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CORRELATION BETWEEN SOIL CHARACTERISTICS AND IRON CONTENT IN ABOVEGROUND BIOMASS OF VIRGINIA TOBACCO

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Abstract

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The study was conducted on alluvial-meadow, maroon-forest soils and vertisols with Virginia tobacco. The total content of iron was measured through decomposition by HF, HClO and HNO acids. A solution of 0.005 M diethylentriaminepentaacetic acid + 0.1 M triethanolamine, pH 7.3 was used for extraction of the elements' mobile forms from soils. The plant sample preparation was made by means of dry ashing and dissolution in 3 M HCl. A Varian Spectra AA 220 Atomic Absorption Spectrophotometer was used for Fe content determination in soil and plant samples. Certified reference materials (three types of soils and tobacco leaves) were also analyzed for accurate determination of Fe concentrations. A correlation and regression analysis was conducted between pH, humus content, total and mobile iron forms in the soil, and the concentration of these elements in aboveground biomass of Virginia tobacco. It was estimated that there were statistically significant relationships between soil pH and iron concentration in Virginia tobacco plant organs. The correlation - regression analysis results showed that there were no statistically significant relationships between humus and iron concentration in aboveground tobacco biomass of Virginia tobacco. Also, the results of the correlation - regression analysis showed that there were no statistically significant relationships between the total element content in soils and iron content in aboveground tobacco biomass of Virginia type. Regression relationships were established between movable iron in the soil and element content in leaves from the lower, middle and upper harvesting zones.

Key words: Fe, uptake, Virginia tobacco, correlation, soils

Abbreviations: ISO - International Organization for Standardization, DTPA - diethylentriaminepentaacetic acid, TEA - triethanolamine, SPSS - Statistical Package for Social Science, CTA-VTL – certified referent material of Virginia tobacco leaves

Introduction

Metabolic functions of Fe in green plants are relatively well studied. Iron is considered the most important metal in the transformation of energy required for synthesis and other vital processes in cells (Tso, 1989; Jones et al., 1999). Fe content in plant leaves ranges from 10 to 1000 $mg.kg^{-1}$ dry matter, its sufficient quantity being from 50 to 75 $mg.\ kg^{-1}$. Its main part is in the form of Fe^{3+} as phosphoprotein, while Fe^{2+} is considered to be in metabolically active form. Iron deficiency affects various physiological processes, which is mainly reflected in growth slowing down and reducing yields. Chlorosis in young leaves presents a typical failure symptom. Symptoms of iron toxicity are not specific and occur differently depending on the type and stage of plant development (Golia et al., 2001). Iron content can often reach several hundred $mg.kg^{-1}$ without toxicity. Its accumulation of large concentrations in tobacco plants is observed in strongly acidic soil reaction. Campbell

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(www.ncagr.com/agronomi/saaesd/fluecure.htm) indicates 50-300 mg.kg^{-1} as an optimal concentration of iron in the early stages and seedling growth as well as in flowering and maturity stage, while in leaves in the technical phase of maturity it is 40 to 200 mg.kg^{-1}. According to Jones et al. (1991) the amount of iron varies greatly in the different stages of plant development ,its content in uppermost leaves decreasing from 430 to 1000 mg.kg^{-1} in a period of 30-45 days, reaching 76-230 mg.kg^{-1} the next 60-80 days. According to Tso (1989) , the iron content varies greatly depending on tobacco type , variety, and place of cultivation. He reported large differences in the values of elements in Virginia tobacco grown in Canada, the United States and Japan.

Iron in soils is usually associated with primary minerals such as clay, oxides and hydroxides. The solubility of iron minerals is very low due to the concentration of soluble iron controlled by amorphous Fe $(OH)_3$. In well-aerated soils trivalent Fe^{3+} ions are dominant, while in over-humid ones - divalent Fe^{2+} ones. The amount of soluble iron is highly dependent on soil acidity, the concentration of Fe^{3+} increasing 1000 times when increasing pH by one unit. Soluble iron reaches plant roots by diffusion of ions in soil solution or in the form of dissolved organic compounds, degradation of plant waste products, microbial metabolism, and oxalic and citric acids contained in soils. The formation of solid organic matter reduces the mobile forms of iron and its absorption by plants (Kabata-Pendias and Pendias, 1992; Uruela, 2005; Petkov et al., 2010; Beev et al., 2011; Pelivanoska, 2011). Iron content in tobacco leaves is variable and depends on the conditions under which tobacco is grown, and mostly on soil composition and properties. One of the major factors influencing trace element concentration in tobacco leaves is soil reaction (Xian and Shokohifard, 1989; King and Hajjar, 1990; Khan et al., 1992; Bell et al., 1992; Mitsios et al., 2005). Other factors influencing the mobility of trace elements are the organic substances in the soil (Adamu et al., 1989; Ross, 1996; Francisco et al., 2010). Mulchi et al. (1991) have found statistically important relations between the extracted from the soil by means of different extragents (DTPA, pH 7.3; DTPA, pH 5.3; 0.05 N HCl + 0.025 N H_2SO_4*)* mobile forms of iron, zinc, manganese, nickel and cadmium, and their concentration in tobacco leaves.

