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Effects of fertilizer with different mineral composition on the absorption of Ca, Cu, K, Mg, Mn, Na and V, by two cultivars of pigeonpea (*Cajanus cajan*, Millsp)

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Seventy-two leaf samples belonging to two cultivars of *Cajanus cajan* Millsp were analysed by instrumental neutron activation analysis (INAA). The samples came from plants treated with two doses of fertilizer containing each of the following elements: B, Co, Cu, Fe, Mn, Mo, V and Zn, which were applied, individually, to the soil. The leaf samples were yielded at two different times. The purpose of this paper is to elucidate the influence of each fertilizer, the dose and leaf harvest time, on the concentrations of Ca, Cu, K, Mg, Mn, Na and V, and the behavior of both cultivars in relation to the concentrations of these elements.

Introduction

Pastures constitute the main component of ruminant diet, particularly, in tropical regions. Animals depend on pasturages and water to obtain energy, proteins, vitamins and minerals to supply their physiological demands. For this reason, the unbalance of minerals in soil or forage, can be identified as one of the most important factors for the low production and reproductive problems of ruminants.[1] A decline in the nutritional value of this food occurs in the dry season, which results in a decrease of crude protein and some macro and micro minerals intake. Providing high quality forage to the animals could be a way to compensate the deficiency in the cattle's diet.[2,3]

Pigeonpea (*Cajanus cajan,* Millsp), an easy cultivation legume species and adapted to Brazilian conditions, has been used as an economic source of proteins for ruminant supplemental feeding during the drought period. However, in spite of the several possibilities and increasing utilization of pigeonpea, data about the composition of microelements and trace elements of this forage species are scarce.

The neutron activation analysis method was applied to determine the concentrations of Ca, Cu, K, Mg, Mn, Na and V in samples of leaves, belonging to two cultivars of pigeonpea, G3 (EPAMIG 1822) and G36 (EPAMIG 1679). These plants were submitted to two doses of fertilizer with different compositions, and harvested in two different times, in a four-month interval.

Experimental

Sample preparation

Leaves of seventy-two samples, belonging to two cultivars of pigeonpea, G3 (or EPAMIG 1822), and G36 (or EPAMIG 1679), were selected for this work by the Southeastern Bovine Research Center (CPPSE-EMBRAPA), in São Carlos, SP, Brazil. The plants were grown on a dark red Latosol (Hapludox), and submitted to two doses of fertilizer, as shown in the Table 1.

A control group of plants were grown on a soil without any mineral fertilizers in order to estimate the effect of the fertilizer addition. The leaves were harvested at two different times, in a four-month interval. The leaves, including veins and sheet, were oven dried at 65 °C, during about 48 hours, under forced air circulation. The dried samples were ground in Willey mills and passed through a 20-mesh sieve (0.84 mm) to prepare a homogeneous material.

For irradiation, 200 mg ground samples were transferred to polyethylene envelopes previously cleaned with a solution of 1:5 p.a. nitric acid.

Standard solutions preparation

Standard solutions were prepared by dissolving spectroscopically pure elements or compounds with acids. Quantities of 25 µl were transferred with micropipettes to a 1 cm^2 surface Whatman Nr. 41 filter paper.

The standards contained the following element masses: Ca-1734.5 µg; Cu-52.85 µg; K-999.7 µg; Mg-522 µg; Mn-3.25 µg; Na-163 µg and V-26.22 µg.

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Table 1. Concentrations of elements present in the fertilizers used to treat the pigeonpea cultivars

Mineral micronutrient in the fertilizer	Concentration, kg/ha	
	Single dose	Double dose
B	1	2
Co	0.2	0.4
Cu	1	2
Fe	3	6
Mn	2	4
Mo	0.1	0.2
V	0.1	0.2
Zn	3	6

For gamma-ray measurements, standards were arranged in three groups: group 1: Cu and V; group 2: Ca and Mg; and group 3: K, Mn and Na.

Irradiation and gamma-radiation measurement

Samples and standards of the elements under investigation were irradiated together in a nylon container in a thermal neutron fluence rate of $1.4 \cdot 10^{11}$ $n \cdot cm^{-2} \cdot s^{-1}$, in the IEA-R1m reactor. The irradiation time was 2 minutes.

