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MICRONUTRIENTS EVALUATION IN *Bidens pilosa* L., A PLANT APPLIED IN DIABETES TREATMENTS

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ABSTRACT

Diabetes mellitus is a disease that has increasingly affected individuals over the last years. World Health Organization estimates that the worldwide number of diabetes cases will rise from 171 million in 2000 to 366 million in 2030. Besides the therapy with pharmaceutical drugs, many diabetic patients use medicinal plants to control the glycemic levels. This herb has anti-diabetic action due to the presence of organic compounds and micronutrients. Among the plants applied in diabetes treatment, *Bidens pilosa* L., popularly known in Brazil as *picão preto*, belongs to the family of Asteraceaes, grows fast and is globally distributed. The aim of this study was evaluate the concentration of Cr, Fe, Mg, Mn, V and Zn in aerial parts of *Bidens pilosa* samples, as well as its growth substrate by means of instrumental neutron activation analysis (INAA). The samples were separated into two groups named control and treatment. During the plant development, micronutrient solution, containing Cr, Fe, Mg, Mn, V and Zn, was added to the treatment group. It was observed that micronutrient solution added to the *Biden pilosa* samples may had contributed to the preferentially absorption of Fe, Mg and V, as well as to decrease Zn absorption.

1. INTRODUCTION

Diabetes mellitus (DM) is an heterogeneous disorders metabolic group witch present hyperglycemia as a common factor. DM can be classified as type I which is resulted of the pancreatic beta cells destruction comprehending 5% - 10% of cases and type II, characterized by defects in insulin action and secretion, representing 90% - 95% of cases, and other specific types [1]. Its main symptoms, described first about two thousand years ago by Aretaeus of Cappadocia, are the constant thirst (polydipsia), excessive urination (polyuria) and weight loss. The disease presents chronic complications such as retinopathy, nephropathy and diabetic neuropathy, which can be fatal to the patients [2].

DM is among the top ten causes of death in western countries, despite the progress in its clinical treatment, it was not possible yet to control their lethal consequences [3]. World Health Organization estimates that the number of worldwide diabetes cases will rise from 171 million in 2000 to 366 million in 2030 [4]. Health care costs associated with diabetes keep growing and require more resources in the economy of the affected countries [2].

Diabetes treatment focuses on keeping blood glucose at normal levels, so that patients adopt joint actions such as nutritional reeducation, the practice of physical activity and the use of medicines [5]. Some drugs are responsible for causing undesirable effects on diabetic patients due to long use and high dosage. The use of medicinal plants is an alternative that can reduce the occurrence of side effects and is a less expensive form of treatment, which favors access by low-income people [6].

A variety of plant species is used by diabetic patients around the world to assist in the maintenance of the glycemic levels. Most of these plants contain phenolic compounds, glycosides, alkaloids, terpenes, flavonoids, among others, that are related to the various mechanisms which produce anti-diabetic action [7]. On the other hand, the influence of the micro-nutrients content in these plants must not be discarded, since changes may occur in the metabolism of some elements as a consequence or cause of the disease [8]. Among the plants applied in diabetes treatment, *Bidens pilosa* L. [2;9-11] (Fig. 1), popularly known in Brazil as *picão preto*, belongs to the family of Asteraceaes, grows fast and is globally distributed [2;12].

Figure 1: *Bidens pilosa* L.

The aim of this study was evaluate the concentration of chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), vanadium (V) and zinc (Zn) in aerial parts of *Bidens pilosa* samples, as well as its growth substrate by means of Instrumental Neutron Activation Analysis (INAA).

2. METHODOLOGY

2.1. Cultivation and collection of samples

Bidens pilosa samples were obtained by growing seeds from field region. The seeds were planted in sowing and the seedlings were transferred to beds encoded as control and treatment. The acidity of the soil was determined after pH analysis carried out in triplicate [13]. This type of analysis is essential for crop production, since the hydrogen ion concentration acts on the elements availability in the substrate [14;15].

During the plants development it were added to the base of the treatment group samples 10 ml of micronutrient solution containing Cr (1 $mg.L^{-1}$), Fe (50 $mg.L^{-1}$), Mg (20 $mg.L^{-1}$), Mn (1 $mg.L^{-1}$), V (1 $mg.L^{-1}$), and Zn (1 $mg.L^{-1}$) once a week during the cultivation in the beds that lasted about two months, then the *B. pilosa* samples were collected. Soil samples were collected in two stages: before transplanting the seedlings (SBC-P and SBT-P, corresponding to the control and treatment soil samples, respectively) and in the moment of plant collection (coded as SBC and SBT, both numbered 1 to 5, corresponding to the control and treatment soil samples, respectively). Plant samples were named according to the bed where they grew,

encoded as BC and BT (control and treatment plant samples, respectively), being these numbered from 1 to 5.