The purpose of this study is to provide information about the relations between pH, humus, total content and mobile forms of Iron in soils, and the concentration of these elements in aboveground biomass of Virginia tobacco.

Materials and Methods

The study was conducted on alluvial-meadow, maroon-forest soils and vertisols with Virginia tobacco. Thirty seven soil samples were taken from a depth of 0–30 cm. The following soil characteristics were determined: pH in water, humus according to Turin (Totev et al., 1987), total content of iron through decomposition by HF, $HClO_4$ and HNO_3 acids, following the ISO-14869-1 (2002) standard. A solution of 0.005 M DTPA + 0.1 M TEA , pH 7.3 was used for the extraction of the mobile forms of the elements from soils. Tobacco samples (stems, leaves, and blossoms) were selected from plants at the same sites where soils were sampled. Leaf samples of cured tobacco at first (lower leaves), second (middle leaves), and third (upper leaves) priming were collected. All samples were washed so as to remove any adhering soil particles and rinsed with distilled water, after which they were dried at 75°C for 12 h and ground. The preparation of plant samples was made by means of dry ashing and dissolution in 3 M HCl. A *Varian Spectra AA 220 Atomic Absorption Spectrophotometer* was used for determination of Fe content in soil and plant samples at the following operating wavelength: Fe – 248.3 nm. SPSS program for Windows was used for statistical data processing.

We calculated the average value, the mode and the median being central features of the empirical distribution function. In order to measure the **asymmetric distribution** and excess, we used coefficients of asymmetry and excess. The differences between the individual indicators were characterized by the statistical dispersion, the variance, the standard deviation and the coefficient of variation, representing a measure of statistical dispersion. The greater the statistical dispersion, the higher the coefficient of variation.

A correlation/regression analysis was conducted between pH, humus content, total and mobile forms of iron in the soil, and the concentration of this element in the aboveground biomass of Virginia tobacco.

Accuracy and precision

Soil and plant materials used for accuracy and precision tests include three certified soil samples corresponding to two main soil types in Bulgaria and one certified reference material of tobacco leaves as follows:

1. Light Alluvial–deluvial Meadow Soil PS-1, SOOMET N°. 0001-1999 BG, SOD N°. 310^a- 98.
2. Light Meadow Cinnamonic Soil PS-2, SOOMET N°. 0002-1999 BG, SOD N°. 311^a-98.
3. Light Alluvial–deluvial Meadow Soil PS-3, SOOMET N°. 0003-1999 BG, SOD N°. 312^a- 98.
4. Polish reference material CTA-VTL-2.

The results from the determination of the total content of iron in the certified samples are presented in Tables 1 and 2. For evaluation of the correctness of the results, three generally accepted criteria are used as follows:

1. $D = X - X_{CRM}$, where X is the measured value and X_{CRM} is the certified value. When D is within the borders of $\pm 2\sigma$, where σ is the standard deviation from the certified value, the result is considered to be good; when it is $-3\sigma \leq D \leq 3\sigma$—satisfactory, and beyond these limits the result is unsatisfactory.

2. $D\% = D / X_{CRM} . 100$ - percentage difference. When the values of $D\%$ are in the limits $\pm 200\sigma / X_{CRM}$, the result is considered to be good; when the value is in the limits $\pm 200\sigma / X_{CRM}$ and $\pm 300\sigma / X_{CRM}$—satisfactory; and when it is out of the limits $\pm 300\sigma / X_{CRM}$, the result is unsatisfactory.

3. $Z = X - X_{CRM} / \sigma$. When $Z \leq 2$, the result is considered to be good; when $2 \leq Z \leq 3$—satisfactory; when $Z > 3$—unsatisfactory. For evaluation of the accuracy of the digestion and measuring procedures, we have used R criterion showing the extent of extraction of the element in percent from the certified value. When the measured value X is within the borders of $X_{CRM} \pm U_{CRM}$, where U_{CRM} is the indefiniteness of the certified value, we accept an extent of extraction to be 100%. In all the remaining cases, the extent of extraction is equal to $X / X_{CRM} . 100$. As can be seen from the tables, the results obtained for all certified materials yield a recovery of 100% for Fe. The precision of the procedures was evaluated by data for $D\%$. In general, the results are "good".

