

Study on the mineral extraction of legume and grass species from various soil types, by instrumental neutron activation analysis

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Instrumental neutron activation analysis (INAA), followed by gamma-ray spectrometry, was used to determine the concentration of K, Ca, Mg, Na, Zn, Fe, Mn, Mo, Co, Cr, La, Eu and Th in six species of legumes and three species of grasses. Each species of forage was cultivated on two different oxisols, that is, a red yellow Latossol and a dark red Latossol, with the aim of comparing the influence of the soils in the mineral extraction. Besides, on each kind of soil, two different limestone concentrations were used in order to verify how the soil pH correction could influence the elemental absorption in each species, and at the same time; to search for an optimum value of limestone concentration for each soil.

Introduction¹²

There is a continuous search for forage species more adapted to the environmental conditions, like soil, climate and vegetation. The adaption study of grasses and legumes to different cultivation regions need, therefore, to consider climatic, soil and management parameters.¹ Besides this, the vegetal species, with their preferential accumulation of certain elements could be used as mineral contamination and depository indicators of the area where they grow, considering that the mineral composition of plants can reflect the chemical composition of the soil.² The extension in which this relation exists, is highly variable and governed by many factors.³ Furthermore, with the appearance of the precision agriculture and the intensification of the agricultural production systems, we need a more precise knowledge of the mineral mobilization and extraction potential of plants used as green manure for cycling minerals, or by forage plants returned as faeces, or of the mineral exportation when plant parts are taken out of the growing place. And this not only with the essential minerals for plants and animals, but also with those introduced in the production systems as impurities in limestones, fertilizers etc., and which could accumulate to toxic levels in the soil.

The aim of this work was to study the influence of soil on the quali and quantitative extraction of minerals by many legume and grass species, cultivated on two types of oxisols, a red yellow Latosol, and a dark red Latosol. Each soil was treated with grinded dolomitic limestone to increase the base saturation to 50% and 75%, to verify how the pH correction of the soil affect

the elemental absorption by each vegetal species, and find the adequate limestone to reach the optimal plant production on each soil, to avoid unnecessary expenditure and eventual damage on mineral absorption.

The analysed plant material could be used as food for animals, as source of organic material and recycled nutrients to soil and/or energy source for microorganisms, or also, to improve the physical, chemical and biological characteristics of the soil and be a mineral source.

In view of the high sensitivity and possibility of multielemental analyses, instrumental neutron activation analysis (INAA) followed by gamma-ray spectrometry was used as a powerful analytic option to verify the chemical composition of different sources,^{4–6} and to determine the K, Ca, Mg, Na, Zn, Fe, Mn, Mo, Co, Cr, La, Eu and Th content of the whole 90-day old plants (leaf, stalk and flowers).

Experimental

Sample preparation

In the experimental site of the Southeastern Bovine Research Center (CPPSE-EMBRAPA), in São Carlos, SP, Brasil six legume and three grass species on two Oxisols namely, a red yellow Latosol (LV) and a dark red Latosol (LE) were grown each corrected to 50% and 75% base saturation, by application of dolomitic limestone 1.0 and 3.2 t/ha in the LV, respectively, 0.5 and 2.7 t/ha in the LE, besides 120 and 180 kg/ha of P₂O₅ as supertriple superphosphate, in both soils, and also 141 and 254 kg/ha in the LV, and 100 and 150 kg/ha K₂O as KCl in the LE.

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The legume species were labe-labe (*Dolichus lablab*), feijão-de-porco (*Canavalia ensiformis*), mucuna-preta (*Mucuna aterrima*), mucuna-cinza (*Mucuna cinerea*), crotalária-juncea (*Crotalaria juncea*), crotalária-espectabilis (*Crotalaria spectabilis*); and the grass species were: sorgo-forrageiro (*Sorghum bicolor*), milheto (*Pennisetum americanum*) and milho (*Zea mays*). At 90 days of development, the fresh matter were harvested and chopped. Samples were taken, put in identified paper bags, oven dried at 60 °C with forced air circulation. These dried samples were ground in Willey mills and passed through 20 mesh sieve (0.84 mm).

For irradiation, 200 mg samples were taken, transferred to polyethylene envelopes, previously treated with p.a. HNO₃ 1:5, to eliminate probable impurities.

Standard preparation

The standards were prepared from spectroscopic pure dissolved elements or their compound solutions. Quantities of 25 or 50 µl, depending on the solution concentration, were transferred, with micropipettes, to 1 cm² surface Whatman N.41 filter paper.

