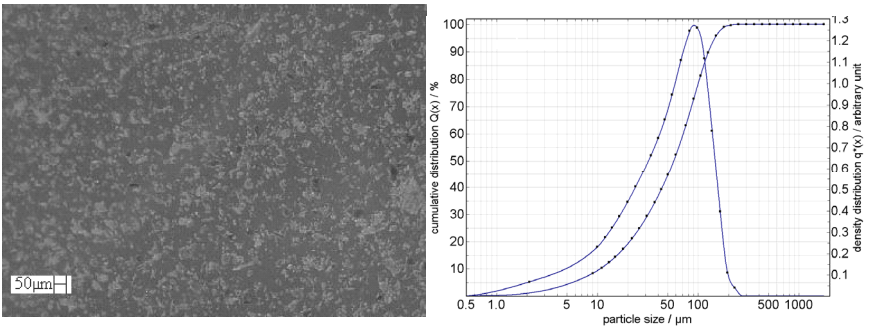

Figure 1: Typical micrograph for milled algae (left) and average particle size distribution obtained using isopropanol as dispersant (3 replicates, right).

During the whole processing, a loss of mass of ca. 85% was evidenced and the final portion recovered (with an average particle size of <125 µm) corresponded to ca. 14% of the total raw material mass.

Water content and activity of the material are key points for the stability of a CRM over long periods of time. Moisture contents above 5% can promote the growth of mould or enzyme activities with possible damage to the material [15]. Thus, the moisture level was decreased to 2.5% by vacuum drying the milled algae before bottling.

A large batch of about 1200 units was produced.

Possible causes of instability for the material generated during the processing were minimised by consistently working under controlled temperature and humidity and by filling the bottles in a glove box with a level of oxygen not exceeding 5%. The degradation due to UV radiation was minimised by using

amber glass bottles, which were later double sealed in aluminium pouches previously flushed with argon.

Finally, the bottles were sterilised by gamma irradiation with a maximum radiation dose of 15 kGy, in order to minimise any remaining bacterial activity.

3 Water content determination

Water content determination plays a significant role in evaluating uncertainties of measurements, as element mass fraction is usually reported dry-mass corrected [16]. Water content can be significantly different according to the method determination used. Oven drying for instance, though being a commonly used method, tends to underestimate the values; while Karl-Fischer titration is more selective for water particles, but less used because of its high costs.

It is therefore essential to establish the best conditions of determining water content in the material which should combine suitability for a large number of users and at the same time reliability.

Milled and sieved algae was checked for water content by using Karl-Fischer titration (765 KF Coulometer Methohm, CH) and five different oven temperatures (85°C, 95°C, 100°C, 105°C and 110°C) in order to establish the most reliable water determination method for the material [17].

As shown in Figure 2, the results obtained demonstrate a consistent increase in the apparent water content by increasing the oven temperature until 105°C. Above 105°C, the water content seems to decrease.

The value obtained with the Karl-Fischer titration was 10.0 ± 0.3 g/100 g (average of 6 replicates measurements ± standard deviation), which was not found to be statistically different (F-test, 95% confidence level) from the value found with oven drying determination by using a temperature of 105°C.

4 Homogeneity and stability tests

In order to perform specific homogeneity and stability tests, several bottles were selected from the whole batch produced, according to a randomly stratified scheme [18] and then stored and analysed in different conditions.

For the homogeneity study, 11 bottles were selected within the whole batch, digested by using HNO_3 and analysed for the total content of As, Cd, Cu, Hg, Pb, Se and Zn with ICP-SFMS (Inductively Coupled Plasma – Sector Field Mass Spectrometry) under repeatability conditions (i.e. in one analytical run).

The results evaluated by using one-way ANOVA, showed a standard deviation between bottles always below 5% (Figure 3), confirming the homogeneity of the material.

Regarding the stability test, an isochronous scheme was followed. Eight bottles were stored at a temperature of 60°C for 0, 1, 2 and 4 weeks (two bottles per time point), in order to simulate harsh environmental conditions. After the testing time, bottles were brought to a reference temperature of -20°C so as to "freeze" their state and later on analysed together under repeatability conditions.

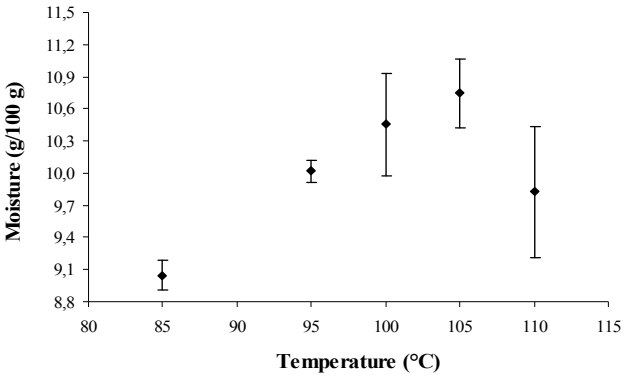

Figure 2: Water content (g/100 g) determination performed by using an oven drying method in brown algae (fucus vesiculosus). Bars correspond to standard deviation of 6 replicate measurements.

