Journal of Experimental Agriculture International



32(1): 1-9, 2019; Article no.JEAI.46995 ISSN: 2457-0591 (Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Corn Cultivation for Silage: Evaluation of Elemental Composition in the Soil and Plants by Neutron Activation Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. Author WFS designed the study, performed the statistical analysis and wrote the protocol. Author MABCM managed the analyses of the study (*k*₀ method). Author DJM managed the bibliographic research. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2019/v32i130092 <u>Editor(s):</u> (1) Dr. Nazimah Maqbool, Professor, Department of Botany, University of Agriculture, Pakistan. <u>Reviewers:</u> (1) Zakaria Fouad Fawzy Hassan, Egypt. (2) Rahul Datta, Czech Republic. (3) Paul Kweku Tandoh, Kwame Nkrumah University of Science and Technology, Ghana. (4) Toungos, Mohammed Dahiru, Adamawa State University Mubi, Nigeria. Complete Peer review History: <u>http://www.sdiarticle3.com/review-history/46995</u>

> Received 15 December 2018 Accepted 28 January 2019 Published 12 March 2019

Original Research Article

ABSTRACT

Corn cultivation for silage requires special soil management and constant applications of fertilizers and agricultural pesticides to achieve satisfactory yield levels. This study was carried out on a farm that has grown corn for several years. The soil does not have adequate management in terms of fertility and fertilization. The matrices collected were soil, roots, leaves and grains in a corn silage area to investigate which chemical elements are present and their concentrations. The neutron

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activation analysis (NAA) by k_0 -standardization method was applied on elemental concentration determination. In this technique, the sample is submitted to a neutron flux, in order to produce radioactive isotopes of the nuclei present in the original sample. In the k_0 method, the sample is irradiated together with a neutron flux monitor, usually gold (Au), in the same irradiation position and standards of the interested element are not necessary. Several samples can be irradiated simultaneously when stacked inside the irradiation vessel, intercalated with neutron flux monitors. The irradiations were carried out in the TRIGA MARK I IPR-R1 research reactor at Nuclear Technology Development Centre/Brazilian Commission for Nuclear Energy (CDTN / CNEN). In the analysis, As, Ba, Br, Ca, Ce, Co, Cr, Cs, Cu, Eu, Fe, Hf, K, La, Mo, Na, Nd, Rb, Sb, Sc, Sm, Ta, Tb, Th, U, Yb and Zn were identified in the samples. Although the site studied lacks adequate management of soil fertility and fertilization, Ca, Cu, K, Mo and Zn were determined and their presences are important because they are essential for corn development. Adequate content for the cultivation of silage corn were verified by assessing these nutrients and their translocation in the plant.

Keywords: Zea mays L; nutrients; neutron activation analysis; corn; silage; absorption; translocation; soil; plants; k₀ method.

1. INTRODUCTION

Corn (*Zea mays* L.) is a major crop in Brazil and is the basis of human foods and animal feeds [1]. Growing corn for silage requires advanced technologies and it is low and irregular in Brazil. According to Martin and Pavinato [2], production of corn forage is mainly affected by the absorption and translocation of nutrients throughout crop development, interfering in the elemental composition of the product.

The Neutron Activation Analysis (NAA) is one analytical technique that analyses chemical elements in samples of different matrices, such as soil, plants and foods. It is a multielemental isotopic technique, specific for quantitative and qualitative determination of chemical elements, and is applied in chemical characterization of samples in the most varied fields of applications [3,4]. The NAA is precise and accurate and capable of determining elemental contents within a wide range, from traces to percentages [5]. It is a non-destructive technique because sample solubilisation is not required, an advantage over other analytical techniques as they require sample solubilisation for analysis. As the NAA does not require procedures, such as chemical separation or samples dissolution, it is a more versatile technique, reducing contamination risks and avoiding fractioning or partial recovery of elements [6].

