

THE EFFECT OF ORGANIC MELIORANTS ON UPTAKE OF HEAVY METALS AND QUALITY OF ORIENTAL TOBACCO KRUMOVGRAD 90

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Abstract

A comparative research on the impact of organic meliorants on the uptake of heavy metals and quality of Oriental tobacco Krumovgrad 90 has been carried out. Experiments have been implemented in controlled conditions. The soil used in this experiment was sampled from the vicinity of the Lead-Zinc Complex near the town of Kardzali, Bulgaria (Ostovitsa village). The pot experiment was a randomized complete block design containing ten treatments and three replications (30 pots). The treatments consisted of a control (no organic meliorants) and compost, vermicompost and peat meliorants (added at 20 t/daa, 30 t/daa 40 t/daa, recalculated based on dry soil weight). Heavy metals were measured in roots, stems and leaves of Oriental tobacco Krumovgrad 90. Application of organic meliorants does not lead to effective immobilization of Pb, Zn and Cd phytoaccessible forms in soil. The effect of the meliorants used for the reduction of the heavy metal content in tobacco is negligible. The best results are obtained with the introduction of 40 t/daa of compost. Organic meliorants influence tobacco quality indicators (ash, protein and reducing sugars). The introduction of organic meliorants leads to increased minerals (pure ash) and protein levels, and reduces the amount of reducing sugars in tobacco leaves compared to the control. Adding compost and peat to soils leads to improved tobacco quality. However, the introduction of 30 t/daa and 40 t/daa of vermicompost into the soil results in lower quality tobacco (Schmuck number below 0.5).

Keywords: heavy metals, organic meliorants, Oriental Tobacco, quality

1. INTRODUCTION

Tobacco is major agricultural crops for many countries, including Bulgaria. Appropriate climatic conditions in the country determine the cultivation of high-quality Oriental tobaccos, which are distinguished by their specific smoking and technological properties. The Bulgarian Oriental Tobacco is a necessary component in the composition of "American blend" cigarettes - a major segment of the global cigarette market. Among the Bulgarian Oriental tobaccos, Krumovgrad origin is the most prominent in the production and best performing on the international markets.

The study of metallic elements in tobacco is associated primarily with its technological qualities, mainly with flammability, but the consumption of tobacco products makes them significant also in terms of their biological impact on human health. This issue is particularly important, given the ability of tobacco to accumulate heavy metals, as well as the possibility of some of them to be emitted in the main and side streams of tobacco smoke (Stoilova & Markova, 2004). In the scientific literature there are numerous examples of the negative impact on human health of tobacco smoke and its major components - nicotine, carbon monoxide, tar, acetaldehyde, nitrosamines, polynuclear aromatic hydrocarbons and many other representatives of the gas and liquid-solid phase of the smoke aerosol. However, insufficient attention is paid to the accumulation of heavy metals in tobacco, their content in the tobacco raw material and the mechanisms of their impact on human health. It is well known that some of the heavy metals contained in tobacco and passing in tobacco smoke are powerful carcinogens (Rodgman & Green, 2003) and their content in cigarette smoke varies depending on the type and variety of tobacco (Stoilova & Markova, 2004). The heavy metal content, due to its biological effects on humans, can be considered as a qualitative sign of the ecological purity of tobacco and tobacco products. Their qualitative and quantitative determination provides important information regarding the introduction of restrictive standards for some of their representatives in commercial tobacco products.

The chemical properties of tobacco depend on a number of factors, including variety, habitat (soil and climate), as well as the impact of agro-technical practices on the physical, chemical and biological properties of the soil. Soil conditions and nutrient content are critical to plant growth and development. In addition to carbon, hydrogen and oxygen, imported from the atmosphere and water, macro and microelements are necessary for plant growth (Kafi et al., 2002). Tobacco yield and quality are strongly influenced by fertilization and especially by the introduction of nitrogen and potassium fertilizers (Spirov & Lukipudis, 1999).

Insufficient or excessive nitrogen fertilization can lead to low yields and lower tobacco quality (Bozhinova & Bozhinov, 2006). The use of organic fertilizers influences the physicochemical properties of soils and improves the conditions for the development of crops. Organic fertilizers can neutralize or reduce the acidity of the soil and increase the content of certain microelements, such as Zn and Cu. The efficiency of organic fertilization is manifested in rapid growth, earlier maturation, improvement in the flammability of leaves, their mass and yield (Hu, 2004). There is evidence that the use of combined inorganic and organic fertilizers can improve the growth and quality of tobacco (Wang et al., 2009).

The increasing application of organic fertilizers improves the soil's ability to supply nutrients, thus providing sufficient amounts of N, P, and K for the development of tobacco (Cao et al., 2004). On the other hand, organic fertilizers can improve the resistance of the tobacco plant to diseases (Li, 2007).

The purpose of this study is to conduct a comparative research that will allow us to determine the influence of organic meliorants on the uptake of heavy metals from Oriental tobacco and the quality of the tobacco raw material.