The standards contained: K (395 µg); Ca (40 mg); Mg (550 µg); Na (67.44 µg); Zn (25.17 µg); Fe (181 µg); Mn (4.49 µg); Mo (50.115 µg); Co (1.115 µg); Cr (2.56 µg); La (2.55 µg); Eu (2.20 µg) and Th (9.52 µg).

For gamma-ray measurement, the standards were taken in four groups: (a) Ca and Mg; (b) K, Mn and Na; (c) Mo and La, and (d) Fe, Zn, Co, Cr, Eu and Th.

Irradiation and gamma-radiation measurements

The samples and the standards of groups (a) and (b) (Ca, Mg, Mn and Na), were irradiated in a nylon container, together with a thermal neutron flux of $0.43 \cdot 10^{12} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, for 3 minutes, in the IEA-R1 reactor.

After irradiation, samples and standards were transferred to proper container for gamma-radiation measurement.

The gamma-spectrum of each sample was measured twice. In the first time, each sample was measured for 4 minutes, after a cooling time of 3 minutes, for each photopeak of the following radionuclides: ⁴⁹Ca in 3083 keV and ²⁷Mg in 1014 keV. After, the standards of group (a) and (b) were measured for 3 and 6 minutes, respectively. The second sample was measured after a cooling time of 15 minutes. In this case each sample was counted for about 12 minutes for each gamma-photopeak.

To analyse the elements of groups (c) and (d) (Mo, Fe, Zn and Co), the samples and standards were irradiated together, in an aluminum container, under a

$1.84 \cdot 10^{12} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ thermal neutron flux, for 8 hours, in the IEA-R1 reactor. After irradiation, the material was left to cool for 3 days, after which the samples and standards were transferred to proper containers for the first measurement of gamma-radiation. The spectra of gamma-rays of each sample was measured for 3 hours for the (c) group; which photopeak is detectable on 140 keV for ⁹⁹Mo and on 1596 keV for ¹⁴⁰La. The gamma-radiation of group (d) was measured after 10 days more cooling of the samples, for 3 hours. This additional cooling time was necessary to get a cleaner spectra, without interfering radionuclides like ⁸²Br, ⁴²K, ²⁴Na, having half-lives up to 35 hours.

Therefore, the second count allows the photopeak measurement of following radionuclides: ⁵⁹Fe on 1099 keV, ⁶⁵Zn on 1115 keV, ⁶⁰Co on 1173 keV, ⁵¹Cr on 320 keV, ¹⁵²Eu on 1408 keV and ²³³Pa on 312 keV, this formed from Th.

After counting, the photopeak area of interest of the sample were compared to that of the standards, to calculate the elemental concentration.

The equipment used for measure the gamma-radiation was a hyperpure Ge detector from EG & Ortec, Model 20195, with a 1.95 keV resolution for the ⁶⁰Co photopeak of 1332 keV. Coupled to the detector was an electronic system with a 8000-channel Buffer-918A, from EG & Ortec, an amplifier, a high tension source and a microcomputer, to analyse the multichannel stored data, with a program in "turbo basic" language. The data obtained by INAA method were submitted to a variance analyses (*F*-test) and a correlation analyses (*r* value) with the results of the same sample by routine methods.⁷

Results and discussion

Tables 1 and 2 present the extraction values, per surface unity (ha), from K, Ca, Mg, Fe (in kg/ha); Na, Zn, Mn, Mo, Co, Cr, La, Eu and Th (in g/ha), calculated from the elemental concentration in dry matter and the dry matter yield per hectare (t/ha) of legume and grass forage crops. It could be seen that:

The correlation analysis between the analytical data obtained by the INAA method and the routine method, showed *r* values of 0.99** for Mn, 0.96** for Fe, 0.95** for Ca, 0.71** for K, 0.69** for Mg and 0.51** for Zn.

From the 13 analysed elements, in both soils, only Mo, Th and Eu appeared in concentration levels below the detection limit in many of the samples. The detection limit values were calculated in accordance with the literature,⁸ and varied from 130 to 610 ng/g for Mo, from 1 to 14 ng/g for Eu and from 7 to 30 ng/g for Th.

The grasses showed and higher extraction potential for K, Mg, Zn, Na, Mo, Co (*P*<0.01), and tendency for

Mn and Cr. The legumes extracted more Ca ($P<0.01$), with tendency for more La.

