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Limestone doses affecting mineral contents in tropical grass forage

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Field trial was performed at the experimental farm of Southeast Embrapa Cattle, São Carlos – SP, Brazil, on a 16 year old *Brachiaria decumbens* pasture, grown on a distrophic Hapludox (Oxisol), recovered by the use of limestone and fertilizer. The experiments were carried out in random blocks, with 6 replications and 5 treatments. The 100 m^2 blocks were established in the pasture. Each block received a sequence of limestone doses of 0, 1, 2, 4 and 8 t/ha. The forage samples were taken one year after limestone application on soil surface. Instrumental neutron activation analysis (INAA) followed by gamma-ray spectrometry was the analytical method used to determine mineral contents. The statistical analysis showed a negative linear correlation of Br, Co, Cr, Mn and Zn contents in forage with the limestone doses, while the uptake of Mg was affected in a positive way.

Introduction

Limestone application is one of the less expensive and effective procedures used to correct soil acidity. Soil acidity is an important issue in agricultural productivity and in soil management because of its direct effect on the development and nutritional constitution of plants, by the decrease or increase of the solubility of some essential nutrients.[1] The acidity correction procedure may consist in limestone distribution on the soil surface without burying it in at an intensively managed pastureland or the so-called direct drill agriculture. However, it is well known from the literature, that the increase of pH in the soil surface may result in undesirable changes of nutrient element levels in forage tissue. CAIRES[2] verified that adequate criterions were necessary to estimate the limestone dose to be applied in the soya culture because a reduction of Zn and Mn absorption by plant was observed.

In the pastures intensively fertilized with nitrogen, limestone could be one of the key inputs to increase the forage yield and to improve its quality. *Brachiaria decumbens* is largely used as forage in the pastures of several Brazilian regions. This forage is adapted to soils with low fertility and relatively high acidity. According to VALLE[3] the pastures of *Brachiaria* occupy more than 40 million hectares in Brazil.

In the present work, instrumental neutron activation analysis (INAA) followed by gamma-ray spectrometry was applied to estimate the concentrations of Br, Co, Cr, Mg, Mn and Zn in the above-ground part of *Brachiaria decumbens*. This forage was grown-up on a degraded pastureland, submitted to a recovery process and in intensive management phase, where different limestone doses were applied to the soil surface, and fertilizer was top dressed after each cutting or grazing in the rainy season. Analysis of variance was used, with partitioning of the orthogonal contrasts, to verify if there are significant differences among treatments and if the element concentrations in B*rachiaria* tissue were affected by the limestone doses, using the GLM procedure of the statistical analysis system.[4]

INAA is a method that involves a minimum sample handling and has been applied to the analysis of biological materials by several researchers during the past years.[5–8]

Experimental

Sampling protocol, collection and treatment of the samples

The field trial was performed at the experimental farm of Southeast Embrapa Cattle, São Carlos-SP, Brazil, on a 16 year old *Brachiaria decumbens* pasture, grown on a distrophic Hapludox (Oxisol), being recovered by the use of limestone and fertilizer.

The experiments were carried out in blocks of 100 m^2 each with 6 replications and 5 treatments. Each block received a sequence of limestone doses of 0, 1, 2, 4 and 8 t/ha indicated as T0, T1, T2, T4 and T8, respectively.

The forages samples were taken one year after the limestone applicaton on the soil surface. The above-ground part of the plant, composed by leaves and slender stems, was analyzed. Samples were collected from 7 cm above the soil surface, at the second cutting after limestone application. The total number of samples was 30.

Parts of the samples destined for analysis, were dried at 60 °C in an oven with forced air circulation. The dried materials were ground in Willey mills and passed through a 20-mesh sieve (0.84 mm).

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For irradiation, 200 mg samples were transferred to polyethylene envelopes, which were cleaned prior to use by leaching with a dilute HNO_3 (1:5).

Preparation of the standards

Standard solutions of Br, Co, Cr, Mg, Mn and Zn (Spex Certiprep) were used to prepare the standards. Aliquots (50–100 µl) were pipetted on small sheets of analytical filter paper (Whatman No. 42) for irradiation. After drying, these filter papers were placed into polyethylene bags. The standards contained: Br (24.5 µg), Co (2.49 µg), Cr (2.49 µg), Mg (495 µg), Mn (4.84 µg), and Zn (24.5 µg).