All the cultivation plant stages were performed at the Instituto de Pesquisas Energéticas e Nucleares (IPEN), located at the University of São Paulo, in São Paulo state.

One sample was separated and deposited in São Paulo Municipal Herbarium where the plant was identified and turned into a voucher specimen.

2.2. Samples preparation and irradiation

Plant samples were initially washed with supra-pure Milli-Q water to remove foreign material, such as dust and insect parts, and then dried in an oven at 40 °C until constant weight. The dried samples were grounded in a mortar previously decontaminated with HNO_3, and sieved to a 0.150 mm grain size. Soil samples were dried in an oven at 60 °C until constant weight, grounded and sieved following the proceeding described to plants.

To determine the concentration of the short half-life elements (Mg, Mn and V), about 70 mg of plant samples and 30 mg of soil samples were packaged in polyethylene bags and the irradiation time was set at 15 seconds (short irradiation). To determine the concentration of the long half-life elements (Cr, Fe and Zn) about 150 mg of plants and 100 mg of soil were used and irradiation time was set at 8 hours (long irradiation). Certified reference material Estuarine Sediment, SRM 1646a (ES) from National Institute of Standards and Technology (NIST), Syenite, Table Mountain (STM) from United Sates Geological Survey (USGS) and the standard solutions (SPEX Certiprep) pipetted on filter paper were irradiated together with samples under the same conditions.

The quality control of the technique was done by evaluation the elementary concentration of certified reference material SRM 1515, Apple leaves (NIST).

The irradiation was performed at the IEA-R1 nuclear reactor under a neutron flux of 10^{12} n cm^{-2} s^{-1}. The cooling time for plant and soil samples, in short irradiation was approximately 5 and 13 minutes respectively and the counting time was set at 3 minutes for both type of samples for the Mg and V determination. To Mn determination, both in plant and soil samples the decay time lasted about 60 minutes and the counting was set in 20 minutes. The standards were counted following the same procedure applied to the samples. The gamma radiation measurement was performed by using an high pure germanium detector EG & G ORTEC model GEM with 20% of efficiency and nominal resolution of 1.9 keV for the 1332 keV gamma ray of ^{60}Co. To the long half-life radionuclides measurement, samples and standards were irradiated for 8 hours, the decay time lasted 7 to 14 days and the counting time was set at 2 hours in the same equipment.

The concentrations were obtained comparing the photopeak area of the interest element in the sample spectrum with the photopeak area of the reference material, using the following expression:

$$C_{ai} = \frac{(A_{ai}\, m_p\, C_{pi})\, e^{\lambda(ta - tp)}}{A_{pi}\, m_a} \qquad (1)$$

Where:

C_{ai} is the elemental concentration in the sample (in μg g^{-1});
C_{pi} is the elemental concentration in the standard (in μg g^{-1});
A_{ai} is the activity from *i* element in the sample (in count per second);
A_{mi} is the activity from *i* element in the standard (in count per second);
w_a and w_p are the weights of the sample and standard (g), respectively;
λ is the decay constant of the *i* element and;
$(t_a - t_p)$ is the difference of time between sample and standard counting.

The results were evaluated by means of basic statistics (mean and standard deviation) and t-test for two pared samples was applied to compare the treatment samples with the control ones at 95% confidence level. The p value of 0.05 was accepted as significant.

3. RESULTS AND DISCUTION

The pH determination of soil samples showed that both studied groups presented values around the neutrality, being 7.12 and 7.16 the mean values to the control and treatment group respectively. A study carried out to evaluate the pH effect on the *Bidens pilosa* development indicated that the plant grow higher and with larger root volume in values between 6.5 and 7.0 than that plants grown in pH between 4.0 and 6.0 [16]. The *B. pilosa* tolerance, at different pH levels, grown with hydroponic technique, showed that the plant presented better development and that the Mg content increase in roots and stems as the pH becomes higher, being the close to neutral values, around 6.0, the best pH value for the plant development according to the regression analysis [15].

The results obtained for the standard reference material SRM 1515, Apple leaves (NIST), analyzed as a sample for quality control purpose are presented in Table 1. The good agreement between the certified and measured values indicates good precision and accuracy of the INAA method.

Table 1: Certified and measured values obtained for SRM 1515.