Pennisetum americanum appeared as the species with the highest extraction of K, Mg, Mn, Zn, Na, Co, as well as of Fe, Mo, La, Eu, Th, due principally to their higher dry matter yield. Under the legume species appeared *Mucuna cinerea* with high extraction of La, Eu, and also Ca, Mn, Zn, Mo and Th.

On the dark red Latosol (LE) happened the highest Ca extraction by plant species ($P<0.01$), with a tendency for higher K, Mg, Zn and Mo uptake. On the red-yellow Latosol (LV) the extraction was higher for Mn ($P<0.01$) and La ($P<0.05$), with tendency for higher Fe, Eu and Th uptake by the species. Also for the same dry matter yield of *Mucuna cinerea*, 2.8 t/ha, at 75% base saturation; the K extraction by plants cultivated on LE was approximately twice as on LV. *Pennisetum americanum* growing on LE showed higher K, Ca, Mg, Na, Zn and Mo extraction as on LV.

Considering the base saturation of soils (V), with 75% saturation, it occurred a higher extraction of Ca ($P<0.05$) and tendency for higher Mg and Mo extraction. *Mucuna cinerea* with 75% base saturation, was between the species with the highest extraction values for the most of the elements, except Cr on LV; and *Crotalaria spectabilis* with V of 50%, presented generally the lowest extraction values, except for K, Mn and La.

The dry matter yield values were higher for species growing on LE.

Qualitatively, the so-called essential elements are indispensable for all vegetal species, but quantitatively the requirements vary. So, as showed by the results in this work, and in literature, the legumes have a bigger need of Ca as grasses; and in the same family, different genders show different requirements from a given element.⁹ The amount of minerals taken up by plants could also preview the amount of reposition need in form of fertilizer or limestone, principally Ca and Mg. The other elements are exported in minimal amounts, considering their presence in soil.¹⁰

Considering the different amounts in limestone used, the species grown on LV showed a slowly higher mineral uptake with increasing limestone. But, this could not allow us to generalize that this favours or reduces the element extraction. It could not be seen the same effect of limestone used on the element extraction by the same species on different soils.

Among the species grown on LE, the legume *Crotalaria juncea* and the grass *Zea mays* showed the biggest benefit from the limestone use; both increasing the extraction of 10 elements, from 13 analysed. *Crotalaria spectabilis* reduced the extraction of the analysed elements, with increase of limestone use.

Among the species grown on LV, the legumes *Crotalaria spectabilis* and *Mucuna cinerea* were the most favoured with the increase of limestone use, extracting more of 12 and 10 minerals, respectively.

Mucuna aterrima was less favoured with the increased use of limestone, changing the base saturation from 50 to 75%.

From the dry matter yield data, it could be seen, that in most cases, the base saturation of 50% on LE, originally richer in nutrients, was sufficient for a normal nutrient absorption by the forage species. On LV, with lower soil fertility, in general, the adequate amount of limestone was that needed to reach a base saturation of 75%.

Conclusions

The instrumental neutron activation analysis (INAA) showed to be useful for essential mineral analysis and/or trace elements in forage plants, in most of the cases with a precision lower than 15%. It also allows a multielement analysis, including other nutrients of interest for agriculture and other correlated areas. With this analytical method it is possible to analyse a big number of samples, with minimal prepared manipulation, and acceptable time use.¹¹

It is advised to search for the plant response to trace elements in soil and air for each particular soil-plant system;³ in view of the dependence of the mineral concentration in forage crops from the interaction of different factors, including, besides soil, the species and maturity stage of the plant, pasture management and climate.¹² An example showing the relation plant-soil-fertilizer use, is the *Crotalaria spectabilis*, favoured with limestone use in LV, increasing the extraction of 12 of 13 analysed elements, and depressed in LE.

Despite of the well understood metabolic sink and the function of each nutrient in plant, additional experiments are needed for many other trace elements. So, the concentration values for La, Eu and Th reinforce the importance of such kind of work and the analytical method used, in view of lack of information on these elements, which could be absorbed, accumulated and be harmful metabolically for plants and animals, an

intensive production systems due the increased use of fertilizers and other plant and animal food.