The results were obtained by digesting the samples with a mixture of HNO_3, H_2O_2 and HF and by using ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry) for As, Cd, Cu, Pb and Zn and GF-AAS (Graphite Furnace – Atomic Absorption Spectrometry) for Hg and Se.

The selection of different digestion and measurement procedures was decided upon, in order to reduce the presence of biased results, related to problems arising from a specific method or procedure.

As for the homogeneity study, data were evaluated by using one-way ANOVA in order to detect any drifting of the elemental concentrations in the material over time. The slope of the linear regression was never found to be statistically significantly different from zero (Figure 4).

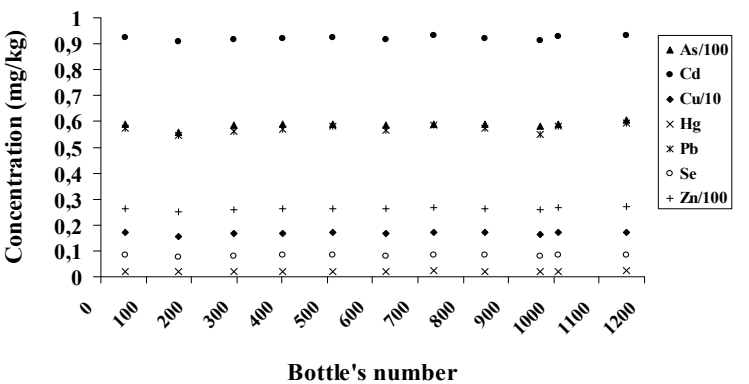

Figure 3: Homogeneity study performed on 11 bottles. Data are reported dry mass corrected in mg/kg. As and Zn are reported divided by a factor of 100 and Cu by a factor of 10.

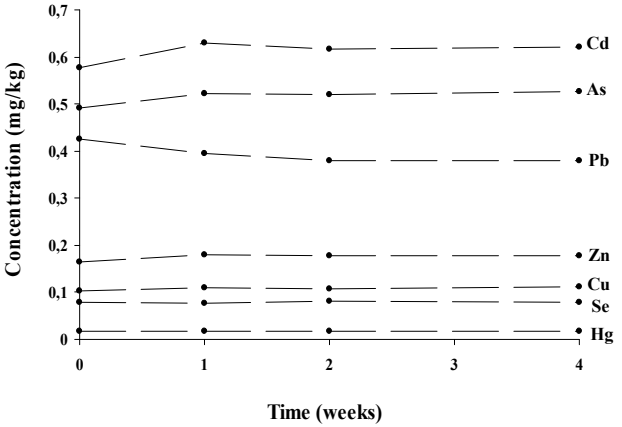

Figure 4: Stability study performed on 4 time points (0, 1, 2 and 4 weeks) at 60°C. Data are reported dry mass corrected in mg/kg. As and Zn are reported divided by a factor of 100 and Cu by a factor of 10.

Alongside this stability test, 14 bottles were stored, by using an isochronous study, for up to 12 months at a maximum temperature of 18°C, with the aim of evaluating the sample stability over a longer period of time and providing an estimation of the future shelf-life of the material.

A preliminary characterisation study on two elements (As and Zn) was performed by using neutron activation analysis (k_0-NAA) as a primary method [19]. The values obtained were in the range of 40-60 mg/kg for As and 20-30 mg/kg for Zn and thus comparable with the ones obtained during the homogeneity and stability tests (Figures 2 and 3).

A number of expert laboratories are currently being selected on the basis of specific quality criteria to take part in an inter-laboratory comparison campaign for the assignment of certified values for As, Cd, Cu, Hg, Pb, Se and Zn.

Key requirements in the selection are the use of different digestion methodology and analytical techniques, so as to minimise method dependant biases.

5 Conclusion

With the aim of producing a CRM for trace metal analysis in brown algae, several aspects concerning homogeneity and stability of the material, were checked. Homogeneity of the material was achieved during processing by employing an extensive milling and sieving procedure; specific preventive measures were taken to ensure stability, such as reducing the moisture content, bottling the material under an inert atmosphere and irradiating with a gamma source.

The homogeneity and stability tests performed so far show that the brown algae material is a good candidate for becoming a CRM.

Along with these studies, characterisation of the total element content, by using an inter-laboratory comparison approach, is in progress, so as to finalise production and release onto the market the CRM for trace metal analysis in brown algae.

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