Irradiation and counting

Two types of irradiation were carried out at the IEA-R1 nuclear research reactor. In the first, the sample and standards (Mg, Mn) were irradiated together in a nylon container for 2.5 minutes. After a decay time of 8 minutes the ^{27}Mg radionuclide (at 1014 keV) was measured in the sample and in the Mg standard afterwards. The ^{56}Mn radionuclide (at 846 keV) was measured after 90 minutes of decay. In the second irradiation, the sample and standards (Br, Co, Cr and Zn) were irradiated together in an aluminum container for 8 hours. The ^{82}Br radionuclide (at 776 keV) was measured after 3 days of decay, while ^{60}Co (at 1332 keV), ^{51}Cr (at 320 keV) and ^{65}Zn (at 1115 keV) were measured after, at least, 10 days. The thermal neutron flux utilized ranged from $5\cdot10^{11}$ to $1\cdot10^{13}$ $n\cdot cm^{-2}\cdot s^{-1}$.

The equipment used to measure the gamma-radiation was Canberra hyperpure Ge detector (Model GX2020) coupled to an Integrated Signal Processor (Model 1510) and MCA System 100, both from Canberra. The detector used had a resolution (FWHM) of 0.9 keV for 122 keV gamma-ray of ^{57}Co and 1.9 keV for 1332 keV gamma-ray of ^{60}Co.

Results and discussion

In this work, the certified reference materials NIST 1515 Apple Leaves and NIST 2710 Montana Soil were also analyzed for quality control. The results obtained in these analyses showed a good agreement with the certified values (Table 1).

Arithmetic mean values and concentration ranges of the elements Br, Co, Cr, Mg, Mn and Zn obtained in *Brachiaria decumbens* forage of 6 blocks are presented in Table 2.

Table 1. Concentrations of Br, Cr, Co, Fe, Mg, Mn and Zn obtained in certified reference materials by INAA

Element, unit	This work (mean ± SD)[a]	Certified values
Br, $\mu g\cdot g^{-1}$	1.76 ± 0.06	(1.8)[b]
Cr, $\mu g\cdot g^{-1}$	36 ± 3	(39)[c]
Co, $\mu g\cdot g^{-1}$	0.096 ± 0.006	(0.09)[b]
Fe, $\mu g\cdot g^{-1}$	77 ± 3	83 ± 5[b]
Mg, $mg\cdot g^{-1}$	2.6 ± 0.2	2.71 ± 0.08[b]
Mn, $\mu g\cdot g^{-1}$	53 ± 4	54 ± 3[b]
Zn, $\mu g\cdot g^{-1}$	12.2 ± 0.5	12.5 ± 0.3[b]

[a] Mean and standard deviation from 4 individual determinations.
[b] NIST 1515 Apple Leaves; [c] NIST 2710 Montana Soil.
Numbers in parentheses are reference values.

Table 2. Mean values and concentration ranges of elements in *Brachiaria decumbens* forage for different limestone doses

Treatment	Element contents					
	Br, $\mu g\cdot g^{-1}$	Co, $\mu g\cdot kg^{-1}$	Cr, $\mu g\cdot kg^{-1}$	Mg, $mg\cdot g^{-1}$	Mn, $\mu g\cdot g^{-1}$	Zn, $\mu g\cdot g^{-1}$
T0	7 (6–10)	50 (23–64)	761 (238–1435)	2 (2–3)	166 (128–259)	26 (19–32)
T1	6 (5–8)	46 (34–55)	743 (211–1056)	3 (2–5)	140 (88–197)	26 (20–32)
T2	6 (5–6)	41 (36–95)	549 (452–625)	3 (2–3)	123 (105–156)	23 (18–32)
T4	6 (5–7)	34 (17–66)	514 (267–635)	3 (2–3)	99 (83–120)	20 (16–26)
T8	5 (5–7)	40 (29–58)	534 (369–989)	3 (2–3)	95 (50–124)	20 (15–23)
F-test	L**	L**	L*	L*	L**	L**

* (P<0.05) and ** (P<0.01) for *F*-test; L = linear component.