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Table 1. Extraction values of analysed elements by legume and grass species grown on dark-red Latosol

Species	V, %	Dry matter	K	Ca	Mg	Na	Zn	$\times 10^2$ Fe	Mn	Mo	$\times 10^2$ Co	$\times 10$ Cr	$\times 10^2$ La	$\times 10^2$ Th	$\times 10^2$ Eu
Lablab	50	4.2	65 ± 5	49 ± 1	13 ± 1	63 ± 13	71 ± 4	144 ± 10	269 ± 8	ND	105 ± 10	19 ± 5	316 ± 2	24 ± 4	ND
	75	5.2	92 ± 8	75 ± 1	13 ± 1	78 ± 10	78 ± 5	138 ± 10	197 ± 5	420 ± 50	66 ± 3	24 ± 2	176 ± 11	27 ± 2	ND
Feijão de porco	50	4.3	100 ± 10	58 ± 1	7 ± 1	56 ± 9	52 ± 4	148 ± 10	292 ± 9	200 ± 20	61 ± 5	33 ± 4	141 ± 1	29 ± 3	12 ± 1
	75	3.4	64 ± 4	62 ± 1	11 ± 1	31 ± 7	61 ± 3	65 ± 10	235 ± 10	ND	79 ± 6	10 ± 3	174 ± 1	13 ± 2	14 ± 1
Mucuna preta	50	2.3	43 ± 6	28 ± 1	6 ± 1	35 ± 7	41 ± 2	39 ± 3	255 ± 7	100 ± 20	49 ± 4	5 ± 2	103 ± 1	5 ± 2	8 ± 1
	75	2.8	59 ± 6	22 ± 1	5 ± 1	ND	36 ± 3	67 ± 1	101 ± 3	210 ± 30	65 ± 5	9 ± 3	162 ± 1	17 ± 2	4 ± 1
Mucuna cinza	50	7.9	92 ± 9	32 ± 1	11 ± 1	87 ± 8	327 ± 8	114 ± 10	261 ± 8	570 ± 80	51 ± 3	30 ± 6	56 ± 1	18 ± 4	3 ± 1
	75	7.5	111 ± 12	49 ± 1	13 ± 1	75 ± 15	146 ± 8	114 ± 10	525 ± 8	320 ± 80	53 ± 3	17 ± 4	170 ± 3	21 ± 4	ND
Crotalaria juncea	50	4.8	75 ± 6	31 ± 1	11 ± 1	62 ± 10	77 ± 5	47 ± 3	394 ± 10	ND	100 ± 10	10 ± 4	184 ± 2	9 ± 3	17 ± 1
	75	6.2	65 ± 5	57 ± 1	18 ± 2	93 ± 12	111 ± 6	78 ± 7	546 ± 12	680 ± 130	177 ± 10	80 ± 8	521 ± 3	ND	58 ± 2
Crotalaria spectabilis	50	2.5	59 ± 5	40 ± 1	10 ± 1	44 ± 8	68 ± 3	57 ± 5	185 ± 5	ND	68 ± 4	15 ± 4	ND	ND	37 ± 2
	75	2.2	54 ± 6	27 ± 1	5 ± 1	18 ± 2	37 ± 2	50 ± 4	121 ± 4	180 ± 30	38 ± 4	9 ± 1	285 ± 1	ND	28 ± 1
Sorgo	50	7.0	127 ± 8	22 ± 2	22 ± 3	70 ± 14	94 ± 7	57 ± 5	581 ± 21	208 ± 130	204 ± 10	28 ± 5	71 ± 1	ND	18 ± 1
Forrageiro	75	9.6	180 ± 13	20 ± 1	18 ± 2	134 ± 29	88 ± 10	51 ± 5	451 ± 10	977 ± 200	203 ± 10	50 ± 7	67 ± 1	ND	10 ± 2
Milheto	50	16.0	440 ± 47	38 ± 2	42 ± 4	496 ± 32	322 ± 16	152 ± 10	2016 ± 48	490 ± 190	251 ± 10	29 ± 6	326 ± 4	32 ± 6	43 ± 2
	75	16.4	418 ± 74	44 ± 3	43 ± 6	361 ± 33	279 ± 16	105 ± 20	1115 ± 33	1370 ± 260	686 ± 50	ND	284 ± 5	ND	57 ± 13
Milho	50	8.3	93 ± 6	9 ± 1	11 ± 1	183 ± 25	108 ± 8	88 ± 5	266 ± 8	ND	110 ± 10	44 ± 5	7.1 ± 0.2	8 ± 2	10 ± 1
	75	9.9	133 ± 12	12 ± 1	23 ± 2	158 ± 20	158 ± 10	125 ± 10	396 ± 10	1010 ± 190	170 ± 10	59 ± 14	22.2 ± 0.4	31 ± 2	ND

ND – Not detected in analytical conditions; - = plant death due to mineral deficiency.
 Extraction values from K, Ca, Mg and Fe in kg/ha; and from Na, Zn, Mn, Mo, Co, Cr, La, Th and Eu in g/ha.
 Dry matter, yield in kg/ha; V = base saturation, in %.

