

## DTPA-extractable micronutrients in soils of Southern Bulgaria

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### Abstract

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A soil survey in oriental tobacco growing regions in Southern Bulgaria was carried out. One hundred thirty-one soil samples were collected. The main soil characteristics: texture, pH, and humus content were determined, as well as DTPA-extractable iron, manganese, copper, and zinc. All indices investigated show that, despite the predominant monocultural practices of growing oriental tobacco, most of the soils in the studied regions were suitable for this type of tobacco, and for obtaining a good quality of tobacco raw materials. The determined values for DTPA-extractable micronutrients were: iron ranging from 1.42 to 85.55 mg.kg<sup>-1</sup>, manganese between 7 and 212 mg.kg<sup>-1</sup>, copper from 0.35 to 12.23 mg.kg<sup>-1</sup>, and zinc from 0.59 to 11.14 mg.kg<sup>-1</sup>. The tobacco soils analyzed were characterized by very high (46%) and high content (21%) of mobile forms of iron, as well as very high content of mobile manganese – 73%. Most of the soil samples had a high to a very high content of DTPA-extractable copper – 41% and 21%, respectively. With regard to mobile zinc content, the majority of the soils were characterized by moderate levels – 63%, followed by soils with low content of the element – 23%.

*Key words:* DTPA-extractable iron; copper; zinc; manganese; soils; oriental tobacco

### Introduction

Iron, manganese, copper and zinc are nutrient elements of high importance in tobacco plants. They are essential in plant metabolism, as they are necessary for maintaining the structure of the cellular plasma membranes, the formation of enzymes and normal enzymatic activity in the organism, and for the transportation of substances between organs. The soil is the main source of microelements for tobacco plants. Soil characteristics, such as pH, humus content, and soil texture, influence the mobility and absorption of microelements. Their content in soils can vary greatly, from levels of deficiency to toxic concentrations. This considerable variation in microelement content in soils necessitates the use of rapid methods for effective control of the concentration of their mobile forms.

For quantitative determination of the mobility and uptake of microelements solutions of salts, dilute strong acids, weak organic acids, and various complexing agents such as EDTA and DTPA have been studied. Salt solutions extract readily soluble and exchangeable, i.e. potentially mobile, forms of the elements. Dilute solutions of strong acids – hydrochloric and nitric – extract not only mobile forms of the soil elements but also the strongly bonded ones, due to the high concentration of active hydrogen ions.

The theoretical basis for the DTPA extraction is the equilibrium of the metal in the soil sample with the chelating agent. A pH level of 7.3 enables DTPA to extract Fe and other metals. The use of DTPA as an extraction reagent was developed by Lindsay and Norvell (1978), and the method was described by Johnson (1992), Liang and Karamanos (1993).

A classification of nutrient availability in soils based on analyzes with DTPA extractable Fe, Mn, Cu and Zn was created and published as part of the Laboratory Guide for Soil Testing and Plant Analysis (Jones, 2001). In 2001, the International ISO Standard 14870 (Soil quality – Extraction of trace elements by buffered DTPA solution) was introduced, based on the method of Lindsay and Norvell (1978). This standard, along with the classification of availability of mobile DTPA-extractable trace elements in soils, allows for valid comparisons of results obtained in surveys in different parts of the world. In recent years, studies on the availability of mobile forms of the elements iron, manganese, copper, and zinc (all extracted following the Lindsay and Norvell method) have been conducted in different countries, and on various types of soils (Cleide Aparecida de Abreu et al. (2005); Jiang et al. (2009); Kumar and Babel (2011); Ibrahim and Abubakar (2013); Kumar et al. (2015); Ammannawar et al. (2017); Askin et al. (2017)). Information on the content of DTPA-extractable microelements in soils from regions of Macedonia, where tobacco was cultivated, is provided by Jordanoska et al., 2018. Golia et al., 2007, 2009 carried out a similar survey in Greece.