The NAA consists of submitting a sample to a neutron flux to produce radioactive isotopes of the nuclei in the original sample, a reaction known as activation [7]. The resulting radioisotopes have half-lives, ranging from

fractions of seconds to several years, with radioactivity measured by gamma-ray spectrometry, with germanium semiconductor detectors. This type of detector is efficient at gamma radiation and has high resolution. Each radioisotope emits gamma rays with characteristic energies, allowing its identification. The produced spectra are analysed by software that locate, identifies and calculates the area under the gamma peak. The amount of events accumulated in a photo pick event of the radioisotope of interest is used to obtain the content of elements in the sample [8].

There are several ways of applying the NAA to obtain contents of chemical elements in the sample. The most commonly used is the comparative method, which analyses standards for each element of interest to be determined. The k_0 method is another analytical method that does not use standards of the elements of interest; but uses neutron fluxes monitors. This method is based on the knowledge of spectral parameters in the irradiation position in the reactor, has to perform gamma spectrometry in an absolutely calibrated gamma spectrometer and uses nuclear constants available in the literature. An advantage of the k_0 method is that it costs of analysis is low, and laboratory operations are reduced [4].

In 2003, the standardized k_0 method was established at the Nuclear Technology Development Centre, Brazilian Commission for Nuclear Energy (CDTN/CNEN), in Belo Horizonte, capital of Minas Gerais State, Brazil. Since then, it has been used to determine the elemental composition of several matrices [9]. In this work, the standardized k_0 method of neutron activation [4,9] was applied to analyse the elemental composition of the soil, roots, leaves and corn samples. The objective was to determine the chemical elements in samples in a corn growing area for silage and evaluate these nutrients in terms of the cultivated area and planted species.

2. MATERIALS AND METHODS

The soil, roots, leaves and corn grains were sampled on a farm in the municipality of Biquinhas, Minas Gerais State (18°46'58" S and 45°30'08" W), at an average altitude of 629 meters in the crop year of 2017.

The systematic sampling was performed, that is, the soil/plant samples were collected in the first crop row and in zigzag. The collection was carried out in plots based on the harvest period (maturation) of the grains and the variety planted (Agroceres 5055).

2.1 Soil Sampling and Preparation

Thirty samples of soil with 150 grams were collected at 15 cm from the roots of the corn plant from 0 to 20 cm. The samples were conditioned in identified plastic bags and sent to preparation at the laboratory at CDTN/CNEN, where the soil was dried at room temperature until reaching a constant weight. Then, the samples were milled in porcelain grains, sieved and packaged in sealed polyethylene bottles, forming a composite sample. Approximately 200 mg aliquots were weighed and placed into polyethylene vials suitable for irradiation.

2.2 Collection and Preparation of Corn Plant Samples

The plants were cut close to the ground and separated into roots, leaves and corn cobs. The samples were stored in plastic bags, tagged, and sent to the preparation at the laboratory at CDTN/CNEN.

The soil aggregated to the roots was removed. The roots were washed in running water, deionized water and dried with paper towel. Then, the samples were packaged in plastic beakers and placed in the freezer for further freeze-drying. Regarding the leaves, whole parts in the opposite position and below the first spike were collected. The samples were stored in plastic bags and tagged. In the laboratory, the samples were washed in running and deionized water to remove any soil particles from the leaves. They were wrapped in plastic beakers and place in the freezer. One corn ear was collected per plant. The samples were then placed in plastic bags, tagged and sent to the laboratory at CDTN/CNEN, where they were threshed, and the grains were washed in running and deionized water and then placed in the freezer.

The samples were kept in the freezer for a minimum of 12 hours at -10°C. After, the samples were freeze-dried and weighed to obtain the moisture percentage. Root and leaf samples were ground in a Grindomix GM 200 knife mill and packed in a bottle with a lid. The corn kernels were packed without crushing.