Table 2. Extraction values of analysed elements by legume and grass species grown on red-yellow Latosol

Species	F, %	Dry matter	K	×10 Ca	×10 Mg	Na	Zn	×10 ² Fe	Mn	×10 Mo	×10 ² Co	×10 Cr	×10 La	×10 ² Th	×10 ² Eu
Lablab	50	2.3	25 ± 2	139 ± 6	26 ± 6	48 ± 7	46 ± 2	590 ± 10	1109 ± 7	19 ± 4	91 ± 10	148 ± 10	200 ± 1	136 ± 3	ND
	75	4.5	75 ± 4	409 ± 16	117 ± 15	77 ± 5	59 ± 5	380 ± 10	2295 ± 36	ND	162 ± 10	73 ± 9	463 ± 3	99 ± 7	ND
Feijão de porco	50	1.0	19 ± 2	72 ± 3	19 ± 3	ND	15 ± 1	39 ± 3	600 ± 19	4 ± 1	30 ± 2	6 ± 1	58 ± 1	8 ± 1	7 ± 1
	75	3.7	76 ± 7	452 ± 13	89 ± 15	ND	44 ± 4	48 ± 5	1735 ± 41	ND	120 ± 10	ND	247 ± 2	13 ± 4	28 ± 2
Mucuna preta	50	2.8	28 ± 2	231 ± 9	51 ± 6	ND	90 ± 3	116 ± 10	1498 ± 28	15 ± 5	96 ± 4	22 ± 3	730 ± 8	30 ± 2	44 ± 1
	75	2.8	31 ± 3	179 ± 5	47 ± 4	42 ± 6	53 ± 3	83 ± 4	658 ± 17	18 ± 2	68 ± 4	21 ± 3	460 ± 5	16 ± 2	32 ± 1
Mucuna cinza	50	2.0	6 ± 1	112 ± 5	19 ± 3	ND	70 ± 2	77 ± 10	1216 ± 24	ND	64 ± 3	14 ± 2	855 ± 5	18 ± 1	ND
	75	6.5	114 ± 11	622 ± 24	215 ± 27	72 ± 33	150 ± 7	12 ± 1	4427 ± 26	ND	190 ± 10	21 ± 4	1353 ± 11	54 ± 4	140 ± 3
Crotalaria juncea	50	0.0													
	75	1.4	17 ± 2	109 ± 3	46 ± 4	15 ± 1	25 ± 1	45 ± 3	98 ± 1	9 ± 3	35 ± 2	12 ± 2	89 ± 1	ND	9 ± 1
Crotalaria spectabilis	50	0.5	14 ± 1	31 ± 2	7 ± 1	7 ± 2	16 ± 1	38 ± 1	195 ± 6	3 ± 1	26 ± 1	6 ± 1	91 ± 2	8 ± 1	10 ± 2
	75	2.0	44 ± 3	250 ± 9	68 ± 8	26 ± 6	36 ± 2	55 ± 10	804 ± 20	ND	68 ± 4	19 ± 3	363 ± 4	15 ± 2	35 ± 1
Sorgo	50	0.0													
	75	1.4	23 ± 2	31 ± 1	32 ± 2	13 ± 3	78 ± 3	84 ± 10	101 ± 3	7 ± 2	45 ± 2	45 ± 3	18 ± 1	12 ± 1	2 ± 1
Forrageiro	50	11.9	273 ± 20	132 ± 13	215 ± 29	381 ± 60	233 ± 12	130 ± 10	2642 ± 60	ND	430 ± 20	51 ± 14	644 ± 4	ND	88 ± 5
	75	15.9	310 ± 37	226 ± 16	251 ± 22	366 ± 48	204 ± 16	200 ± 10	2926 ± 95	89 ± 21	150 ± 10	47 ± 6	442 ± 8	41 ± 5	59 ± 1

ND – Not detected in analytical conditions; - = plant death due to mineral deficiency.

Extraction values from K, Ca, Mg and Fe in kg/ha; and from Na, Zn, Mn, Mo, Co, Cr, La, Th and Eu in g/ha.

Dry matter, yield in kg/ha; F = base saturation, in %.