2. MATERIALS AND METHODS

2.1. Materials

Pot experiments were carried out with soils contaminated with heavy metals from the Kardzhali region. The soils were collected from the surface soil horizon (0-20 cm) from sites situated at a distance of 7 km from the source of pollution (Lead-Zinc Complex, Kardzhali). After sieving the soils through a 2 cm² mesh sieve, organic soil meliorants (peat, vermicompost obtained from the processing of natural fertilizer and other organic waste from Eisenia Fetida and compost) were added at 20 t/daa, 30 t/daa 40 t/daa (recalculated based on dry soil weight) and gently mixed with the soil by hand. The total amount of mix used in all variants was 9 kg. All treatments were performed in three reps. Additionally, three test vessels were prepared for the control samples (no additives). Table 1 presents the agrochemical characteristics of the organic meliorants used.

Parameter	Compost	Vermicompost	Peat
pH	6.9	7.5	5.8
Electrical conductivity, dS/m	0.2	2.2	0.3
Organic matter, %	72.9	38.6	93.8
N Kjeldal, %	1.49	1.67	0.93
Available P, mg/kg	1000	3650	350
Available K, mg/kg	2776	556	1612
Total P, mg/kg	11884.6	9588.2	999
Total K, mg/kg	3780.7	15614.9	1115.8
Total Pb, mg/kg	29.8	34.4	6.0
Total Zn, mg/kg	165.8	233.0	73.2

Total Cd, mg/kg	0.789	0.100	0.588
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Table 1. Agrochemical characterization of organic meliorants used

As the Oriental tobacco's leaves ripe consecutively, harvesting takes place in stages /harvests/, keeping the leaf maturation sequence. The harvested technically mature leaves were strung into a single needle and dried under natural conditions, respecting sun drying technology of Oriental tobacco.

2.2. Methods

The content of Pb, Zn and Cd was determined in the different parts - roots, stems and leaves. Since Pb, Zn and Cd are less accumulated in the leaves of the lower harvesting belt compared to the leaves of the middle and upper harvesting belt, only the leaves of the middle harvesting belt were analyzed. The content of substances that are directly related to the quality and smoking properties of tobacco - total nitrogen, proteins, reducing substances and mineral substances, was also determined in the dried leaves. Analyzes were carried out according to conventional methods, expressed as a dry matter percentage.

The total heavy metal content of the soil was determined in accordance with the requirements of ISO 11466. Mobile heavy metal forms were extracted with AB-DTPA solution (1 M NH_4HCO_3 and 0.005 M DTPA, pH 7.8) (Soltanpour & Schwab, 1977). "JobinYvon Emission - JY 38 S" atomic emission spectrometer was used to determine the heavy metal content in the samples.

3. RESULTS

The tested soils are characterized by a neutral reaction ($\text{pH} = 7.0$) and an average organic carbon content (1.76%). Soil properties are a prerequisite for low to moderate metal mobility, as confirmed by the results of DTPA-extracted Pb, Cd and Zn (mean for Pb and Zn, and high for Cd). The total Pb and Cd content is high and exceeds the limit concentration values (Table 2).

Element	Total content mg/kg	DTPA-extractable mg/kg	DTPA-extractable/ total content, %	MPC g/kg
Pb	114.2±1.9	23.4±0.7	20.5	80
Cd	9.9±0.6	4.0±0.1	40.4	3.0
Zn	252±2.5	36.8±1.0	14.6	340

Table 2. Total content and DTPA – extractable mobile forms of Pb, Zn and Cd in soils

The results presented in Figure 1 show that the influence of soil meliorants on the Zn and Cd mobile forms is relatively poorly expressed and practically does not influence the amount of mobile Zn and Cd. The trend of reduction of mobile forms under the action of soil meliorants is more pronounced with respect to Pb. Adding compost and vermicompost results in a decrease in the amount of mobile Pb. Only in the peat the amount of mobile Pb is slightly increased.

The trend of reduction of mobile forms under the action of soil meliorants is also expressed in terms of Cd. In most cases, the amount of Cd decreases. Only with peat variants the amount of Cd remains unchanged.