2.3 Preparation of Samples for Analysis

For irradiation, aliquots of 150 mg of root and leaves were weighed and packed in a polyethylene vial. The corn samples were weighed in triplicate and stored in the sample containers with masses of 2.5 g. These sample masses were in agreement with the methodology of analysis of large samples, recently established at CDTN [10].

For the application of the k_0 method, the samples were conditioned in a larger sample vial, intercalated by neutron monitors, disks (6 mm diameter and 1 mm thick) [11] of Al-Au (0.1%) alloy, IRMM-530RA, supplied by the Institute for Reference Materials and Measurements (IRMM), Belgium.

2.4 Irradiation

The irradiations were carried out for 8 hours in the TRIGA MARK-I IPR-R1 nuclear research reactor at CDTN/CNEN, operating at 100 kW. The samples were irradiated at the carousel in the irradiation channel IC-7. In this position, the spectral parameters *f* (ratio of thermal and epithermal neutron fluxes) and *a* (distance from the epithermal neutron profile) were 22.32 and - 0.0022, respectively, and the thermal neutron flux was 6.35×10^{11} cm⁻² s⁻¹ neutrons [9].

2.5 Gamma Spectrometry

After irradiation, the necessary time was waited for the decay of radionuclides with shorter and interfering half-lives. Then, the gamma spectrometry was performed on a HPGe coaxial detector, with 50% of nominal efficiency, model GC 5019 CANBERRA, associated to appropriate electronics and the Genie 2K spectra acquisition program, CANBERRA. The counts were performed on the characteristic gamma energies of the radioisotopes produced.

For the analysis of the gamma spectra, the HyperLab program [8,12], was used. The Kayzero for Windows[®] program [13] was applied to calculate the elemental concentrations.

2.6 Quality Control

To verify the method performance, two certified reference materials were analysed: Tomato Leaves (SRM 1573a) and Channel Sediment (BCR-320R) using the same method applied to the samples. The results are displayed in Tables 1 and 2.

To evaluate the method efficiency, the E_{n} -score [14] test was applied, which takes into account for the calculations, the expanded uncertainty of the experimental and certified values with a coverage factor k = 2 (95% confidence interval). This means that true results are 95% likely to be within the confidence interval.

The following equations were used in the E_n : calculations:

$$E_n = \frac{Valor_{exp} - Valor_{certified}}{\sqrt{U_{exp}^2 + U_{certified}^2}}$$
(1)

where exp means experimental, U_{exp} means the expanded uncertainties with k = 1, of the experimental results and $U_{certified}$ is the certified values, k = 2.

$$U_{\exp}=2 \cdot U_{exp_Con}$$
 where: $U_{\exp_Comb}=\sqrt{u_{AREA}^2+u_{method}^2}$ (2)

 u_{AREA} is the net area uncertainty of the peak gamma studied and u_{method} is the total uncertainty of method k_0 established as 3.5% in the CDTN.

The method performance was evaluated by the criterion $|E_n| \le 1$, meaning that the performance was satisfactory, that is, the results of the method are within the 95% confidence interval. If $|E_n| > 1$, indicating the unsatisfactory performance of the method.

Table 1. Experimental results and certified values for SRM 1573a, Tomato Leaves, and statistical evaluation, E_n -score

Elements	SRM 1573a			
	Experimental Results, k=1	Certified Values, k=2	E _n -score	
	mg kg ⁻¹	mg kg ⁻¹		
Са	47180 ± 1922	50500 ± 900	0.84	
Со	0.55 ± 0.02	0.57 ± 0.02	0.43	
Fe	378 ± 12	368 ± 7	0.41	
К	27190 ± 492	27000 ± 500	0.17	
Na	133 ± 2	136 ± 4	0.49	
Rb	15 ± 1	14.89 ± 0.27	0.22	
Sb	0.063 ± 0.003	0.063 ± 0.006	0.03	

Table 2. Experimental results and certified values for BCR-320R, Channel Sediment, and
statistical evaluation, E_n -score