The gamma-ray spectra of the samples were measured twice in the conditions given in the Table 2.

Table 2. Experimental conditions

Irradiation time (thermal neutron fluence rate)	Decay time, min	Measurement time, min	Radioisotope measured, gamma-ray energy, keV
2 min $1.4 \cdot 10^{11}$ $n \cdot cm^{-2} \cdot s^{-1}$	2	4	^{49}Ca, 3085 ^{66}Cu, 1039 ^{27}Mg, 1014 ^{52}V, 1434
	90	15	^{42}K, 1525 ^{56}Mn, 1811 ^{24}Na, 1369

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

The gamma-ray spectra were measured using an EG&G Ortec, model GEM 20195, HP Ge detector with a 1.95 keV FWHM resolution for the ^{60}Co photopeak at 1332 keV. The detector was coupled to an electronic system with an EG&G Ortec 8000-channel BUFFER-918A. Data analysis was carried out using an IBM/PC microcomputer and a VISPECT2 software in Turbo Basic language.

Results and discussion

The concentrations of the elements investigated were evaluated as the mean of 3 independent determinations. The data are shown in the Tables from 3 to 9. The precision of the analysis expressed as the relative standard deviation ranged from 5% to 20% for most of the results. In the seventy-two samples analyzed, including the control plants, the concentrations of the elements of interest varied in the following ranges: from 3890 to 12315 μg/g for Ca; from 10 to 26 μg/g for Cu; from 9 to 23 mg/g for K; from 1304 to 2623 μg/g for Mg; from 46 to 235 μg/g for Mn; from 7 to 29 μg/g for Na and from 48 to 410 μg/kg for V.

To evaluate if there are significant changes occurred of the concentrations of Ca, Cu, K, Mg, Mn, Na and V in the leaves of both cultivars of pigeonpea, which were treated with two doses of the fertilizers (B, Co, Cu, Fe, Mn, Mo, V and Zn) and harvested in different times, the analysis of variance test at the 5% significance level[4] was applied to all sets of the results obtained in this work. To verify if there is any significative alteration in the absorption of the elements under investigation by the fertilized plants compared to the control plants, DUNNETT's test[5] at the 5% significance level was used. From the results obtained by the above statistical treatments, the following conclusions on the behavior of the elements studied were drawn:

Calcium

The fertilization with the single or double dose of Fe and V increased the Ca concentration of the first harvest leaves of the cultivar G3, whereas for the cultivar G36 the fertilization with the single dose of B resulted in a decrease of the Ca concentration in the first harvest leaves (Table 3).

Table 3. Ca mean concentrations (in µg/g) for 3 independent determinations in pigeonpea leaves

Cultivar	G3				G36			
Harvest time	1		2		1		2	
Fertilizer dose	1	2	1	2	1	2	1	2
Fertilizer	Pigeonpea samples under investigation							
B	6427 ± 353	5997 ± 397	5357 ± 150	5821 ± 242	6314 ± 666	5715 ± 201	5251 ± 232	5300 ± 193
Co	6700 ± 152	6002 ± 587	5821 ± 410	5424 ± 522	10228 ± 1065	12315 ± 659	6253 ± 1016	8322 ± 632
Cu	8138 ± 1141	6542 ± 477	5502 ± 277	5616 ± 433	8038 ± 740	8246 ± 1276	6960 ± 1371	4010 ± 362
Fe	10753 ± 585	11658 ± 290	4529 ± 478	6280 ± 595	8953 ± 1404	9441 ± 882	5509 ± 717	6184 ± 463
Mn	9095 ± 867	8146 ± 378	3890 ± 438	4529 ± 538	7634 ± 471	8898 ± 1397	7688 ± 784	4233 ± 351
Mo	6255 ± 116	6254 ± 691	5304 ± 558	4135 ± 307	8964 ± 1313	11884 ± 1768	5157 ± 123	4680 ± 676
V	9634 ± 767	9018 ± 627	5772 ± 1268	4480 ± 703	7534 ± 435	9448 ± 1613	6560 ± 1193	7079 ± 764
Zn	6145 ± 350	6430 ± 146	5354 ± 601	5500 ± 875	7642 ± 1026	6149 ± 676	6288 ± 603	5560 ± 660
Control	7295 ± 1003	6693 ± 724	6950 ± 536	5447 ± 173	10547 ± 1398	9501 ± 966	5824 ± 697	6251 ± 378