In Bulgaria studies on the availability of DTPA-extractable microelements in soils were conducted in the regions of Plovdiv, Pazardzhik, Asenovgrad, where broad-leaved varieties of tobacco are grown (Zaprjanova et al., 2010, 2014). Zaprjanova et al. (2016), assessed the agro-environmental features of soils from southern Bulgaria and compared three tobacco-producing regions where oriental tobacco is cultivated. An overall assessment of the availability of DTPA-extractable elements in Bulgarian soils, based on a large number of soil samples, was not performed previously. Most of the soils traditionally used for oriental tobacco cultivation are severely eroded and located on sloped terrains. Preservation of soil fertility in the long term is an issue of particular relevance in oriental tobacco monocropping.

The aims of this study were to determine the content of DTPA-extractable essential microelements (Fe, Mn, Cu, Zn) in soils from tobacco growing regions in southern Bulgaria, to assess the availability of these elements, and to ascertain the main properties of soil affecting the quality of tobacco raw materials.

## Materials and Methods

A total of 131 soil samples were collected in several oriental tobacco-producing regions in southern Bulgaria: the municipalities of Satovcha, Hadjidimovo, Garmen, Gotse Delchev, Kardzhali, Kirkovo, Momchilgrad, Krumovgrad,

Chernochene, Ardino, Dzhebel, Banite, Haskovo, Stambolovo, Ivaylovgrad, Aytos, Ruen, Burgas. The soils were sampled at a depth of 0-30 cm.

The following indices were determined:

1. Soil texture – pipette method (by Wigner);
2. pH in aqueous extract – potentiometric (ISO 10390);
3. Humus content – in Turin;
4. Content of mobile forms of iron, manganese, copper, and zinc – 0.005M DTPA + 0.1M TEA, pH 7.3 (ISO 14870).

Element Concentration was determined in accordance with ISO 11047 using Spektra AA 220 atomic absorption spectrometer by Varian, Australia, at the following wavelengths: Fe – 259.9 nm, Mn – 257.6 nm, Cu – 324.8 nm, Zn – 213.9 nm.

## Results and Discussion

### Soil properties

#### Soil texture

The main types of soils occurring in the studied area were: Leptosols, Fluvisols, Gleyic Colluviosols, Planosols, Rendzic Leptosols, Cambisols, and small patches of Vertisols. Suitable for the cultivation of oriental tobacco is loamy sand and sandy clay soils with silt and clay content of 10-50% (Tanov et al., 1978). The results obtained for soil separates (Fig. 1) show that 34% of the soil samples were loamy sandy, 24% were sandy, and 15% were medium textured sandy clay. About 12% of the soils were characterized by a heavy texture. Such were some of the soils collected from the regions of Momchilgrad, Haskovo, Stambolovo, where the oriental tobacco is grown on Vertisols. In general, it was confirmed that most of the studied soils were suitable for oriental tobacco cultivation and met the production requirements for this type of tobacco.