Elements	**This work (μg g^{-1})**	**Certified value (μg g^{-1})**
Fe(%)	86 ± 28	83 ± 5
Mg	2780 ± 117	2710 ± 80
Mn	52 ± 2	54 ± 3
Zn	15 ± 2	12.5 ± 0.3

INAA results, presented in Table 2, showed the presence of Cr, Fe, Mg, Mn, V and Zn for both plant and soil from control and treatment group. The element concentrations were higher in soil than in plant samples. Comparing control and treatment groups it was observed the same order of magnitude for elemental concentrations according to the matrix it was being evaluated (soil or plant).

Table 2: Elemental concentration of *B. pilosa* and its growing soil

Soil Control	Elemental concentration Cr (µg g^{-1})	Fe (%)	Mg (%)	Mn (µg g^{-1})	V (µg g^{-1})	Zn (µg g^{-1})	Plant control	Elemental concentration Cr (µg g^{-1})	Fe(%) (µg g^{-1})	Mg(%) (µg g^{-1})	Mn (µg g^{-1})	V (µg g^{-1})	Zn (µg g^{-1})
SBC-P	59 ± 2	4.95 ± 0.05	1.8 ± 0.2	193 ± 3	110 ± 4	142± 12							
SBC-1	112 ± 3	4.89 ± 0.05	1.6 ± 0.1	158 ± 2	92 ± 7	110 ± 10	**BC-1**	3.1 ± 0.1	0.066 ± 0.001	0.26 ± 0.02	21.6 ± 0.6	1.1 ± 0.3	52 ± 2
SBC-2	115 ± 3	4.51 ± 0.05	2.0 ± 0.1	153 ± 2	48± 2	122 ± 10	**BC-2**	3.0 ± 0.1	0.074 ± 0.001	0.23 ± 0.02	20 ± 1	0.66 ± 0.02	63 ± 3
SBC-3	106 ± 3	4.32 ± 0.05	2.3 ± 0.1	148 ± 2	83 ± 3	104 ± 9	**BC-3**	3.3 ± 0.1	0.076 ± 0.001	0.34 ± 0.03	21.1 ± 0.6	Nd	57 ± 3
SBC-4	106 ± 3	4.97 ± 0.05	1.2 ± 0.1	164 ± 2	27 ± 3	Nd	**BC-4**	3.2 ± 0.1	0.083 ± 0.001	0.21 ± 0.02	21.8 ± 0.4	1.82 ± 0.2	53 ± 2
SBC-5	122 ± 3	5.43 ± 0.06	1.6 ± 0.1	177 ± 2	105 ± 7	188 ± 15	**BC-5**	6.0 ± 0.2	0.081 ± 0.001	0.24 ± 0.01	20.7 ± 0.5	1.01 ± 0.2	60 ± 3
Soil treatment	**Cr (µg g^{-1})**	**Fe (%)**	**Mg (%)**	**Mn (µg g^{-1})**	**V (µg g^{-1})**	**Zn (µg g^{-1})**	**Plant treatment**	**Cr (µg g^{-1})**	**Fe (%)**	**Mg (%)**	**Mn (µg g^{-1})**	**V (µg g^{-1})**	**Zn (µg g^{-1})**
SBT-P	55 ± 1	3.88 ± 0.04	1.7 ± 0.1	228 ± 3	24 ± 4	232 ± 6							
SBT-1	121 ± 3	3.65 ± 0.04	1.5 ± 0.2	201 ± 2	95 ± 4	200 ± 5	**BT-1**	3.7 ± 0.2	0.115 ± 0.002	0.35 ± 0.02	24.5 ± 0.5	3.2 ± 0.2	44 ± 2
SBT-2	109 ± 3	3.63 ± 0.04	1.3 ± 0.1	180 ± 2	92 ± 8	193 ± 5	**BT-2**	4.0 ± 0.2	0.198 ± 0.002	0.17 ± 0.01	47 ± 1	1.8 ± 0.2	58 ± 3
SBT-3	113 ± 3	3.98 ± 0.04	1.22 ± 0.04	157± 2	83 ± 4	219 ± 6	**BT-3**	4.5 ± 0.2	0.235 ± 0.003	0.30 ± 0.03	39.1 ± 0.8	5.3 ± 0.8	56 ± 3
SBT-4	132 ± 3	3.97 ± 0.04	1.6 ± 0.2	188 ± 3	79 ± 2	210 ± 17	**BT-4**	4.3 ± 0.2	0.148 ± 0.002	0.31 ± 0.03	12.7 ± 0.7	3.2 ± 0.5	43 ± 2
SBT-5	118 ± 3	3.65 ± 0.04	0.6 ± 0.1	182 ± 2	43 ± 5	221 ± 6	**BT-5**	5.9 ± 0.3	0.211 ± 0.003	0.23 ± 0.02	28.4 ± 0.6	3.0 ± 0.4	52 ± 3

Nd = not determined

Among the plant samples, the elements were found in the following order, from highest to lowest concentration: Mg > Fe > Zn > Mn > Cr > V. In the soil samples the highest concentrations were observed for Fe and Mg, followed by Zn > Mn > Cr > V.