Results and Discussion

Soils

The soil reaction (pH) is within the limits from slightly acid to slightly alkaline (Table 3). The average arithmetic value is

Table 1
Analytical results of the certified materials for Fe in tobacco

Element	Sample	$X_{CRM} \pm U_{CRM}$ mg.kg^{-1}	$X \pm \sigma_x$ mg.kg^{-1}	D	D, %	Z	R
Fe	CTA-VTL-2	1083 ± 33	1070 ± 34	-13**	-1.20**	-0.39**	98.8

Table 2
Analytical results of the certified materials for Fe (as Fe_2O_3) in soil

Element	Sample	$X_{CRM} \pm U_{CRM}$ g.kg^{-1}	$X \pm \sigma_x$ g.kg^{-1}	D	D, %	Z	R
Fe_2O_3	PS-1	39.7 ± 2.4	37.8 ± 2.3	-0.19**	-4.79**	1.58**	95.2
	PS-2	41.2 ± 1.7	40.2 ± 2.4	-0.10**	-2.43**	0.83**	97.6
	PS-3	45.6 ± 1.9	44.3 ± 2.6	-0.13**	-2.85**	1.18**	97.1

* "Satisfactory" result; ** "Good" result

Table 3
Soil properties, content of Fe in soil and aboveground biomass of Virginia tobacco (n = 37)

Statistical Index	Content of Fe in soil				Content of Fe in aboveground biomass, mg.kg^{-1}				
	pH	Humus	Total, g.kg^{-1}	Mobile forms, mg.kg^{-1}	Stems	Lower leaves	Middle leaves	Upper leaves	Blossoms
Mean	6.65	1.82	44.5	28.54	49.62	439.20	233.42	124.04	175.59
Minimum	5.25	1.07	34.6	0.31	16.2	51.4	45	40.2	59.7
Maximum	7.73	2.45	68.8	137.7	113.8	1378	568.3	262.3	419.9
Standard deviation	0.75	0.41	1.13	35.64	20.78	288.96	132.93	56.30	96.72
Sample variance	0.57	0.17	1.29	1270.41	432.01	83499.46	17671.44	3169.84	9354.84
Kurtosis	-0.97	-1.18	5.28	-0.08	1.29	2.63	0.15	-0.28	0.46
Skewness	-0.19	-0.15	-0.22	1.98	0.89	1.38	0.92	0.37	1.25
Range	2.48	1.38	4.9	132.68	97.6	1326.6	523.3	222.1	360.2
Median	6.66	1.81	45.1	13.06	48.4	398	185.5	127.5	126.5
Mode	6.11	1.39	58.1	-	-	-	-	169	105.4
Count	37	37	37	37	37	37	37	37	37
CV, %	11.34	22.70	25.46	124.87	41.89	65.79	56.95	45.39	55.08

6.65, as pH of most of the soils is close to this value, i.e., they are very suitable for Virginia tobacco growing. The humus content is within the limits from 1.07 to 2.45 (low to medium), as most of the soils have low humus content and they are suitable for the Virginia variety group (Tanov et al., 1978).

The total iron content in soils ranges - from 34.6 to 68.8 g. kg^{-1}. The arithmetic mean is 44.5 $g.kg^{-1}$, its value is less than the mode and the median, i.e., there is a negative asymmetry (Table 3). This is proved by the coefficients of asymmetry (- 0.22) and kurtosis (- 0.08). Lower measure dispersion of Fe, compared to the average value, leads to a standard deviation (1.13) and a coefficient of variation reaching 25.46%. (Table 3).

Tobacco

The average values of iron concentration in aboveground biomass of tobacco from different locations are presented in Table 3. If we trace the iron content in different parts of tobacco plants we can see that it is the lowest in stems, followed by blossoms, and the highest in leaves. Its general, the content in the tested samples ranges from 16.2 to 1378 $mg.kg^{-1}$, being within the average values for the country (Brashnarova, 1981; Koinov et al., 1998). The amount of iron in tobacco vegetative organs is the highest in leaves (40.2 - 1378 $mg.kg^{-1}$), lower in flowers (59.7 – 419.9 $mg.kg^{-1}$) has the lowest value in stems (16.2 - 113.8 $mg.kg^{-1}$). If the results are compared to leaves from upper, middle and lower harvesting layers, it appears that the lower harvesting layer leaves accumulate more iron (51.4 - 1378 $mg.kg^{-1}$), compared with leaves from the middle (45 - 568.3 mg. kg^{-1}) and upper harvesting layers (40.2 - 262.3 $mg.kg^{-1}$). Although the iron in plants is slightly movable (only a small part of it is in soluble form, while 80-90t% of it is related to stable organic structures), in blossoms it accumulates in greater quantity than at the top harvesting zone. The probable reason for this is that the flowering and butonization stages appear to have significant consumption of Fe.