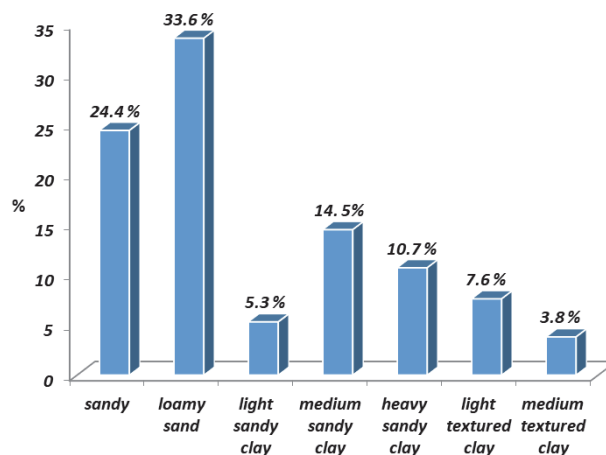


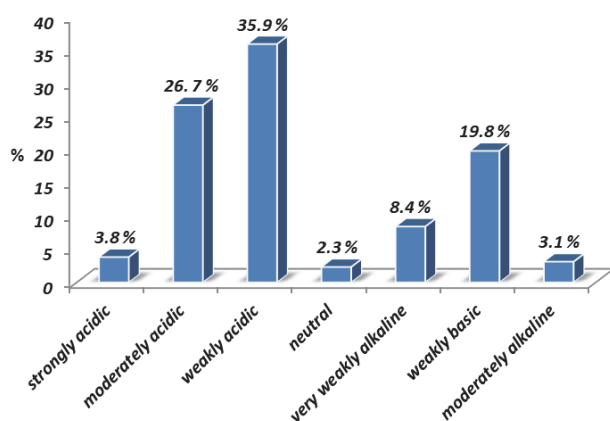
Fig. 1. Soil texture

### Soil reaction

The data shown in Table 1 indicates that the soil reaction ranged between 4.90 and 8.26. Most of the soil samples were characterized by weakly acidic reaction – 36%, followed by the samples with moderately acidic reaction – 24% (Fig. 2). 20% of the soils had a weakly alkaline reaction. With regard to pH, almost all soils analyzed met the requirements for normal growth and development for tobacco cultivation. Only 4% of the soils had a pH of 5, and for 3% of the samples, the pH was higher than 8.2.

**Table 1. Soil properties**

Statistical indices	Humus, %	pH	Silt+Clay (< 0.02 mm), %
Mean	1.79	6.60	34.20
Std. Deviation	0.89	0.92	17.24
Range	5.29	3.36	71.00
Minimum	0.59	4.90	11.00
Maximum	5.88	8.26	82.00
CV, %	49.72	13.94	50.41



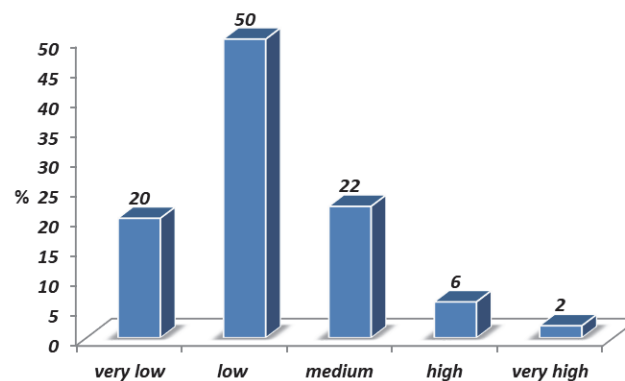
**Fig. 2. Soil reaction**

### Humus content

It was determined that for half of the soils, the humus content was low, according to the classification by Artinova (Gurov and Artinova, 2015). 20% of the soils had very low humus content, and medium humus content was determined in 22% of the samples (Fig. 3).

With regard to humus content, most of the analyzed soils from the tobacco-producing regions met the requirements for oriental tobacco production. 6% of the samples were characterized by high humus content, and very high values were determined in only 2%. The highest values were measured in soils from the municipalities of Stambolovo, Haskovo, Aytos, Ruen, and Banite. The soil samples collected in the municipalities of Satovcha, Gotse Delchev and Garmen,

were characterized by low humus content. The determined humus content in soil samples collected in the rest of the municipalities studied ranged between low, moderate and high.



**Fig. 3. Humus content**

### Dtpa-extractable Micronutrients

#### + Iron

In soils, iron occurs in divalent and trivalent form. Salts of trivalent iron are dissolvable in strongly acidic conditions only. Therefore their occurrence in soil solutions is rare. In soils with acidic reaction (4-6), divalent iron is common, and it is easily available for plants (Gorbanov, 2010).

The determined content of mobile iron was in the range of 1.42 to 85.55 mg.kg<sup>-1</sup>. The lowest values were measured in soil samples collected in the municipalities of Aytos, Stambolovo, Dzhebel, and Chernoochene. Higher values were determined in samples from the municipalities of Bourgas, Ruen, Krumovgrad, Satovcha, Gotse Delchev, Kirkovo (the villages of Chakalarovo and Erovete). The mean value was 27.50 mg.kg<sup>-1</sup>, and the variation coefficient was 66.22% (Table 2).