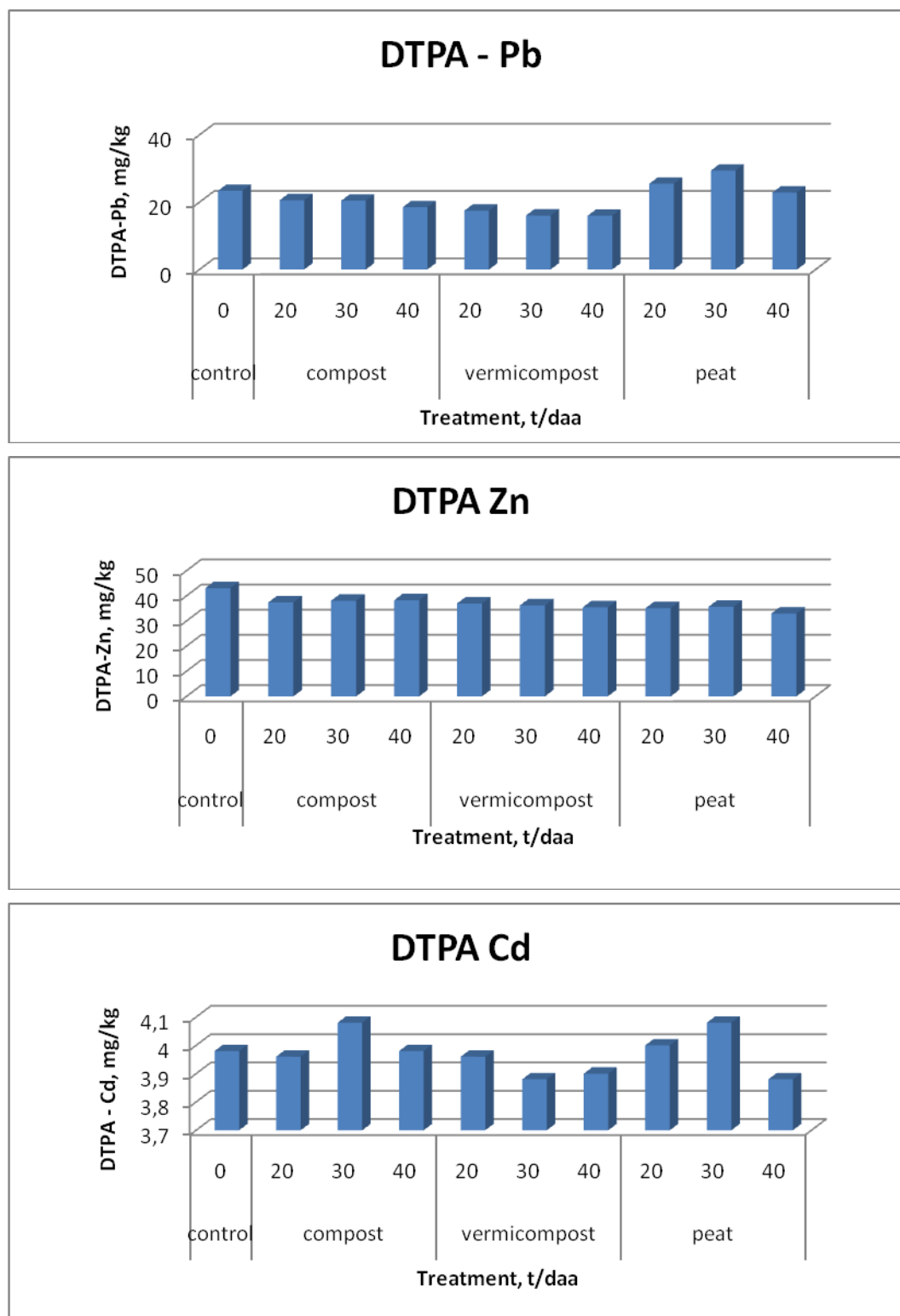
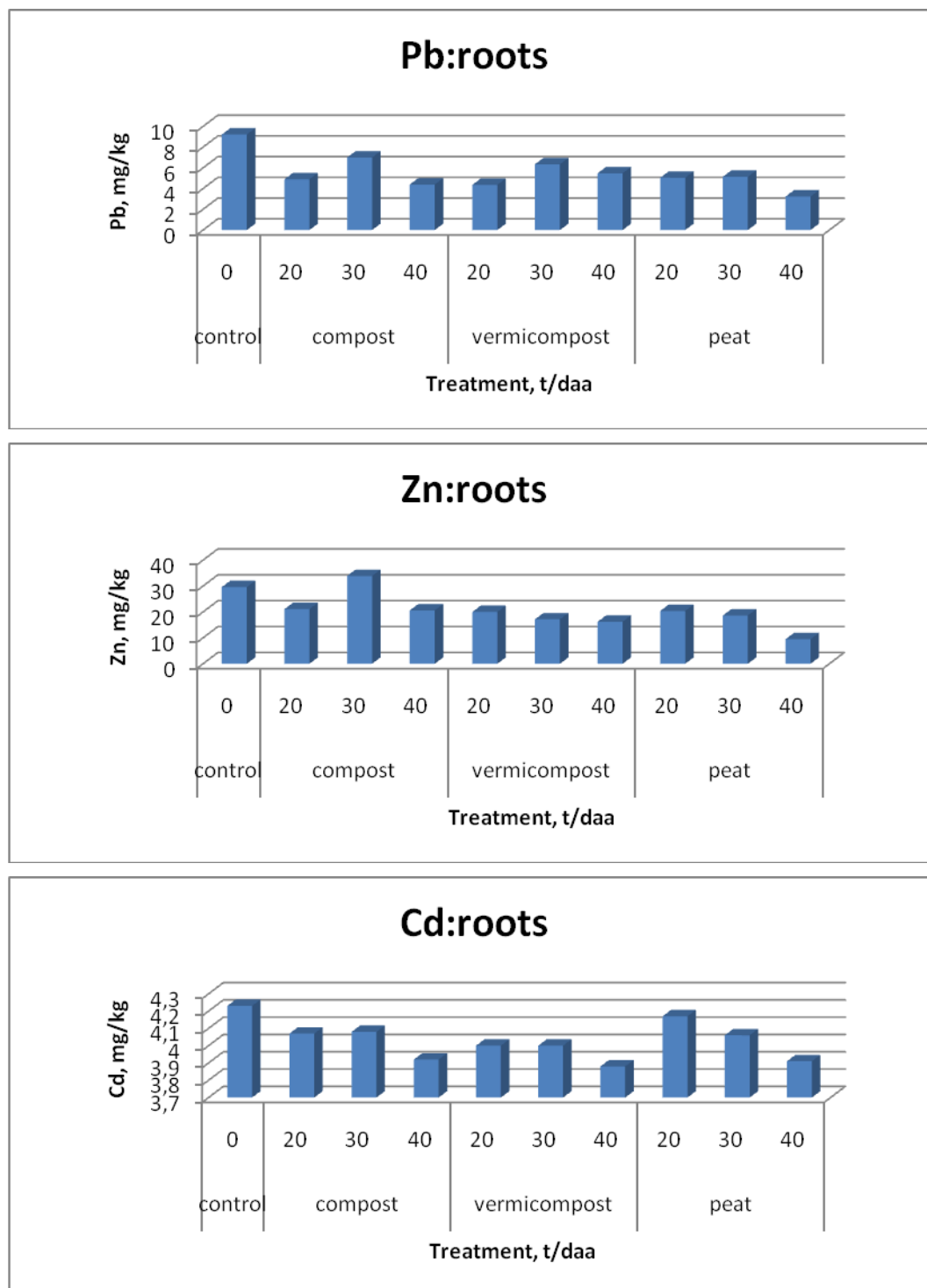