Elements	BCR-320R, Channel Sediment			
	Experimental Results, k=1	Certified Values k=2	E _n -score	
	mg kg ⁻¹	mg kg⁻¹		
As	24 ± 1	21.7 ± 2	0.94	
Со	11 ± 1	9.7 ± 0.6	0.61	
Cr	67 ± 6	59 ± 4	0.58	
Fe	27825 ± 6833	25700 ± 1300	0.28	
Hg	1.01 ± 0.07	0.85 ± 0.09	0.95	
Sc	6 ± 1	5.2 ± 0.4	0.21	
Th	6 ± 2	5.3 ± 0.4	0.08	
U	1.6 ± 0.5	1.56 ± 0.2	0.00	
Zn	352 ± 124	319 ± 20	0.13	

The values of E_n -score pointed out that the method produced results with 95% of possibility to be inside a range of values that correspond to the true values.

3. RESULTS AND DISCUSSION

The results for the elements in the different matrices studied (soil, roots, leaves and corn grains) with their respective uncertainties are shown in Table 3. Six essential elements for plant growth, namely Cu, Fe, Mo and Zn as well as Ca and K were determined (Table 3). Co and Na, beneficial elements for the plants, were also analysed.

3.1 Elements in Soil Samples

Fe content in the soil samples was higher than in the other samples (root, leaves and corn grains). Fe is considered an important element for energy transformation in plants [15]; however, it is not usually available for absorption due to the low solubility in its oxidized form. In the plants, Fe is related to several metabolic activities, participating in the formation of some enzymes, besides it is indispensable in the processes of respiration, photosynthesis, N₂ fixation and electron transfer through the cycling between Fe^{2+} and Fe^{3+} . Thus, Fe availability is linked to crop yield. However, in Brazilian soils, responses of corn crops to Fe applications are practically non-existent. Fe contents in soils of the Cerrado region in Brazil are satisfactory for the development of the plants [16].

Zn content was $42 \pm 4 \text{ mg kg}^{-1}$ in the soil sample. According to Broadley and Partners [17], its share in soil contents ranges from 60 to 89 mg kg⁻¹ depending on the rock of origin and deposition sources. Zn deficiency is increased by prolonged cultivation, especially in sandy soils and in the Cerrado region [18].

Table 3. Chemical elements determined in so	il, root, leaf and corn	grains samples (dry mass)
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	Concentration (mg kg ⁻¹)			
Elements	Soil	Root	Leaf	Corn Grains
Ag	< 1	< 0.2	< 0.08	< 0.02
As	6.9 ± 0.3	< 0.1	< 0.08	< 0.08
Ba	267 ± 12	< 10	< 4	< 2
Br	5.9 ± 0.2	20 ± 1	7.0 ± 0.3	0.30 ± 0.06
Ca	< 2*	< 1.0*	7.0 ± 0.6*	< 0.2*
Ce	81 ± 3	2.0 ± 0.1	0.36 ± 0.06	< 0.1
Со	4.5 ± 0.2	0.30 ± 0.02	< 0.008	0.010 ± 0.001
Cr	100 ± 4	3.8 ± 0.2	0.71 ± 0.07	< 0.1
Cs	7.5 ± 0.3	0.2 ± 0.01	0.08 ± 0.01	< 0.005
Cu	< 3	< 1	< 0.7	388 ± 31
Eu	1.2 ± 0.1	< 0,01	< 0.004	< 0.002
Fe	44 ± 2*	0.78 ± 0.03*	83 ± 5	13 ± 1
Hf	17 ± 1	0.20 ± 0.01	< 0.008	< 0.006
Hg	< 0.7	< 0.1	< 0.08	< 0.3
K	16.9 ± 0.6*	24 ± 1*	21 ± 1*	$3.0 \pm 0.4^*$
La	21.9 ± 0.8	0.60 ± 0.02	0.60 ± 0.02	< 0.001
Мо	< 1	< 0.8	< 0.5	0.30 ± 0.01
Na	0.50 ± 0.02*	0.105 ± 0.004*	50 ± 2	6 ± 1
Nd	16.7 ± 2	< 0.9	< 0.8	< 0.4
Rb	124.0 ± 5.0	27 ± 1	26 ± 1	4.4 ± 0.4
Sb	1.2 ± 0.05	0.07 ± 0.01	< 0.02	< 0.002
Sc	17.4 ± 0.6	0.31 ± 0.01	0.010± 0.001	< 0.0003
Se	< 2,0	< 0.2	< 0.2	< 0.08
Sm	4,2 ± 0,2	0.089 ± 0.004	0.030 ± 0.001	< 0.001
Та	2.0 ± 0.1	0.04 ± 0.01	< 0.01	< 0.003
Tb	0.70 ± 0.03	< 0.02	< 0.01	< 0.003
Th	17.9± 0.6	0.31 ± 0.01	< 0.02	< 0.01
U	4.4 ± 0.2	< 0.5	< 0.3	< 0.01
Yb	3.8 ± 0.2	< 0.05	< 0.04	< 0.01
Zn	42± 4	8.5 ± 0.6	14 ± 1	22 ± 4

