Embrapa's experience in the production and development of agriculturereference materials.

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Palavras-chave: forneça de três a cinco palavras-chave.

Abstract: The main challenge of Embrapa is to develop a model of genuine Brazilian tropical agriculture and livestock. To get this task, the quality of laboratories results is mandatory, increasing the demand for reference materials. Projects were proposed to produce reference materials to support the national agriculture laboratories and consolidate a network able to perform reliable and reproducible analytical testing laboratory within the internationally standards required. Reference materials were produced and available to interested laboratories and collaborative tests were conducted to obtain consensus values. The results and statistical evaluations were performed with the use of software developed by Embrapa Southeast Livestock.

Keywords: reference material; inorganic; proficiency test; analytical chemistry.

1. INTRODUCTION

Appropriate compositionally-similar reference materials, a way of transferring accuracy from well-defined methods of analysis to the laboratory are a difficult task. It is observed especially for agricultural products produced in a tropical climate with different characteristics of those produced in temperate areas, the usual suppliers of reference materials. The COMAR database has registered more than 11000 RMs for various applications produced by 25 countries, including Brazil, which is represented by INMETRO. Otherwise, less than 150 of these samples are related to agriculture and livestock materials, none of them a Brazilian material [1]. Since the assurance of the traceability of measurements became indispensable to acquire accurate analytical information, get conclusions and make decisions, mainly in research and regulatory areas, the developed national reference materials is required.

The variety of raw and processed agricultural products, foods, and feedstuffs normally constitute a broad range of chemical composition, and inconsistent values are normal [2]. In attempt to obtain most appropriate control of analytical results, Embrapa starts developed agriculture

reference materials with a close correspondence, with respect to analyte and matrix, suitable for data quality control for inorganic constituents.

This report describes the preparation of five candidate agriculture reference materials: mineral mixing (to feed bovine milk cattle); tropical forage; tropical soil; bovine liver; and phosphate rock. The sample preparation and proficiency tests were performed at Embrapa Southeast Livestock with supplementary input by outside expert laboratories. Volunteers cooperating laboratories was essential to obtain the materials.

2. EXPERIMENTAL

2.1. *Sources of material*

Mineral mixing and phosphate rock were commercial products. Forage, soil, and bovine liver were prepared in-house. The tropical forage (RM-Agro E1001a) (Brachiaria brizantha Staf. cv. Marandu) was obtained with the harvest in 400 pots containing 25 kg of a previously artificially contaminated oxisol. Arsenic, Cd, Cr, and Pb (25 mmol/pot) solutions prepared from nitrate salts were added in the corrected oxisol.

The first crop of the forage has been made approximately 60 days after planting and the second one after 120 days. The material was oven-dried at 45°C for 72 h and ground in a cutting mill with 500 µm sieve. A second ground was performed in a high centrifuge mill with 250 µm sieve. It was obtained approximately 20 kg of dry and ground material, only with the aerial part of the forage plant, with particle sizes lower than 280 µm. The material was homogenized in a Y type homogenizer coated with polypropylene, and divided into 100 mL amber glass bottles, resulting 360 bottles each one with 50 g of the sample.

The tropical soil (RM-Agro E2002a) was the same oxisol that was previously prepared to forage. Next the end of forage experiment, the samples from the 400 pots were mixed, dried at

ambient temperature and sieved, to avoid the presence of injurious materials, such as forage roots. After 12 months, the soil was ground in a mortar grind and homogenized in a concrete mixer coated with epoxy resin. It was obtained approximately 65 kg of dry sieved soil, with particle sizes lower than 390 µm, which was divided into 645 amber glass bottles, each one with 100 g the soil.

The bovine liver (RM-Agro E3001a) was obtained from 57 bovines. Processing including bulk tissue separation, freeze-drying, milling, sieving, and packing resulted in 330 amber glass bottles with 100 g of the bovine liver with particle sizes lower than 145 µm.

The mineral mixing (RM-Agro E2001a) was a commercial product acquired by Embrapa Southeast Livestock to feed milk cattle. After ground, sieve and homogenizing process, it was obtained 300 polypropylene vials with 50 g of product with particle sizes lower than 80 µm.

The phosphate rock (RM-Agro E2003a) was also a commercial product, a phosphate rock from Morocco. After ground, sieve and homogenizing process, it was obtained 100 amber glass bottles with 100 g of product with particle sizes lower than 150 µm. The particle sizes measurements were performed in an Analysette 22 (MicroTec Plus, Fritsch, Germany).

To enhance long-term stability, all the prepared materials were bulk sterilized by cobalt-60 irradiation to 20-25 kG (IPEN, São Paulo, SP, Brazil) and were additionally individually sealed in aluminium-nylon pouches.

2.2. Chemical Characterization

A series of preliminary in-house experiments was undertaken to evaluate the adequates sample preparation and quantification methodologies, to define the materials homogeneity and stability.