Comparing the results obtained for the *B. pilosa* elemental concentration with plants applied in diabetes treatment found literature [17] the obtained values is relatively concordant with that reported for magnesium and manganese concentration in *Foeniculu vulgare* (Mg = 2774 μg g^{-1}, Mn = 27.8 μg g^{-1}), *Salvia officinalis* (Mg = 2143 μg g^{-1}; Mn = 32.6 μg g^{-1}) and *Cassia anqustifolia* (Mg = 3321 μg g^{-1}; Mn = 23.0 μg g^{-1}).

Magnesium content found in the present work is higher than that reported by Souza et al. [18] (750 μg g^{-1} of Mg) determined in *B. pilosa* samples in dray weight basis. Manganese and Zn concentration are in accordance in both studied groups (control and treatment) and Fe is in agreement only when compared to the control group with the results reported by those authors (34 μg g^{-1} of Mn, 44 μg g^{-1} of Zn and 684 μg g^{-1} of Fe). Adongo [19] determined concentrations of Cr (1.24 μg g^{-1}), Fe (7.60 μg g^{-1}), Mg (30.67 μg g^{-1}) and Zn (1.38 μg g^{-1}) in *B. pilosa* samples being those concentrations all below the ones found in the present study.

The concentrations of Cr (6.3 μg g^{-1}), Fe (317 μg g^{-1}), Mg (0.28%), Mn (102 μg g^{-1}) and V (0.15 μg g^{-1}) measured in *B. pilosa* by Franscisconi [20] presented higher concentrations for Cr and Mn than that reported in the present work, whereas for Fe and V the opposite was observed. The Mg concentrations were consistent in both studies.

The comparison of the results obtained for the elemental concentration from *B. pilosa* with data present in the literature has shown agreement for most investigated elements. Nevertheless, the plant growing conditions should be considered, since this factor has important influence on the content of nutrients in plant [21].

Mean values and standard deviation for plant and soil samples elemental concentration are presented in Figures 2 and 3. The average concentrations were compared by means of t-test. It was verified (Fig. 2) that there was statistically significant difference ($p < 0.05$) for the concentrations between control and treatment samples. Iron presented higher concentrations in the control while Mn and Zn presented higher concentrations in the treatment group. For mean concentrations observed in plant samples (Fig. 3), the t-test indicated statistically significant difference for Cr?, Fe, V and Zn ($p < 0.05$). The higher concentrations for Cr, Fe and V were observed in the treatment samples and, for Zn, in the control group.

The higher concentrations of Fe, Mg, and V in the plant samples of treatment group (Fig. 3) must probably be a result of the higher absorption due to the micronutrient solution addition to the soil. For Zn, it was observed lower concentration in samples of the treatment group. The competition for the micronutrients absorption may have caused the decrease of Zn absorption. In a study conducted to evaluate Zn in rice, it was found that high Fe and Mn concentrations may be related to the reduction of Zn absorption [22].