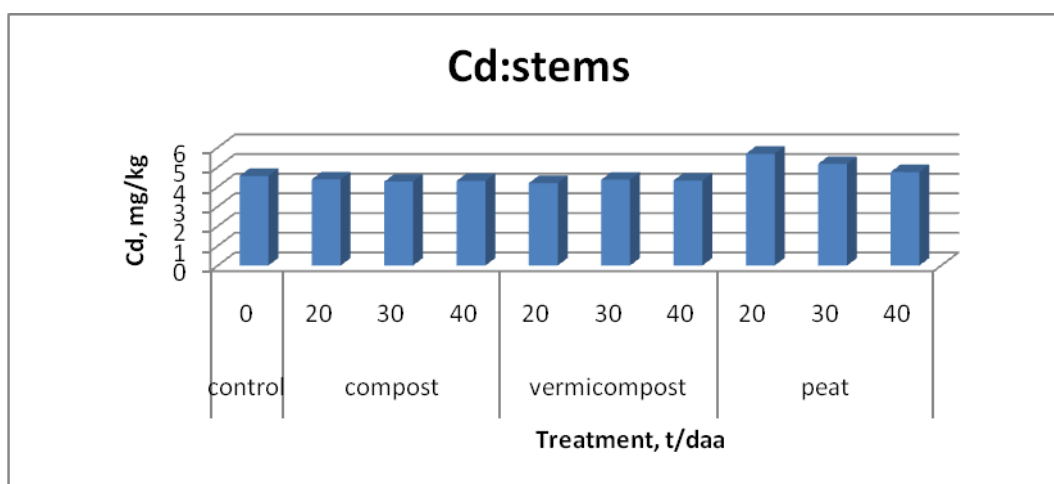
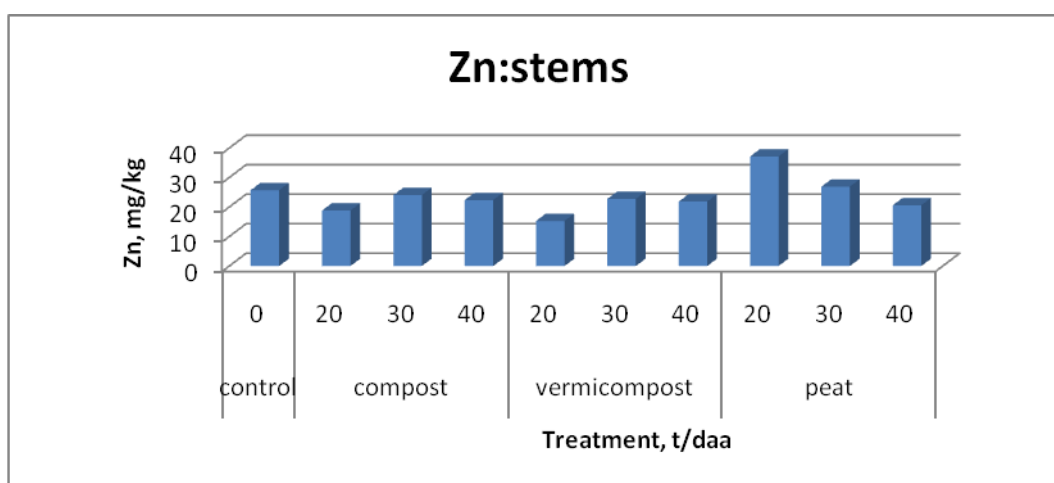
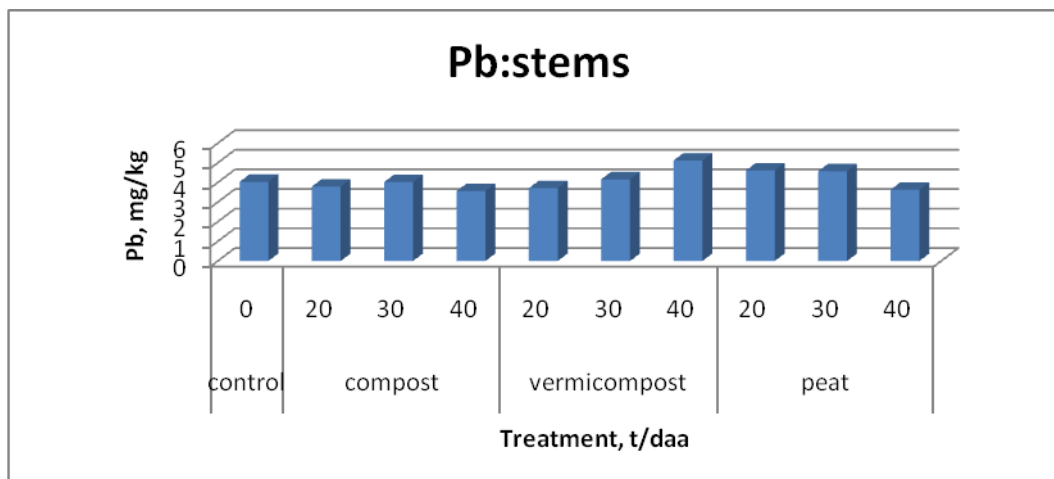
Fig. 1. Effect of organic meliorants on DTPA –extractable forms of Pb, Cd and Zn