According to Golia et al. (2009), mobile iron content in soils in Greece ranged from 2.2 to 56 mg.kg<sup>-1</sup>, with an average of 25.6 mg.kg<sup>-1</sup>. The content of mobile forms of iron in soils from several regions of Macedonia is similar to the

**Table 2. Microelement content in soils**

Statistical indices	Fe	Mn	Cu	Zn
	Mobile forms, mg.kg <sup>-1</sup>			
Mean	27.50	62.34	2.08	2.08
Std. Deviation	18.21	40.25	1.77	1.69
Range	84.13	205.03	11.88	10.55
Minimum	1.42	6.98	0.35	0.59
Maximum	85.55	212.00	12.23	11.14
CV, %	66.22	64.57	85.10	81.25

concentrations determined in Bulgarian soils, traditionally used for oriental tobacco cultivation: from 3 to 96 mg.kg<sup>-1</sup> (Jordanoska et al., 2018). A comparison with data for soils in Turkey showed that the iron content there is higher: from 10.1 to 189.6 mg.kg<sup>-1</sup> (Aşkın et al., 2017).

Of the 131 soils analyzed, 46% were characterized by a very high content of DTPA-extractable iron (according to the DTPA Extraction classification, Jones, 2001). High content was determined in 21% of the samples, 16% had moderate content, and in 13% the content was low (Fig. 4). According to O'Hallorans et al. (2004), iron deficiency in tobacco occurs at a soil content of mobile iron of less than 5 mg.kg<sup>-1</sup>. A vast majority of the surveyed soils had a good availability of mobile forms of iron, and only 4.5% were found to have a very low iron content of <5 mg.kg<sup>-1</sup>. Iron deficiency causes interveinal chlorosis in newly formed organs of the plant, as well as a condition called “grey tobacco” in Virginia varieties, which is unfavourable.

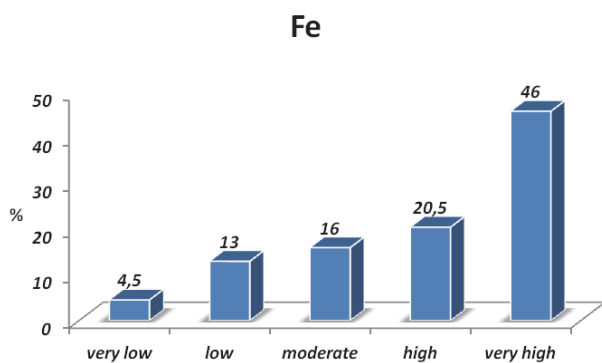


Fig. 4. Mobile Fe availability

#### +Manganese

In the soil, manganese can occur as a constituent of various compounds, due to the oxidation-reduction conditions. The availability of manganese to plants is increased when the element occurs in compounds as a divalent ion, whereas compounds with trivalent and tetravalent manganese ions decrease the availability to plants (Gorbanov, 2010).

According to the classification for DTPA-extractable elements (Jones, 2001), 73% of the studied soils had a very good availability of mobile forms of manganese, and 18% were characterized by good availability of the element (Figure 5). It is likely that the typically high content of total manganese in Bulgaria could explain the high content of mobile forms of the element determined in the samples. According to Brashnarova, 1981, the average Mn content in the country is 1200 mg.kg<sup>-1</sup>, whereas the average value for world's soils is about 545 mg.kg<sup>-1</sup> (Kabata-Pendias and Pendias, 1989).

Only 2% of the soil samples were low in the availability of the element (Fig. 5).