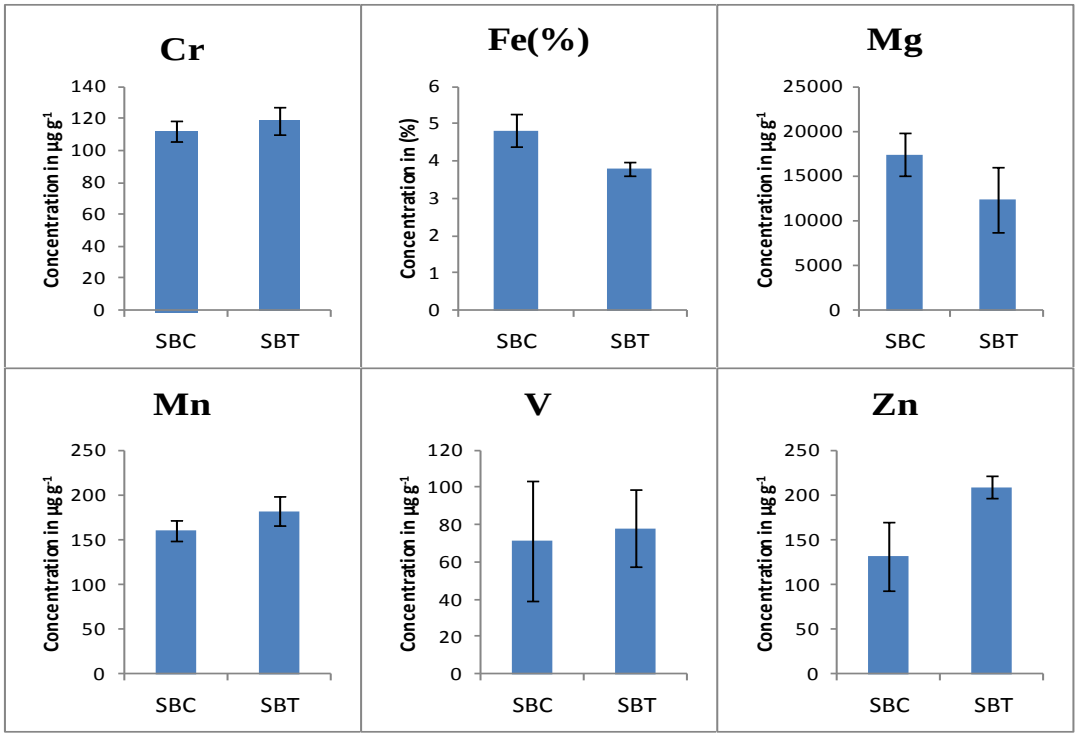

Figure 2: Mean concentrations of micronutrients in soil samples

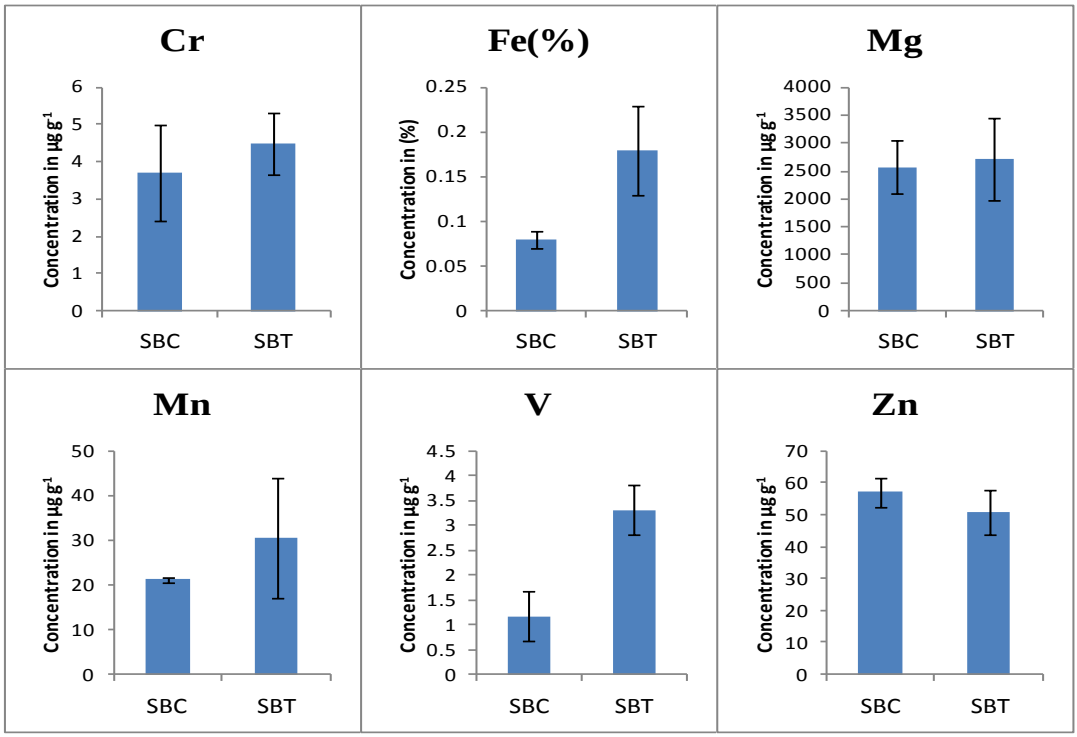

Figure 3: Mean concentrations of micronutrients in plant samples

The preferential Fe, Mg, and V absorption by the plants, as well as the decrease of Zn absorption can be also noted by means of bioconcentration factor (BCF) which is presented in

Table 3. The BCF is defined as the ratio between the concentration of a substance in the plant and the concentration of that substance in soil [23]. These results are illustrated by the graphs of Fig. 4

Table 3: Bioconcentration factor from Cr, Fe, Mg, Mn, V and Zn micronutrients in samples from control and treatment groups