4. DISCUSSION

4.1. Accumulation of heavy metals in vegetative organs of Oriental tobacco (without meliorants)

The root system is a major route for the introduction of heavy metals into plants. Once they have entered the roots, they can be stored or moved to the stems. The results obtained show that a significant portion of Pb, Zn and Cd is accumulated at the roots of the tobacco, and their content depends primarily on the amount of heavy metals in the soil. The Pb content reaches 9.2 mg/kg, Zn - 29.6 mg/kg, and Cd - 4.2 mg/kg (Figure 2).





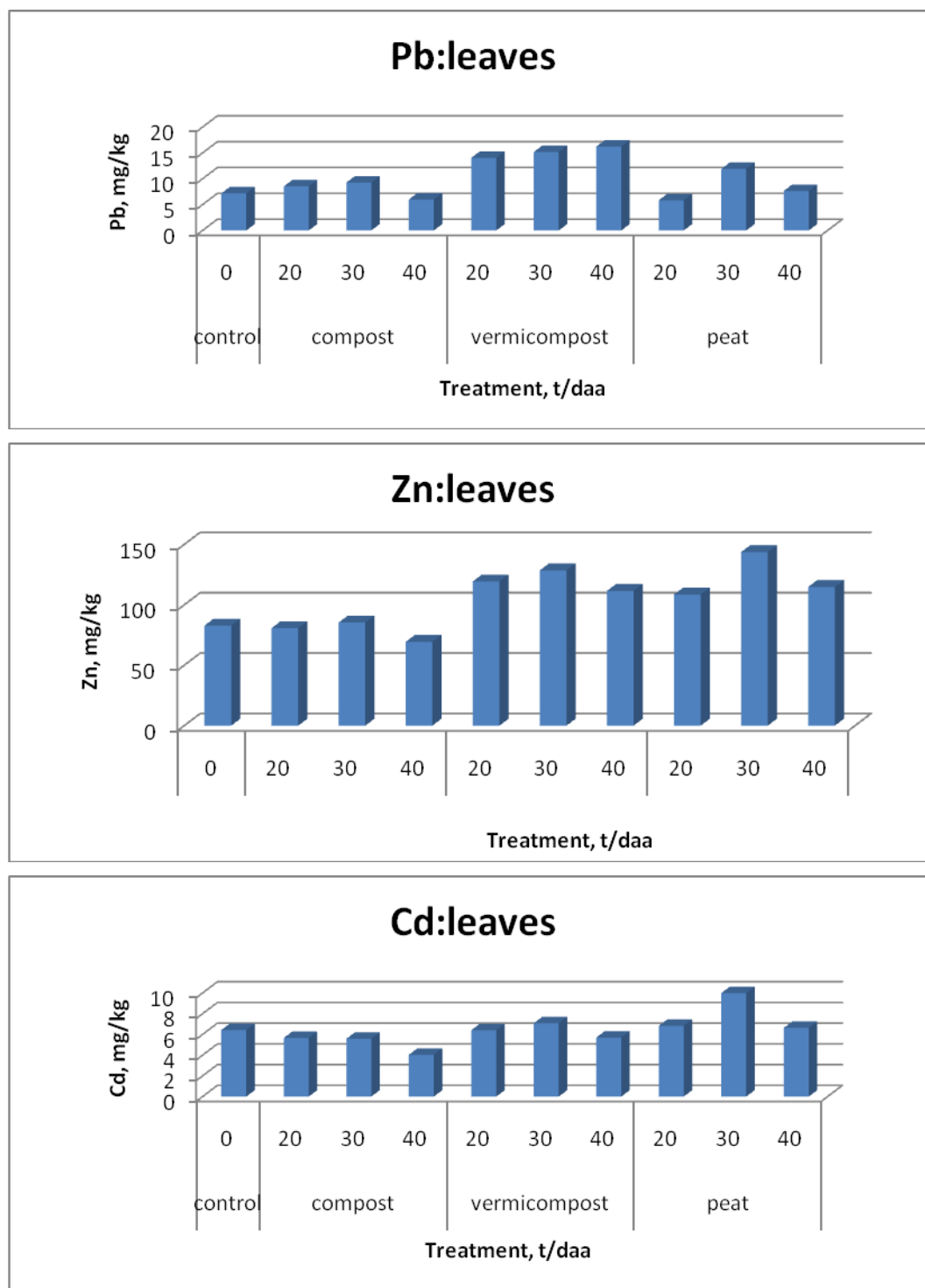


Fig. 2. Effect of organic meliorants on content of Pb, Zn and Cd (mg/kg) in vegetative parts of Oriental tobacco

The amount of heavy metals in the tobacco stems is significantly higher compared to the root system, indicating that their movement along the conduction system is greatly increased. The Pb content in tobacco stems reaches 4.0 mg/kg, Zn – up to 25.7 mg/kg, and Cd – up to 4.6 mg/kg (Figure 2). The results obtained show that the ratio between the heavy metals in the stem and in the roots in the tobacco grown in vessel experiments is about 1.0.

The content of heavy metals is higher in the tobacco leaves than in the root system and stems, which is in line with the results of other authors (Wagner & Yergan, 1986, Mench & Martin, 1991, Mench et al., 1994). The greater accumulation of heavy metals in tobacco leaves is probably due to their absorption from the soil through the root system of the plant and their movement through the conductive system. This is consistent with what was found by Yeargan et al. (1992), who found that tobacco had an extraordinary ability to digest Cd as compared to other plants when grown on Cd contaminated soils.

The Pb content in tobacco leaves reaches 7.2 mg/kg (Fig. 2). The results obtained are consistent with those of Tso (1972), according to whom the Pb content of tobacco leaves varies widely from 0 to 200 mg/kg and largely depends on the soil characteristics, the type and variety of tobacco, as well as its place of cultivation (Lugon-Moulin et al., 2004).

The zinc content in tobacco leaves reaches 25.7 mg/kg. According to Jones et al. (1991) and Campbell (2000), the optimum amount of Zn in the tobacco is in the range 20 - 60 (80) mg/kg, and excess is observed at values above 80-100 mg/kg.