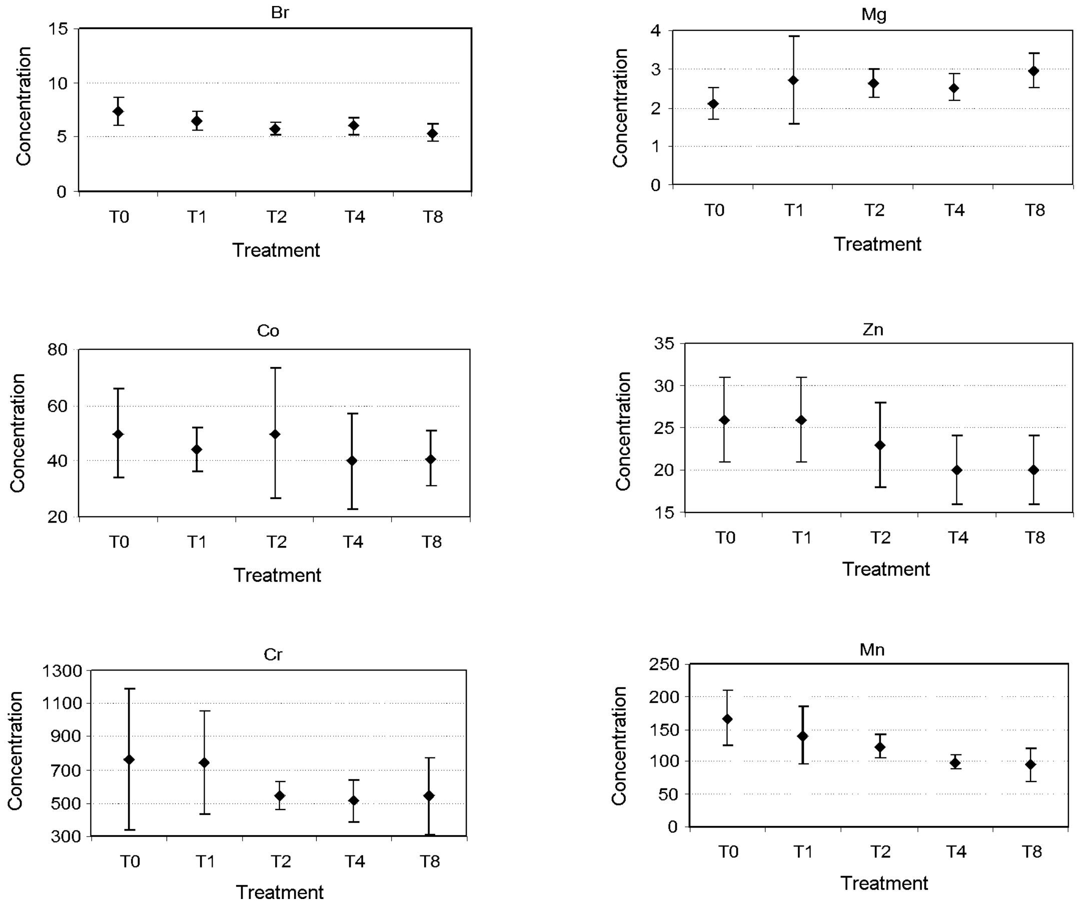

Fig. 1. Mean concentrations of elements and their variation for each treatment

Figure 1 shows the variation of element concentration determined in *Brachiaria decumbens* forage within each treatment. The uncertainty of individual determinations, considering the statistical nature of counting, varied from 1 to 7% according to the sensitivity and concentration of the element in the forage. The observed variation of the element concentration within each treatment, in excess to the statistical counting error, was presumably on account of the biological variations of the element uptake and the variations of the soil composition among the different blocks. Nevertheless, differences in the Br, Co, Cr, Mg, Mn and Zn absorption by *Brachiaria decumbens* versus limestone doses could be evaluated from results of analysis of variance (P<0.01) and (P<0.05) applied to all analytical results. A negative linear correlation was found for Br, Co, Cr, Mn and Zn contents in forage with the limestone doses, due to the increase of soil-pH decreasing their availability for plants for Mn and Zn,[2] while a reverse trend for Mg was observed due to its input with limestone.To confirm these trends of element absorption by the *Brachiaria decumbens*, and the influence on forage yield, other experiments are in progress.

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