Table 4. Cu mean concentrations (in µg/g) for 3 independent determinations in pigeonpea leaves

Cultivar	G3				G36			
Harvest time	1		2		1		2	
Fertilizer dose	1	2	1	2	1	2	1	2
Fertilizer	Pigeonpea samples under investigation							
B	13 ± 1	13 ± 4	12 ± 2	21 ± 7	14 ± 3	16 ± 2	14 ± 3	12 ± 2
Co	17 ± 3	18 ± 2	18 ± 5	16 ± 2	18 ± 1	23 ± 2	13 ± 4	18 ± 4
Cu	25 ± 7	16 ± 9	18 ± 1	16 ± 5	16 ± 5	21 ± 7	23 ± 3	10 ± 2
Fe	15 ± 3	13 ± 2	20 ± 7	18 ± 3	14 ± 3	14 ± 3	14 ± 2	12 ± 3
Mn	17 ± 2	26 ± 8	17 ± 5	20 ± 4	16 ± 2	17 ± 4	15 ± 2	14 ± 2
Mo	15 ± 4	14 ± 2	23 ± 9	14 ± 4	17 ± 2	17 ± 4	13 ± 5	17 ± 2
V	17 ± 5	13 ± 2	17 ± 4	16 ± 8	13 ± 4	13 ± 1	15 ± 3	17 ± 2
Zn	20 ± 7	12 ± 2	15 ± 1	23 ± 10	20 ± 2	15 ± 1	17 ± 4	12 ± 2
Control	22 ± 9	16 ± 2	21 ± 3	16 ± 2	17 ± 2	17 ± 3	15 ± 4	15 ± 3

Table 5. Mg mean concentrations (in µg/g) for 3 independent determinations in pigeonpea leaves

Cultivar	G3				G36			
Harvest time	1		2		1		2	
Fertilizer dose	1	2	1	2	1	2	1	2
Fertilizer	Pigeonpea samples under investigation							
B	1715 ± 259	1937 ± 125	1429 ± 136	1702 ± 47	1648 ± 234	1701 ± 122	1620 ± 53	2048 ± 86
Co	1610 ± 57	1932 ± 62	1538 ± 189	1791 ± 150	1737 ± 51	2071 ± 261	1772 ± 215	1787 ± 300
Cu	1786 ± 187	1890 ± 146	1624 ± 147	1450 ± 150	1863 ± 199	2315 ± 254	2070 ± 323	1764 ± 335
Fe	1824 ± 138	2623 ± 376	1571 ± 71	1903 ± 114	1961 ± 218	1653 ± 183	1530 ± 138	1812 ± 242
Mn	1958 ± 241	1927 ± 212	1680 ± 240	1695 ± 74	1941 ± 151	1990 ± 182	1625 ± 179	1671 ± 157
Mo	1905 ± 104	2002 ± 67	1487 ± 187	1599 ± 118	1759 ± 159	1477 ± 90	1670 ± 290	1680 ± 142
V	1485 ± 155	1749 ± 180	1426 ± 21	1304 ± 225	1930 ± 210	1740 ± 349	2037 ± 198	1661 ± 128
Zn	1739 ± 236	1872 ± 46	1643 ± 52	1687 ± 197	1862 ± 184	1685 ± 116	1787 ± 113	1658 ± 196
Control	1808 ± 184	1771 ± 193	1602 ± 34	1551 ± 115	2045 ± 85	2033 ± 453	1938 ± 15	2062 ± 150

Table 6. Mn mean concentrations (in µg/g) for 3 independent determinations in pigeonpea leaves