The cadmium content of tobacco leaves reaches 6.4 mg/kg. According to Golia et al. (2007), the Cd content in tobacco varies from 0.5 to 3.5 mg/kg. Tso (1972) reported a Cd concentration in tobacco leaves up to 11.6 mg/kg. According to Mench et al. (1994) and Sappin-Didier et al. (1997), Cd content in tobacco leaves varies from 40 to 120 mg/kg depending on the soil characteristics. The results obtained are consistent with the values reported in other literature sources (Adamu, 1989; Bell et al., 1992) and lower than the concentrations considered critical in plants (5-30 mg/kg) (Kabata Pendias, 2001). It has been found that cadmium can be accumulated in tobacco leaves in an amount 10 times higher than that in the soil (Anonymous, 1995).

Tobacco is a crop that has an exceptional ability to accumulate more Cd in its leaves than any other crop (Mench & Martin, 1991; Mench et al., 1994). So far, no direct relationship has been established between the Cd content in tobacco leaves and the amount of Cd taken by smokers. However, there is a hypothesis that the reduction of Cd content in tobacco leaves will lead to a decrease in the amount of Cd in cigarettes and cigarette smoke.

The results obtained show that the distribution of heavy metals in Oriental tobacco organs is selective, which is specific for the individual elements, which decreases in the following order: lead and zinc: leaves > roots > stems; cadmium: leaves > stems > roots.

4.2. Effect of organic meliorants on accumulation of heavy metals in vegetative organs of Oriental tobacco

The results for the influence of organic meliorants on the accumulation and distribution of Pb, Zn, and Cd in the Oriental tobacco test crop are shown in Figure 2. The addition of compost results in a decrease in the Pb content of the tobacco roots and stems compared to the control, this decrease being more pronounced at 40 t/daa of compost. Adding a vermicompost also leads to a decrease in the Pb content in the roots and stems relative compared to the control, this decrease being more pronounced at 20 t/daa of additive. Similar are the results obtained when adding peat, with the lowest content being in the roots and stems when adding 40 t/daa of peat.