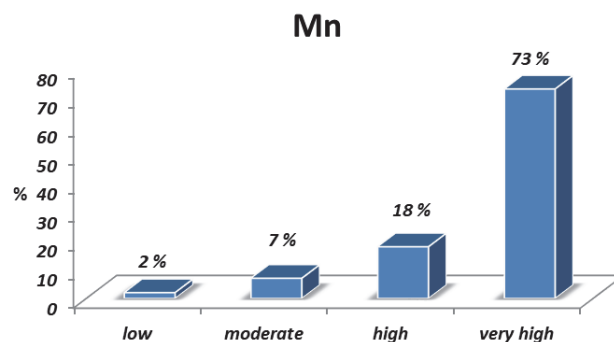


Fig. 5. Mobile Mn availability

The content of mobile manganese in the studied soil ranges from 7 to 212 mg.kg<sup>-1</sup>, with an average of 62.34 mg.kg<sup>-1</sup> (Table 2). Similar values, ranging between 5 and 172 mg.kg<sup>-1</sup>, were reported for soils used for tobacco cultivation in Macedonia (Jordanoska et al., 2018). The content of DTPA-extractable manganese in soils in Turkey is in the range from 1.2 to 136.9 mg.kg<sup>-1</sup>, with an average value of 38.8 mg.kg<sup>-1</sup> (Aşkın et al., 2017). Even lower values were reported for soils in Greece used for cultivation of tobacco: from 2.3 to 25 mg.kg<sup>-1</sup> (Golia et al., 2009). High manganese content was reported in publications by Cleide Aparecida de Abreu et al. (2005) for soils in Brazil (1–325 mg.kg<sup>-1</sup>), and by Kumar et al. (2015) for soils in Tanzania (0.59 to 266.28 mg.kg<sup>-1</sup>).

#### +Copper

In the soil, copper occurs in the form of different compounds with different solubility and plant availability. Decreased copper mobility and plant availability occur when the element has undergone non-exchangeable absorption when it is bound in the form of oxides, hydroxides, carbonates, and phosphates, as well as if it is a constituent of organic compounds. The exchangeable copper cations found on the surface of clay minerals are “accessible” to plants (Gorbanov, 2010).

The results show that most of the soils from the tobacco growing regions were characterized by a good availability of mobile forms of copper – 41% of the samples had a high mobile copper content, and in 21% the content was determined to be very high (Fig. 6). The content of the element ranges from 0.35 to 12.23 mg.kg<sup>-1</sup> (CV – 85%), and the highest values were measured in samples from the villages of Ognyanovo and Dolno Voden. High content of copper was determined in samples from the municipalities of Bourgas,

Ruen, Aitos, Haskovo, Stambolovo. The arithmetic mean value was 2.08 mg.kg<sup>-1</sup> (Table 2). Golia et al. (2009) reported an average value for copper content in soils in Greece of 3.2 mg/kg, with a minimum value of 0.3 mg.kg<sup>-1</sup>, and a maximal value of 6.5 mg.kg<sup>-1</sup>. Data for soils used for tobacco cultivation in Macedonia showed that the content of mobile copper ranged between 0.5 and 7.4 mg.kg<sup>-1</sup>, with average values in the range of 1.23 and 2.9 mg.kg<sup>-1</sup> for different surveyed regions (Jordanoska et al., 2018).

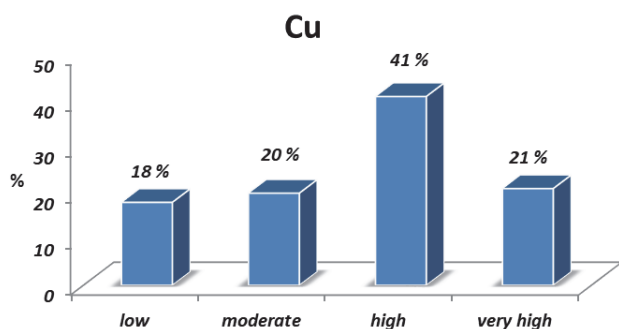


Fig. 6. Mobile Cu availability

#### +Zinc

Wind erosion of the minerals biotite, amphibole, pyroxene, and others, improves zinc availability to the plants, as this process aids the formation of “absorbable” forms of the element. Zinc exchange cations are more readily available to plants (Gorbanov, 2010). Zinc hydroxides, carbonates and phosphates are poorly soluble and with limited availability to the roots of plants.

