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Study on the absorption of Fe, Mn, Mo and Zn by two cultivars of pigeonpea (*Cajanus cajan*, Millsp) submitted to two doses of fertilizers using INAA

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Leaf samples of two varieties of *Cajanus cajan* Millsp were analysed by instrumental neutron activation analysis (INAA). The samples were taken from plants grown under two fertilizer dose conditions, making use of the following microminerals: Fe, Mn, Mo and Zn, which were applied individually to the soil. The samples were collected in two cutting times. We verify the variation in the absorption of each element, considering its availability in the soil.

Introduction

In the animal nutrition area, the imbalance of minerals, deficiencies or excesses, in soil or forage, can be identified as one of the most important parameters for the low production and reproductive problems of ruminants in tropical areas.[1] Animals depend on pasturages and water to obtain energy, proteins, vitamins and minerals to supply their physiological demands. Considering the climatic variability, there is a high disposability of forages in the rainy period, but a low availability of food during the dry season, which results in a lack of proteins, energy and some macro and micro minerals in this season. Providing high quality forage to the animals by means of grazing or its supply in feed bunks could be a way to compensate for the lack of minerals in the cattle's diet.[2,3]

Pigeonpea (*Cajanus cajan*, Millsp) is an easy cultivation legume species, originated from Africa and adapted to the Brazilian conditions, which has been used as an economical source of proteins for ruminant supplemental feeding during the drought period. However, despite the wide-ranging utilization of pigeonpea, data about the composition of micronutrients and trace elements of this forage species are still scarce in the literature.

Leaf samples of two pigeonpea cultivars, which were submitted to two doses of Fe, Mn, Mo and Zn fertilizers, applied individually, and harvested in two cutting times, were analysed by INAA. The interest in these elements is that they play an important role in animal and plant nutrition or, on the other hand, they could be toxic, as for example, Mo under some practical feeding situations.[4] The purpose of this work was to verify the influence of these minerals on the absorption of each element by the leaves, compared to the test plants.

Instrumental neutron activation analysis (INAA), followed by gamma-spectrometry, offers a powerful option to verify the chemical composition of the most different matrixes,[5–7] due to its high sensitivity and multielemental analysis capability. INAA is also an attractive technique because it meets the requirement of minimum sample manipulation, avoiding eventual contamination, mainly when the goal is to analyse trace elements.

Experimental

Sample preparation

The first three mature leaves of two cultivars of pigeonpea, EPAMIG 1822 (G3; adapted to low fertility soils) and EPAMIG 1679 (G36; adapted to medium to high fertility soils), selected for this study, were produced by the Southeast Cattle Research Center (CPPSE-EMBRAPA), in São Carlos, SP, Brazil. The plants were grown on a dark red Latosol (Hapludox), submitted to no dose, one dose or two doses of fertilizer containing Fe, Mn, Mo and Zn.

The leaves were harvested in two cutting times, the first on 6 May 1992 (116 days old plants) and the second at 2 March 1993 (211 days old regrowth), from biomass produced 30 cm above ground produced biomass. Fresh leaves, including nervure and limbo, were oven dried at 65 °C for about 48 hours with forced air circulation. These dried samples were ground in Willey mills through a 20-mesh sieve (0.84 mm), making a homogeneous material. For irradiation, 200-mg ground samples were transferred to polyethylene envelopes, which had been cleaned previously with a solution of 1:5 p.a. nitric acid.

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Standard solutions preparation

Standards were prepared from spectroscopically pure elements dissolved in acid or their compound solutions. Quantities of 25 µl were transferred with micropipettes to a 1 cm^2 surface Whatman N.41 filter paper. The standards contained the following mass: Fe (181 µg), Mn (4.49 µg), Mo (50.115 µg) and Zn (25.17 µg). For gamma-ray measurement, standards were taken in three groups: (a) Mn; (b) Mo and (c) Fe and Zn.

Irradiation and gamma-radiation measurement

Samples and the standards of group (a) (Mn) were irradiated together in a nylon container, with a thermal neutron flux of $1.43 \cdot 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$, for 2 minutes, in the IEA-R1m reactor. After irradiation, samples and standards were transferred to a container for gamma-radiation measurements. The samples were measured after a decay time of 90 minutes. Each sample was counted for about 15 minutes for measurement of the photopeak corresponding to ^{56}Mn gamma-radiation, at 847 keV.