Quite complicated and controversial are the trends in the change in Cd and Zn content in tobacco roots and stems. The Zn and Cd content in the root system decreases in all variants of the experiments. A similar but less pronounced trend is observed for Zn and Cd in stems and leaves. The only exception is the use of additive 20 t/daa and 30 t/daa of peat, in which the Cd content in stems increases while in the 40 t/daa peat variant its content in stems remains close to that of the control. The reason is probably the significant increase in the total biomass.

The influence of the meliorants used on the accumulation of heavy metals in tobacco leaves is more complex and depends substantially on their quantity. Adding vermicompost leads to an increase in the Pb content of leaves, whereas in the introduction of 40 t/daa of compost and 20 t/daa of peat the decrease in Pb content is relatively well expressed. The trend of increasing the Zn and Cd content in tobacco leaves after the introduction of peat is preserved. The results obtained may be explained by the

amount of heavy metal mobile forms. The addition of peat to the soil leads to the increase of mobile forms, resulting in greater accumulation of these elements in the leaves of the plants.

Similar results are obtained for the influence of the vermicompost on the accumulation of heavy metals in tobacco leaves. As its quantity increases, the Zn content in leaves increases. Highest values were reported in the 40 t/daa vermicompost variant.

The compost influence on the heavy metal content the tobacco leaves is not unidirectional. With regard to Pb, Zn and Cd, there is a clear correlation between the amount of additive added and their content in leaves. By increasing the amount of compost the elements content in leaves is reduced. The decrease in Cd content in leaves is more pronounced in the 40 t/daa compost variant, suggesting that the introduction of 20 t/daa of meliorant is not enough to reduce the amount of Cd. Higher doses of meliorant may be more effective but economically unjustified.

The results obtained by us show that the effect of the meliorants used for the reduction of the Cd content in tobacco is negligible. This contradicts the results obtained by other authors. A possible reason for this is the fact that in many of the experiments the plants are not grown to the phase of technological maturity. Conclusions on the influence of meliorants are most often based on the results obtained after conducting a one-month experiment.

The difference in the results obtained by us and those obtained by Chlopecka & Adriano (1997) is probably due to the fact that tobacco has the ability to move Cd into the leaves to a much greater extent compared to corn (Mench et al., 1989; Mench & Martin, 1991). Mench et al. (1994) and Sappin-Didier & Gomez (1994) found that the Cd concentration in the above-ground mass of plants is always lower and the percentage of reduction is higher in the ryegrass compared to that in tobacco. A greater reduction in Cd content in plants was observed in the use of zeolites and phosphates in highly contaminated soils (Boisson et al., 1999; Gworek, 1992), although Basta et al. (2001) did not establish a positive effect of phosphates on the Cd content in lettuce grown in contaminated soils.

4.3. Effect of organic meliorants on the chemical indicators determining the quality of tobacco

Table 3 presents the results obtained for the influence of organic meliorants on the chemical indicators (ash, sugars and total nitrogen content).

Variants	Ash	Soluble carbohydrates	Proteins	Schmuk number
Control	14.2	22.3	7.35	3.0
20 t/daa compost	8.43	19.0	7.7	2.5
30 t/daa compost	17.69	16.66	1.55	10.8
40 t/daa compost	15.52	17.82	7.41	2.4
20 t/daa vermicompost	19.9	13.44	8.96	1.5
30 t/daa vermicompost	22.68	4.12	16.28	0.25
40 t/daa vermicompost	22.47	3.21	18.81	0.17
20 t/daa peat	13.9	23.69	6.85	3.5
30 t/daa peat	17.46	18.36	10.76	1.7
40 t/daa peat	17.63	13.54	9.66	1.4

Table 3. Chemical composition (%) of dried Oriental tobacco leaves, variety Krumovgrad 90

Organic meliorants also influence the tobacco quality indicators - ash, proteins and reducing sugars. The introduction of organic meliorants to the soil leads to increased minerals (pure ash) and protein levels, and reduces the amount of reducing sugars in tobacco leaves compared to the control.