Samples	Elements						Samples	Elements					
	Cr	Fe	Mg	Mn	V	Zn		Cr	Fe	Mg	Mn	V	Zn
BC1/SBC1	0.028	0.014	0.161	0.137	0.012	0.472	**BT1/SBT1**	0.030	0.031	0.239	0.121	0.034	0.219
BC2/SBC2	0.026	0.016	0.118	0.133	0.014	0.514	**BT2/SBT2**	0.037	0.054	0.130	0.263	0.019	0.303
BC3/SBC3	0.031	0.018	0.147	0.143	Nd	0.549	**BT3/SBT3**	0.040	0.059	0.246	0.248	0.065	0.256
BC4/SBC4	0.030	0.017	0.177	0.133	0.067	Nd	**BT4/SBT4**	0.033	0.037	0.200	0.067	0.041	0.205
BC5/SBC5	0.049	0.015	0.148	0.117	0.010	0.319	**BT5/SBT5**	0.049	0.058	0.362	0.156	0.071	0.238
average	0.033	0.016	0.150	0.133	0.026	0.464	**average**	0.038	0.048	0.235	0.171	0.046	0.244

Nd = not determined

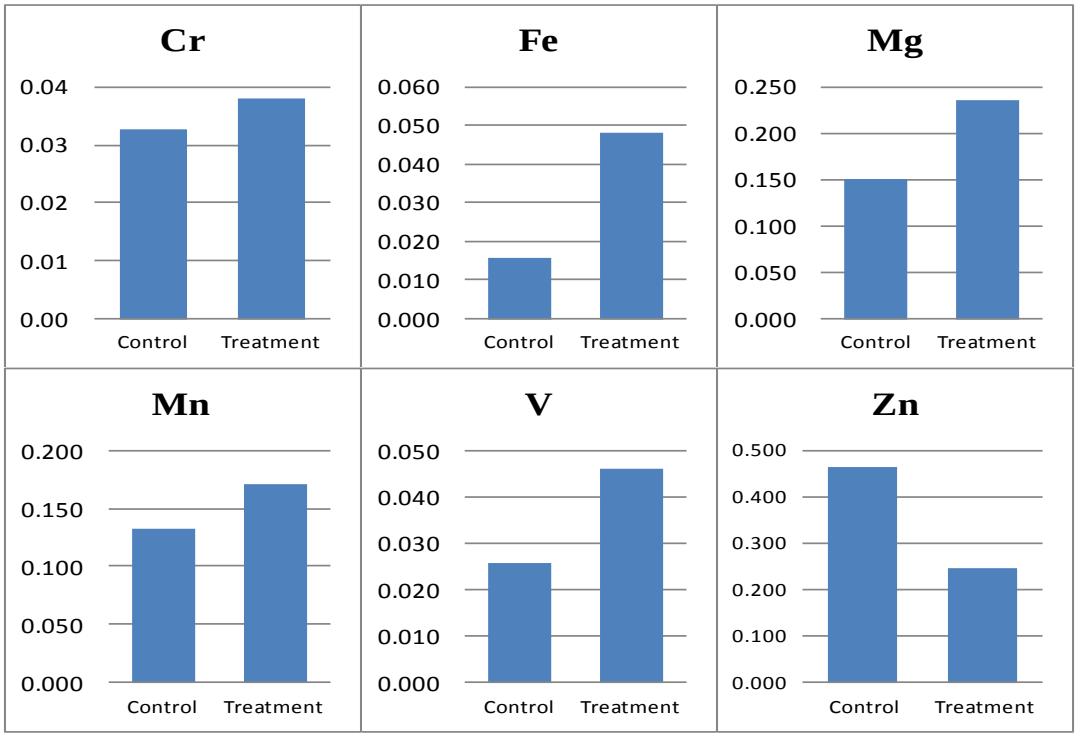

Figure 4: Mean values of BCF for Cr, Fe, Mg, Mn, V and Zn in control and treatment plant samples

It is believed that the investigated elements in this work have some relationship with diabetes mellitus [24-26]. It is knew that decrease of magnesium content occur in 13.5 to 47.7% of patients with DM type 2. There is a possibility that hypomagnesemia induces to insulin resistance due to three factors: tyrosine kinase inhibition, reduction in the use of cellular

glucose, and decreasing the phosphorylation of proteins that act on glucose membrane transporters (GLUT) [27].