To analyse the elements of groups (b) and (c) (Mo, Fe, and Zn), samples and standards were irradiated together in an aluminum container, under a $1.84 \cdot 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$ thermal neutron flux for 8 hours in the IEA-R1m nuclear reactor. After irradiation, the material was left to cool down for 3 days, then samples and standards were transferred to proper containers for the first measurement of gamma-radiation. The radiation of each sample was measured for a 6 hour count time for (b) group, whose photopeak was detectable at 140 keV for ^{99}Mo. The gamma-radiation of group (c) was measured for a 6 hour count time, after a decay period of more than 10 days. Therefore, the group (c) count allowed the photopeak measurements of the following radionuclides: ^{59}Fe at 1099 keV and ^{65}Zn at 1115 keV.

The equipment used to measure gamma-radiation was an EG&G Ortec, model GMX 20190, hyperpure Ge detector with a 1.95 keV full-width-at-half maximum (FWHM) resolution for the ^{60}Co photopeak at 1332 keV. This detector was coupled to an electronic system with an EG&G Ortec 8000-channel BUFFER-918A. Data reduction was carried out using IBM/PC microcomputer and VISPECT2 software in Turbo Basic language for spectral analysis.

Results and discussion

Tables 1 and 2 show the concentrations of Fe, Mn, Mo and Zn, obtained in leaves from the cultivars G3 and G36, respectively. The results correspond to the plants which grew on a soil containing simple or double doses of the elements, and to the test plants without fertilizer. All the plants were harvested at two cutting times. Each result is an average of 2 determinations, followed by its standard deviation.

Table 1. Concentrations of the elements absorbed by pigeonpea samples belonging to cultivar G3, submitted to the distinct fertilizer doses, and the test plants, harvested in two different times

Element	Younger plants			Older plants		
	Test plants	Fertilized plants simple dose	Fertilized plants double dose	Test plants	Fertilized plants simple dose	Fertilized plants double dose
Fe, µg/g	80 ± 2	144 ± 37	153 ± 25	81 ± 6	77 ± 29	54 ± 6
Mn, µg/g	94 ± 18	114 ± 8	129 ± 4	59 ± 1	46 ± 1	64 ± 1
Mo, µg/kg	566 ± 154	983 ± 276	1831 ± 271	965 ± 117	882 ± 199	1704 ± 235
Zn, µg/g	26 ± 3	25 ± 4	25 ± 1	18 ± 1	19 ± 4	19 ± 1

Table 2. Concentrations of the elements absorbed by pigeonpea samples belonging to cultivar G36, submitted to the distinct fertilizer doses, and the test plants, harvested in two different times

Element	Younger plants			Older plants		
	Test plants	Fertilized plants simple dose	Fertilized plants double dose	Test plants	Fertilized plants simple dose	Fertilized plants double dose
Fe, µg/g	129 ± 11	125 ± 5	142 ± 2	66 ± 7	79 ± 4	61 ± 4
Mn, µg/g	151 ± 57	80 ± 7	108 ± 17	72 ± 23	46 ± 3	47 ± 6
Mo, µg/kg	552 ± 102	479 ± 148	506 ± 9	688 ± 96	1956 ± 40	2407 ± 99
Zn, µg/g	26 ± 1	33 ± 1	24 ± 1	20 ± 3	16 ± 1	19 ± 1

The results obtained with the addition of Fe, Mn and Mo as mineral fertilizer show an increase of their concentrations in the leaves from younger plants of the G3 cultivar, and the fertilizer dose was effective only for Mo, since a double dose provided a significant increase of the absorption of this element. On the other hand, the older plants show a smaller absorption of these elements, in the case of a positive reaction with fertilization. The Zn concentrations found in the leaf samples show that there is no variation in the absorption of this element, due either to the plant age or the fertilizer dose.

The younger plants of the G36 cultivar show a decreased absorption or negative reaction, when they received the simple dose of Mn and Mo fertilizer. When a double dose of fertilizer is used, just the Mn absorption is decreased. The older plants show an increase in the absorption of Fe and Mo, and negative reaction for Mn, when the simple dose of fertilizer is used. The double doses of fertilizer provide the highest Mo absorption, a decrease in the Mn absorption and almost no variation in the Fe and Zn levels.

From the obtained results it seems to be clear that the mineral absorption resulting from the fertilization depends on the plant species and its age, on the fertilizer dose and the element studied. As a rule, the dry matter yield show that the G36 cultivar responds less than G3 to the micronutrient fertilization. This could be explained by the soil fertility conditions for which these cultivars were selected.

Conclusions

Although the number of analysed samples in this work is not large, it has permitted the establishment of a general behavior of these species of pigeonpea, in relation to the mineral absorption, when different doses of mineral fertilizer are used. Neutron Activation Analysis method is a very satisfactory monitor for the absorption variations of Fe, Mn, Mo and Zn.

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