(g kg⁻¹) *; <, smaller than

Cr was also identified in the soil at content $100 \pm 4 \text{ mg kg}^{-1}$. For [19] reported that lettuce plants grown in a soil supplemented with 200 mg kg⁻¹ Cr³⁺ showed a content of 11.1 mg kg⁻¹ in the tissue and a 60% reduction in dry matter weight 60 days after application, in relation to the control samples.

Br contents varied according to samples with the highest value found in the roots at $20 \pm 1 \text{ mg kg}^{-1}$. Most toxic elements are found in the roots, as the plants attempt to contain the toxic effect they cause to metabolism. Br is not considered an essential chemical element for plants and animals; however, its excessive consumption can be harmful to human health [20]. In the soil, Br contents range from 5 to 40 mg kg⁻¹, confirming the data obtained in this study.

Ce, Eu, La, Nd, Sc, Sm, Tb, Yb are rare elements and were detected in the soil samples. Ce, La, Sc and Nd had the highest contents, below the toxic limit for the plants, also confirming the data obtained by Uchida and partners, 2007 [21].

3.2 Elements in Root Samples

Table 3 shows the elements found in the roots of corn plants. Fe, K and Na showed higher content, indicating greater absorption of these elements by the roots.

Br, Co, Cr, Cs, Hf, Rb, Sb, Ta, Th, and Zn were also found in the roots and the number of elements absorbed by the roots is smaller than in the soil. This is because the plant may have blocked these toxic elements to avoid absorption. In addition, plants have root exclusion mechanisms and when none of these mechanisms is sufficient, they have physiological mechanisms to contain their toxic effect on metabolism [22]. There was Br accumulation in the root, $20 \pm 1 \text{ mg kg}^{-1}$, since Br content in the soil is smaller, $5.9 \pm 0.2 \text{ mg kg}^{-1}$.

Rare earth elements, namely Ce, La, Sc and Sm, were also identified in the root samples, at contents much lower than in soil samples.

3.3 Elements in Leaf Samples

Fe, K and Na were detected in the leaf samples. Ca, which was not detected in the other samples, was found in the leaf sample at a content $6.8 \pm$ 0.6 g kg⁻¹. For [23] states that the suitable range for Ca, in nutritional terms, for corn plants is 2.5 to 8.0 g kg⁻¹.

K and Ca had the highest contents in the leaf samples and Ca accumulation in leaves is due to its low mobility in the phloem [24].

Br, Cr, Cs, Rb and Zn were also detected in the leaf samples; however, the elements were detected at lower contents than in the root samples. Except for Zn, which showed a larger translocation from roots to leaves.