Vanadium is the element that presented expressive positive difference in relation to BCF from treatment group compared to control group. Reports indicating the formation of compounds able to normalize the glucose concentration by means of the synthesis of glycogen stimulation in the liver, skeletal muscle and adipocytes are found in [28]. A review referent to vanadium states that this element has considerable antidiabetic action. Among the findings of this review are: the restoration of the glucose levels in blood of diabetic rats after administration of drinking water containing vanadium, the reduction in blood glucose, cholesterol and triglycerides in a variety of diabetic models due to application of vanadyl sulfate, the non-toxicity pointed by long-term studies with control and diabetic mice and applying vanadyl sulfate doses which decreased glucose levels in the blood, the reduction of insulin requirements by up to 75% in insulin-dependent rats after application of vanadyl sulfate, the efficacy of this element when administered orally and intraperitoneally, the production of a organic compound, bismaltolato-oxovanadium IV (BMOV), which is more potent than vanadyl sulfate, and the suggestion of improved diabetes frame due to an insulin-mimetic effect, but vanadium mechanism of action is still being investigated [29].

A positive correlation between high concentration of Fe and glucose intolerance development in type 2 diabetes may occur. The mechanism related with diabetes development when patients presenting hemochromatosis is the high concentration of iron in the liver which conduces to an insulin resistance, and iron accumulation in pancreatic beta cells, resulting in damage to those cells and consequent reduction in insulin secretion [30].

4. CONCLUSIONS

The obtained results allowed to concluding that elemental content obtained in *Bidens pilosa* in this work was in agreement with most of the investigated elements when compared with literature values related to plants applied to diabetes treatment. It can also be concluded that addition of micronutrient solution may has contributed to the preferably absorption of Fe, Mg and V by the plant, as well as a decreasing Zn absorption. Future studies are required to evaluate the elemental content in prepared tea from this herb which has antidiabetic properties.

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REFERENCES

1. “Sociedade Brasileira de Diabetes: Diretrizes da sociedade brasileira de diabetes,” http://www.diabetes.org.br/images/2015/area-restrita/diretrizes-sbd-2015.pdf (2015).
2. W. C. Yang, “Botanical, pharmacological, phytochemical, and toxicological aspects of the antidiabetic plant *Bidens pilosa* L.” *Evidence-Based Complementary and Alternative Medicine*, **vol. 2014**, p. 14 (2014).

3. M. C. L. Neto, "Avaliação de atividade hipoglicemiante do extrato hidroalcoólico das folhas de Calotropis procera (Aiton) W. T. Aiton.**"** Dissertação (Mestrado). Universidade Federal de Pernambuco. Recife, Pernambuco, p. 72 (2011).
4. L. R. S. Freitas, L. P. Garcia, "Evolução da prevalência do diabetes e deste associado à hipertensão arterial no Brasil: análise da pesquisa nacional por amostra de domicílios, 1998, 2003 e 2008," *Epidemiologia e Serviço de Saúde*, **vol. 21**, n. 1, pp. 7-19 (2012).
5. K. A. Lima, "Análise do processo de construção do conhecimento dietoterápico de pacientes diabéticos atendidos no Programa Saúde da Família do município de Araras-SP." Dissertação (Mestrado). Universidade Estadual Paulista. Araraquara, São Paulo (2004).
6. A. Karim, M. Nouman, S. Munir, S. Sattar, "Pharmacology and Phytochemistry of Pakistani and herbal drugs used for treatment of diabetes." *International Journal of Pharmacology*, **vol 7**, n. 4, pp. 419-439 (2011).
7. D. Patel, R. Kumar, D. Laloo, S. Hemalatha, "Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects." *Asian Pacific Journal of Tropical Disease*, **vol. 2**, n. 3, pp. 239-250 (2012).
8. "Repositório aberto: Zinco, crómio e diabetes mellitus," http://hdl.handle.net/10216/54608 (2000).
9. S. C. Chien, P. H. Young, Y. J. Hsu, C. H. Chen, Y. J. S. Y. Tien, L. T. H. Shiu, C. W. Yang, P. Marimuthu, L. F. L. Tsai, W. C. Yang, "Anti-diabetic properties of three common *Bidens pilosa* variants in Taiwan." *Phytochemistry*, **vol. 70**, pp. 1246-1254 (2009).
10. Y. J. Hsu, T. H. Lee, C. L. T. Chang, Y. T. Huang, W. C. Yang, "Anti-hyperglycemic effects and mechanism of *Bidens pilosa* water extract." *Journal of Ethnopharmacology*, **vol. 122**, pp. 379-383 (2009).
11. H. Tag, P. Kalita, P. Dwivedi, A. K. Das, , N. D. Namsa, "Herbal medicines used in the treatment of diabetes mellitus in Arunachal Himalaya, northeast, India." *Journal of Ethnopharmacology*, **vol. 141**, pp. 786-795 (2012).
12. M. F. Agra, P. F. Freitas, J. M. Barbosa-Filho, "Synopsis of the plants known as medicinal and poisonous in northeast of Brazil." *Revista Brasileira de Farmacognosia*, **vol. 17**, pp. 114-140 (2007).
13. "Empresa Brasileira de Pesquisas Agropecuárias. Centro Nacional de Pesquisa do Solo: Manual de métodos de análise de solo," http://www.agencia.cnptia.embrapa.br/Repositorio/Manual+de+Metodos_000fzvhotqk02wx5ok0q43a0ram31wtr.pdf (1997).
14. F. J. Garbuio, "Atributos químicos e biológicos do solo, nutrição e produção de grãos de soja influenciados pela calagem e pela cobertura de aveia preta em sistema de plantio direto." Tese (Doutorado). Universidade de São Paulo. Piracicaba, São Paulo, 2009.
15. S. Y. Obara, A. J. Bezutte, P. L. C. A. Alves, "Development and mineral content of the hairy beggar-ticks under different pH levels." *Planta Daninha*, **vol. 12,** n. 1, pp. 52-56 (1994).
16. S. Y. Obara, P. L. C. A. Alves, "Efeito do pH do solo sobre o desenvolvimento de picão preto." In: *Congresso de iniciação cientifica*, Botucatu, p. 183 (1990).
17. S. Başgel, S. B. Erdemoğlu, "Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey." *Science of the Total Environment*, **vol. 359**, pp. 82-89 (2006).
18. L. S. Souza, E. D. Velini, R. C. S. Maimoni-Rodella, D. Martins, "Teores de macro e micronutrientes e a relação C/N de várias espécies de plantas daninhas." *Planta Daninha*, **v. 17**, n. 1, pp. 163-167 (1999).