The content of DTPA-extractable zinc was found to be relatively low, unlike the obtained results for the other trace elements determined in soils from tobacco growing regions of Bulgaria. Predominant were the soils with moderate zinc availability – 63%, followed by soils with a low mobile zinc content – 23% (Fig. 7). It should be noted that low mobile zinc content was determined even in soils with acidic reaction. 10% of the sampled soils had a high mobile zinc content, and only 4% were characterized by a very high DTPA-extractable zinc content. The highest values were determined in soils from the municipalities of Ivailovgrad – in the village of Kazatsite; in Kardzhali municipality – in the villages of Gluhar and Shiroko pole, as well as in Krumovgrad municipality.

The content of mobile zinc ranged from 0.59 to 11.14 mg.kg<sup>-1</sup>, with an arithmetic mean of 2.08 mg.kg<sup>-1</sup>, and a variation coefficient of 81.25% (Table 2). Very similar values were reported for soils from tobacco growing regions

of Macedonia. In the Pelagonia region, the content of the element ranged between 0.15 and 8.62 mg.kg<sup>-1</sup> (Jordanoska et al., 2018). Data for the South Eastern Production Region was in the range of 0.4 to 9 mg.kg<sup>-1</sup>, and in the Vardar Valley Production Region – between 1 and 11 mg.kg<sup>-1</sup> (Jordanoska et al., 2018). In soils used for tobacco growing in Greece, the reported zinc content ranged from 0.2 to 5.5 mg.kg<sup>-1</sup>, with an arithmetic mean of 1.1 mg.kg<sup>-1</sup> (Golia et al., 2009). Aşkın et al. (2017) ascertained even lower concentrations of DTPA-extractable zinc in soils in Turkey, ranging between 0.16 and 2.64 mg.kg<sup>-1</sup>, with an arithmetic mean of 0.77 mg.kg<sup>-1</sup>.

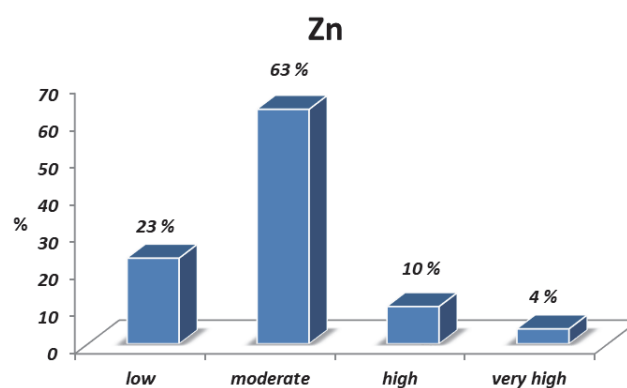


Fig. 7. Mobile Zn availability

## Conclusions

A soil survey in oriental tobacco growing regions in Southern Bulgaria was carried out. One hundred thirty-one soil samples were collected. It was found that:

The determined contents of DTPA-extractable micronutrients were: Fe in the range from 1.42 to 85.55 mg.kg<sup>-1</sup>, Mn – 7 to 212 mg.kg<sup>-1</sup>, Cu – from 0.35 to 12.23 mg.kg<sup>-1</sup>, and Zn from 0.59 to 11.14 mg.kg<sup>-1</sup>.

Soils from the studied tobacco producing regions were characterized by a very high (46%) and high content (21%) of mobile forms of iron, and a very high content of mobile manganese – 73%. Most of the soils had a high and very high content of DTPA-extractable copper, 41% and 21%, respectively. A majority of the soils sampled had a moderate availability of mobile forms of zinc – 63%, followed by soils with a low content of the element – 23%.

All indices investigated show that, despite the predominant monocultural practices of growing oriental tobacco, most of the soils in the studied regions were suitable for this type of tobacco, and for obtaining a good quality of tobacco raw materials.



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