In scientific literature the optimal iron content in tobacco leaves is 50 to 300 $mg.kg^{-1}$ (Campbell, www.ncagr.com/agronomi/saaesd/fluecure.htm ; Metsi et al., 2002; Husnjak et al., 2009). 40-50 $mg.kg^{-1}$ is considered low concentration depending on the stage of plant development (Jones et al., 1991). According to Tso (1989), iron concentration in Virginia tobacco varies from 132 to 595 $mg.kg^{-1}$, and in Burley tobacco- from 200 to 650 $mg.kg^{-1}$. Radojicic et al.(2003) state that in Virginia tobacco values range from 170.72 to 995.87 $mg.kg^{-1}$. The average concentrations found in technically mature leaves of Virginia tobacco are within the same range. The minimum values are 40-50 $mg.kg^{-1}$, i.e. they are at the lower limit, while the maximum content measured in the lower zone leaves reaches 1108 $mg.kg^{-1}$. Similar values for iron are referred to in other literature sources (Radojicic et al., 2003; Golia et al., 2007; Pelivanovska, 2007). Applying leaf diagnostics, it is assumed that the leaves belonging to suffering from iron deficiency tobacco plants contain less than 40-50 $mg.kg^{-1}$ dry weight of Fe, the normal amount being between 60-350 $mg.kg^{-1}$, the surplus - more than 1100 $mg.kg^{-1}$. The symptoms of iron toxicity are expressed as chlorosis and necrosis at the edges of leaves, vein chlorosis in young leaves, and the so-called „gray tobacco" in Virginia type, growth arrest of plants as a whole, as well as root damages.

Correlation between pH, humus, total quantities and mobile forms of Fe in soils and concentration of elements in tobacco aboveground biomass

Correlation coefficients between soil parameters and iron concentration in stems, leaves and colors of Virginia tobacco are summarized in Table 4. Correlation and regression analysis results show that there are statistically significant relations between soil pH and iron content in aboveground tobacco biomass of Virginia type. The exponential model adequately reflects the relation between soil pH and iron concentration in the three harvesting zone leaves. These results are consistent with the data published by Golia et al. (2001) about the same type of tobacco, concerning the relation pH - leaf element concentration. In many literature sources pH is indicated as one of the main factors influencing the receipt of iron in plants, because the highest element content is in acidic soil reaction (Kabata

Table 4
Correlation among soil parameters and concentration of Fe in the aboveground biomass of Virginia tobacco (n = 37)

Element	Soil parameters	Lower leaves	Middle leaves	Upper leaves	Stems	Blossoms
	pH	0.442**	0.397*	0.462**	ns	ns
Fe	Humus	ns	ns	ns	ns	ns
	Total	ns	ns	ns	ns	ns
	Mobile	0.786**	0.865**	0.651**	ns	ns

ns - no significant correlation; * correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level

Pendias and Pendias, 1989; Xian and Shokohifard, 1989; Khan et al., 1992; Sekin et al., 2002; McNeill et al., 2006). The correlation - regression analysis results show that there is no statistically significant relationshipbetween soil and humus content of iron in aboveground tobacco biomass of Virginia tobacco. Also, the correlation - regression analysis results show that there is no statistically significant relationship between the total element content in soils and the iron content in aboveground tobacco biomass of Virginia type. Regression relationships are established between the movable iron in soils and element content in leaves from the lower, middle and upper harvesting zones. The relations are linear. Correlation coefficients differ reliably from 0 at a 0.01 level of significance.

Conclusions

A correlation and regression analysis was performed between pH, humus, total and mobile forms of iron in the soil and the concentration of this element in aboveground biomass of Virginia tobacco. The results are:

- The experimental model adequately reflects the relationship between soil pH and iron concentration in leaves of three harvesting zones.
- No statistically significant correlations were found between soil pH and iron concentration in Virginia tobacco plant organs.
- No statistically significant correlations were found between humus and iron concentration in Virginia tobacco plant organs.
- No statistically significant relationship was established between total quantity and iron content in Virginia tobacco plant organs.
- Regression relationships were shown between movable iron in soils and leaf element concentration in the three harvesting zones.
- The correlation and regression analysis showed that the preliminary determination of mobile forms of iron can be used in area and fertilization selection in order to produce high quality Virginia tobacco.

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