19. O. S. Adongo, "Medicinal plants of Chuka Community in Tharaka Nithi County, Kenya and some of their selected essential elements." Dissertação (Mestrado). Kenyatta University (2013).
20. L. S. Francisconi, "Determinação dos constituintes inorgânicos em plantas medicinais e seus extratos." Dissertação (Mestrado). Instituto de Pesquisas Energéticas e Nucleares, São Paulo (2014).
21. A. Kasrati, C. A. Jamali, K. Bekkouche, H. Wohlmuth, D. Leach, A. Abbad, "Plant growth, mineral nutrition and volatile oil composition of *Mentha suaveolens* subsp. *timija* (Briq.) Harley cultivated under salt stress conditions," *Industrial Crops and Products*, **vol. 59**, pp. 80-84 (2014).
22. P. M. Giordano, J. C. Noggle, J. J. Mortvedt, "Zinc uptake by race, as affected by metabolic inhibitors and competing cations." *Plant and Soil*, **vol. 41**, pp. 637-646 (1974).
23. E. Olivares, E. Peña, "Bioconcentración de elementos minerales en *Amaranthus Dubius* (bledo, pira), creciendo silvestre en cultivos del Estado Miranda, Venezuela, y utilizado en alimentación." *Interciencia*, **vol. 34**, n. 9, pp. 604-611 (2009).
24. T. G. Kazi, H. I. Afridi, N. Kazi, M. K. Jamali, M. B. Arain, N. Jalbani, G. A. Kandhro, "Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients." *Biological Trace Element Research*, **vol. 122**, pp. 1-18 (2008).
25. K. H. Thompson, D. V. Godin, "Micronutrients and antioxidants in the progression of diabetes." *Nutrition Research*, **vol. 15**, n. 9, pp. 1377-1410 (1995).
26. H. I. Afridi, T. G. Kazi, N. Kazi, M. K. Jamali, M. B. Arain, N. Jalbani, R. A. Sarfaraz, A. Shah, G. A. Kandhro, A. Q. Shah, J. A. Baig, "Potassium, calcium, magnesium, and sodium levels in biological samples of hypertensive and nonhypertensive diabetes mellitus patients." *Biological Trace Element Research*, **vol. 124**, pp. 206-224 (2008).
27. , C. H. Sales, "Avaliação do status de magnésio em pacientes com diabetes mellitus tipo 2." Dissetação (Mestrado). Universidade de São Paulo, São Paulo (2008).
28. R. M. L. Müller, "Podem ser metais os utilizados no diagnóstico do diabetes mellitus tipo 2?" Dissertação (Mestrado). Instituto de Pesquisas Energéticas e Nucleares, São Paulo (2008).
29. P. Poucheret, S. Verma, M. D. Grynpas, J. H. McNeill, "*Vanadium and diabetes. Molecular and Cellular Biochemistry*, **vol. 188**, pp. 73-80 (1998).
30. M. Silva, "Participação e homeostase do ferro no diabetes tipo 1 em modelos animais." Tese (Doutorado). Universidade Federal de Ouro Preto, Ouro Preto (2011).