Cultivar	G3				G36			
Harvest time	1		2		1		2	
Fertilizer dose	1	2	1	2	1	2	1	2
Fertilizer	Pigeonpea samples under investigation							
B	93 ± 11	90 ± 2	47 ± 4	57 ± 8	121 ± 10	235 ± 8	68 ± 8	95 ± 3
Co	122 ± 9	107 ± 13	73 ± 5	61 ± 8	145 ± 20	112 ± 15	62 ± 4	63 ± 9
Cu	102 ± 5	73 ± 7	48 ± 5	55 ± 7	234 ± 3	154 ± 34	107 ± 20	52 ± 5
Fe	112 ± 15	117 ± 3	55 ± 2	65 ± 4	113 ± 4	121 ± 4	47 ± 5	63 ± 9
Mn	124 ± 9	135 ± 10	49 ± 1	65 ± 11	84 ± 8	117 ± 18	50 ± 3	51 ± 7
Mo	148 ± 13	127 ± 17	95 ± 13	70 ± 17	164 ± 12	124 ± 21	77 ± 2	60 ± 2
V	177 ± 16	114 ± 4	86 ± 4	53 ± 4	101 ± 12	90 ± 13	63 ± 7	58 ± 3
Zn	90 ± 3	87 ± 2	46 ± 4	52 ± 4	108 ± 8	113 ± 5	57 ± 3	62 ± 5
Control	88 ± 6	115 ± 3	64 ± 2	62 ± 5	121 ± 27	208 ± 18	61 ± 3	96 ± 4

Table 7. K mean concentrations (in µg/g) for 3 independent determinations in pigeonpea leaves

Cultivar	G3				G36			
Harvest time	1		2		1		2	
Fertilizer dose	1	2	1	2	1	2	1	2
Fertilizer	Pigeonpea samples under investigation							
B	20 ± 2	21 ± 2	21 ± 1	16 ± 1	22 ± 4	17 ± 1	17 ± 2	9 ± 1
Co	22 ± 1	21 ± 1	14 ± 3	15 ± 1	16 ± 2	15 ± 1	13 ± 1	10 ± 1
Cu	20 ± 2	15 ± 3	16 ± 1	16 ± 3	15 ± 3	15 ± 3	14 ± 1	13 ± 1
Fe	16 ± 1	13 ± 2	20 ± 2	17 ± 1	16 ± 3	15 ± 2	15 ± 2	10 ± 1
Mn	17 ± 1	16 ± 1	17 ± 2	16 ± 1	17 ± 4	17 ± 3	14 ± 1	17 ± 1
Mo	23 ± 3	22 ± 2	21 ± 4	17 ± 1	17 ± 2	16 ± 2	21 ± 1	21 ± 1
V	18 ± 1	17 ± 2	20 ± 2	17 ± 1	18 ± 3	22 ± 5	16 ± 3	16 ± 1
Zn	16 ± 1	18 ± 2	16 ± 2	17 ± 2	21 ± 3	20 ± 2	16 ± 1	12 ± 2
Control	22 ± 1	16 ± 2	16 ± 2	15 ± 1	17 ± 3	15 ± 2	12 ± 4	14 ± 1

Table 8. Na mean concentrations (in µg/g) for 3 independent determinations in pigeonpea leaves

Cultivar	G3				G36			
Harvest time	1		2		1		2	
Fertilizer dose	1	2	1	2	1	2	1	2
Fertilizer	Pigeonpea samples under investigation							
B	8 ± 2	13 ± 3	10 ± 1	15 ± 2	16 ± 3	12 ± 4	12 ± 3	10 ± 1
Co	11 ± 3	10 ± 1	10 ± 4	14 ± 1	20 ± 3	12 ± 1	10 ± 1	15 ± 2
Cu	18 ± 8	16 ± 1	11 ± 2	9 ± 2	13 ± 3	14 ± 2	13 ± 2	10 ± 2
Fe	20 ± 2	20 ± 4	29 ± 3	17 ± 3	18 ± 1	11 ± 2	8 ± 2	12 ± 3
Mn	15 ± 3	15 ± 3	14 ± 4	10 ± 2	17 ± 4	12 ± 2	11 ± 2	10 ± 1
Mo	13 ± 3	16 ± 3	16 ± 2	11 ± 3	14 ± 3	20 ± 1	12 ± 2	7 ± 1
V	11 ± 2	12 ± 1	10 ± 1	8 ± 1	15 ± 2	22 ± 3	11 ± 1	10 ± 2
Zn	13 ± 3	14 ± 1	12 ± 1	9 ± 2	14 ± 2	12 ± 2	8 ± 2	11 ± 2
Control	11 ± 1	10 ± 2	16 ± 3	12 ± 2	15 ± 3	12 ± 5	8 ± 2	12 ± 1