The materials were produced following the internationally accepted ISO Guides 30-35 norms

[3-5] considering the preparation and packaging, material characterization, homogeneity tests (inter- and intra-bottle analysis of variance (ANOVA)), short and long term stability, and four collaborative campaigns.

Chemical characterization analyses to establish reference elemental concentrations were performed by microwave digestion assisted. Arsenic, Cd, Cr, and Pb were determined by inductively coupled plasma mass spectrometry (ICP-MS). Calcium, Cu, Fe, K, Mg, Mn, Na, P, and Zn were determined by inductively coupled plasma optical emission spectrometry (ICP OES). For quality control purposes, certified reference materials (SRM – NIST) were included in the analytical determination and were based on reference and validated methodologies.

For the intra-bottle homogeneities evaluation, 10 subsamples were collected from one bottle of each prepared material. For inter-bottle homogeneity evaluations, 10 bottles of each one of material were randomly taken and analyzed. The variation represents the randomly variation of the prepared materials batches. Statistical evaluation of results was obtained using the single factor analysis of variance (ANOVA). The homogeneity was evaluated by the Fisher test (F-test).

Short stabilities were assessed by submitting the samples (three bottles randomly taken from each one of prepared material) to extremes humidity and temperature conditions. The determination of the elements was performed in triplicate before and at the end of the experiment, after 30 days. Long term stabilities were also performed with the use of three bottles randomly selected, and the quantification of the analytes has been monthly performed.

Each one of collaborative campaign was establishment with the chosen and invitation to laboratories specialized that performed the selected analysis. A bottle of prepared material and a form with the instructions to carry out the determinations and returned the results were sent to laboratories that accepted the invitation to participate of the campaign. An attempt was consciously made to get the diverse analytical procedures, including different sample preparation steps, including no decomposition as in instrumental neutron activation analysis (INAA), as well as different detection techniques. The results were statistically evaluated to define the reference values by a software previously developed [6]. The uncertainties related to each step of the prepared materials were calculated to obtain the expanded uncertainty for each one of the analytes and to the final drafting of the control chart containing the results of the reference materials.

3. RESULTS

The list of agricultural reference materials arising from this research is presented in Table 1. The four collaborative chemical characterization campaign, together with contribution from initiating laboratories (Embrapa, CENA/USP, and IPEN) produced a great number of submitted individual concentration results. The bovine liver and phosphate rock are in the final data treatment and statistical evaluation.

Table 1. Agricultural reference materials for inorganic chemical composition.

Tropical forage	RM-Agro E1001a
Mineral mixing	RM-Agro E2001a
Tropical soil	RM-Agro E2002a
Bovine liver*	RM-Agro E3001a
Phosphate Rock*	RM-Agro E2003a

*In final stage of development.

Table 2 presents a summary list of actual concentrations with associated uncertainties in the three firstly prepared reference materials, tropical forage, mineral mixing, and tropical soil.

Table 2. Reference concentrations with associated uncertainties of constituent elements in three agricultural reference materials

Element	E1001a	E2001a	E2002a
As (mg/kg)	1.69±0.70	0.48±0.20	59.3±7.2
Cr (mg/kg)	3.30±1.66	-	120±30
Pb (mg/kg)	4.0±1.8	18.5±9.6	173.8±18.8
Cd(mg/kg)	19.9±5.1	0.43±0.08	94.0±11.4
Ca (g/kg)	4.37±0.58	187.8±36.3	0.25±0.07
Mg (g/kg)	2.95±0.44	10.8±2.7	0.33±0.06
Na (g/kg)	0.19±0.10	80±15	0.08±0.02
Fe (mg/kg)	0.09±0.01	2.73±0.21	32.9±12.7
Cu(mg/kg)	4.0±0.7	804±87	8.8±4.0
Zn (mg/kg)	9.9±1.6	-	-
K (g/kg)	12.0±2.4	35.6± 4.3	0.24±0.07
Mn (g/kg)	0.08±0.02	1.57±0.14	0.13±0.02
P (g/kg)	-	84.5±17.3	0.21± 0.04

Values based on a dry material basis, obtained from independent collaborative campaigns. Uncertainties associated with reference values are estimates expressed typically as 95% confidence intervals for a single future determination based on a sample weight of at least 200 mg, and include de expanded uncertainties related with homogeneities, characterizations, and long-term stabilities.

4. CONCLUSIONS

The experience in reference material production led to the extensive elemental characterization of new agricultural materials for many macro and micronutrients, as well as contaminants constituents of nutritional, toxicological and environmental significance. The research projects, supported by CNPq and Embrapa (Rede-Agro and REPENSA), consolidate a network able to perform reliable and reproducible analytical testing laboratory within the internationally standards required. It is also important to observe that the research allows the students formation in metrological aspects [7-9] and new analytical developments.

These reference materials are available to the analytical community from: (www.embrapa.br/pecuariasudeste/busca-de-produtos-processos-e-servicos/-/produto-servico/1263/materiais-de-referencia).

5. REFERENCES

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