Cr changed its content from $3.8 \pm 0.2 \text{ mg kg}^{-1}$ in the roots to $0.71 \pm 0.07 \text{ mg kg}^{-1}$ in the leaves, in agreement with Moral and Partners [25]. The authors explain that, in plants, most Cr is retained in the roots and only a small portion is transported to the shoots.

Rare elements (Ce, La, Sc and Sm) continued to be translocated to the leaves at levels much lower than in the root samples. As these elements are absorbed by the plants, their distribution among different organs differs considerably. Research has shown that contents of rare elements in the roots are higher than in the other plant organs. Cunha and Partners [26] showed that rare earth elements decrease towards roots> leaves> stems> flowers> fruits or grains in various crops, such as corn, wheat and rice.

3.4 Elements in Corn Grains

Fe, K and Na were also detected in samples of corn grains, however, Ca was determined at detection limit. K content was lower in soil, root and leaf samples, the opposite to data obtained by Marschner and Partners [15]. The authors reported that higher Ca contents increase grain production. Higher transport and storage of photo assimilation in corn grains increase K content since K participates in the transport of sucrose and photo assimilation from the source to the drain [15].

Na contents ranged from 500 mg kg⁻¹ in the soil to 6 mg kg⁻¹ in the grains. No reference value was found for this mineral in the literature; however, it is known that Na can partially replace K when Na contents are low in the soil. Still, Na is essential for some species, usually grasses, which photosynthesize via C-4 metabolism [27].

Trace elements were also found in samples of corn grains, such as Co, Mo, Rb and Zn. Co was

detected at a low content. Figliolia and Partners [28] reported that Co could be absorbed as Co^{2+} , transported from the roots to shoots by the xylem, via the transpiratory current. The authors report that the highest contents are found in the roots, followed by the leaves, and the lowest contents are found in the stems. Mo also had a low content at 0.30 ± 0.01 mg kg⁻¹. Pereira and Partners [29] showed that approximately 0.08 mg kg⁻¹ of Mo in corn seeds are sufficient to allow normal growth and development of plants.

3.5 General Evaluation of the Elements Found

The high Zn contents found in the samples must be related to its greater absorption and storage in plant organs with later translocation to the grains. According to Fahad and Partners [30], the Zn concentration in plant organs are proportional to translocation and accumulation of this element in grains. Foliar applications become a more effective strategy to increase Zn contents in the grain [31] since Zn is absorbed by the leaf epidermis, remobilized and transferred to the grain through the phloem.

Rare earth elements were not detected in corn samples, as expected, due to their low solubility and bioavailability to reproductive organs. Oliveira [32], describes that not all elements are equally retained in the roots of different species, suggesting that tolerance to an element does not necessarily guarantee tolerance to another.

Therefore, the most important elements, based on contents found in the soil, root, leaves and corn grains, were Fe, with high content in the soil, along with Br, K, Na, Rb and Zn, since all these elements were detected in all the samples studied. Several toxic elements and rare earth elements were also detected in soil samples, roots and leaves; nevertheless, their concentrations were below the threshold established by the Brazilian legislation [33].

4. CONCLUSION

The neutron activation analysis, k_0 -method, determined many elements, namely As, Ba, Br, Ca, Ce, Co, Cr, Cs, Cu, Eu, Fe, Hf, K, La, Mo, Na, Nd, Rb, Sb, Sc, Sm, Ta, Tb, Th, U, Yb, and Zn in the samples, despite the different matrices.

Although the studied site did not have adequate management of soil fertilization, six essential elements were identified for crop development. The evaluation of contents of these nutrients and translocations in the plant showed the nutrients have adequate contents for the cultivation of corn for silage.

ACKNOWLEDGEMENTS

The authors thank the Nuclear Technology Development Centre, Brazilian Commission for Nuclear Energy (CDTN/CNEN) for providing the laboratories and all the necessary structure for the development of this work. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior -Brasil (CAPES) - Finance Code 001". The authors also thank FAPEMIG and CNPq for support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle3.com/review-history/46995