Table 9. V mean concentrations (in µg/kg) for 3 independent determinations in pigeonpea leaves

Cultivar	G3				G36			
Harvest time	1		2		1		2	
Fertilizer dose	1	2	1	2	1	2	1	2
Fertilizer	Pigeonpea samples under investigation							
B	226 ± 66	133 ± 21	50 ± 11	66 ± 1	195 ± 47	205 ± 57	162 ± 18	80 ± 12
Co	186 ± 42	255 ± 27	102 ± 25	96 ± 2	380 ± 38	362 ± 32	92 ± 17	125 ± 53
Cu	274 ± 17	233 ± 161	67 ± 14	93 ± 17	410 ± 93	343 ± 70	63 ± 7	77 ± 21
Fe	295 ± 21	368 ± 28	143 ± 24	107 ± 25	291 ± 36	516 ± 15	48 ± 15	130 ± 18
Mn	238 ± 49	328 ± 73	122 ± 20	66 ± 8	222 ± 24	349 ± 33	95 ± 13	72 ± 12
Mo	314 ± 4	209 ± 13	62 ± 9	78 ± 21	396 ± 62	352 ± 58	101 ± 23	116 ± 3
V	410 ± 39	297 ± 39	86 ± 14	52 ± 5	351 ± 59	228 ± 30	65 ± 12	113 ± 13
Zn	266 ± 35	290 ± 84	201 ± 22	66 ± 23	366 ± 48	266 ± 37	157 ± 50	116 ± 28
Control	177 ± 32	168 ± 45	97 ± 12	86 ± 13	312 ± 53	367 ± 91	92 ± 13	85 ± 16

Copper

The concentration of Cu in both cultivars did not show any variation with the treatment with different fertilizer doses and different harvesting times (Table 4).

Magnesium

It was the only element, whose concentration was influenced by the fertilizer dose disregarding either the plant age or the cultivar. The Mg concentration of the first harvest leaves of the cultivar G3 increased with the double dose of the Fe fertilizer. In general, the first harvest of this cultivar yielded a higher Mg concentration when the fertilizers was given in the single dose. When both cultivars were compared, it appeared that the second harvest leaves, of the cultivar G36, in general, showed a higher Mg concentration, with the single fertilizer dose (Table 5).

Manganese

The Mn concentration in the first harvest leaves of both cultivars was significatively higher. The Mn absorption of the cultivar G3 increased mainly with the use of Mo and V fertilizers in the single dose. In the cultivar G36 the most important increase occured with the single dose of Cu (Table 6).

Potassium

The application of Mo fertilizer in the single dose increased the K concentration in the second harvest leaves of the cultivar G3. The use of B and Mo in the single dose increased the absorption of K in the second harvest of both cultivars (Table 7).

Sodium

The Na concentration increased in the first harvest of the cultivar G3 when either the Fe or Cu fertilizers were used in both doses or when the double dose of Mo was used. For the second harvest the fertilization with Fe in the single dose also increased the Na concentration. The cultivar G36 showed more changes in the Na concentration, considering the plant age. In general, the Na concentration increased in the first harvest, when the single dose of the fertilizers was applied (Table 8).

Vanadium

For both cultivars, the first harvest leaves yielded a higher V concentration independent of the fertilizer dose. However, the leaves of the cultivar G36 appeared to contain a higher V concentration in relation to the cultivar G3, when the double fertilizer dose was used disregarding the plant age (Table 9).

Conclusions

The results obtained in this work were helpful to elucidate the influence of the mineral fertilizer treatment by the comparison of the concentration values of the elements analysed in the leaf samples, with those of the control plants and in the element absorption behavior of both cultivars as well.

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