Ash

Mineral substances have no direct influence on tobacco smoke properties, as only insignificant quantities pass into the smoke when burning. The total mineral content (ash) has a well-correlated correlation with the content of tobacco leaves and hence with their quality. The ash content in dried leaves of Oriental tobacco varies from 12% to 15 % (Gyuzelev, 1983). The increase in ash content compared to the control is strongly expressed when vermicompost and peat are introduced into the soil. As the amount of meliorant increases, the ash content increases and in the experiment with 40 t/daa of vermicompost it reaches 22.47% and in the experiment with peat it reaches up to 17.63%. The compost influence on the ash content is not unidirectional. Higher ash content was reported for variants with the addition of 30 t/daa and 40 t/daa of compost - 17.69% and 15.51%. In the variant with 20 t/daa of compost there was a slight decrease - 8.43%

Protein substances

The protein influence on the consuming properties of tobacco is generally negative, as in the pyrolysis of proteins nitrogen compounds with unpleasant odor and irritating taste are produced in tobacco smoke. The correlation between the protein content and the tobacco quality is negative. The protein content in tobacco leaves varies from 4% to 16%, while in Oriental tobaccos it reaches 8.5 %. The introduction of organic meliorants to the soil also has an impact on this indicator. The type and quantity of the additive used are also influential, the increase in the protein content compared to the control is very pronounced when the vermicompost is introduced into the soil. With an increase in the quantity of the meliorant their content increases, while in the experiment with 40 t/daa of vermicompost they reach 18.81%. The peat influence on the protein content is not unidirectional. Higher content was reported with the addition of 30 t/daa and 40 t/daa of vermicompost. In the variant with 20 t/daa of vermicompost, there is a slight decrease. The introduction of compost to the soil does not significantly affect the protein content compared to the control.

The nitrogen supply is the most important factor influencing tobacco quality (Mylonos et al., 1981; Jancheva, 2002; Ju et al, 2008). A significant negative correlation has been established between yield and protein content. The use of organic fertilizers leads to an increase in the nitrogen and nicotine content in leaves of Oriental Tobacco and Virginia (Juet et al., 2008). Controlled nitrogen release from organic fertilizers can be a useful practice for increasing the nicotine content in tobacco. It has been reported that nitrogen mineralization in later stages is an important factor affecting its accumulation and, consequently, the nicotine content in leaves (Wang et al., 2008).

Soluble carbohydrates

Oriental tobaccos contain significant amounts of reducing sugars, with the average content ranging from 10 to 18% (Ghiuselev, 1983). Very high levels of soluble carbohydrates (over 16%) and low nitrogen content result in unidirectional, incomplete taste, enhancing the burning effect. The amount of soluble carbohydrates is strongly influenced by the conditions of tobacco growing. Higher soil moisture and higher K and P content in the soil promote their accumulation while the lower moisture and higher nitrogen content is associated with lower carbohydrate content. The correlation between the amount of soluble carbohydrates and the tobacco quality is positive. The introduction of compost to the soil leads to a decrease in the content of soluble carbohydrates in tobacco leaves.

Substantial changes in the chemical composition of tobacco leaves are observed with the meliorants used. The introduction to the soil of organic meliorants leads to a decrease in the soluble carbohydrates content. The type and quantity of the meliorant used is influential. Reduction in the soluble carbohydrate content compared to the control was observed in all the meliorants used. The most significant is the decrease in the introduction of vermicompost to the soil. As the amount of melilant increases, the soluble carbohydrates decrease, and in the experiment with 40 t/daa of vermicompost they reach 3.21%.

Tobacco quality determination

Tobacco quality determination through its chemical composition is becoming increasingly widespread in practice. With the help of the Schmuck number, the main qualitative categories of Oriental tobacco

can be objectively characterized. Quality tobacco has a value above 1.0, the average quality tobacco is between 0.5 and 1.0, and the low quality tobacco is below 0.5.

Organic meliorants also influence tobacco quality. Adding compost to the soil does not cause the tobacco quality to deteriorate compared to the control. The introduction of compost to the soil produces high quality tobacco - low ash content, sufficient amount of soluble carbohydrates and a Schmuck number above 1.0. Quality tobacco is also produced as a result of the addition of peat. However, the introduction of 30 t/daa and 40 t/daa of vermicompost to the soil results in lower quality tobacco (Schmuck number below 0.5) (Table 3).

In the control, the ratio between total soluble sugars and proteins is the highest and reaches 3. The ratio between total soluble sugars and proteins is slightly lower for the compost variants - 2.4. In the variants with introduction of 30 t/daa and 40 t/daa of vermicompost, however, the ratio between total soluble sugars and proteins is very low, below 0.5, which would lead to deterioration of the smoking qualities of tobacco.

5. CONCLUSIONS

On the basis of the results obtained, the following conclusions can be drawn:

1. Tobacco is a crop tolerant to heavy metals and it develops normally when grown on soils contaminated with heavy metals.
2. The use of organic meliorants does not lead to effective immobilization of Pb, Cu, Zn and Cd phytoaccessible forms in soil. This contradicts the results of other authors who worked with slightly or moderately contaminated soils. Explaining this contradiction requires the accumulation and analysis of more experimental results.
3. The effect of the meliorants used for the reduction of the heavy metal content in tobacco is negligible. The best results are obtained with the introduction of 40 t/daa of compost.
4. Organic meliorants influence tobacco quality indicators (ash, protein and reducing sugars). The introduction of organic meliorants leads to increased minerals (pure ash) and protein levels, and reduces the amount of reducing sugars in tobacco leaves compared to the control.
5. Organic meliorants influence tobacco quality. Adding compost to soils leads to improved tobacco quality compared to the control. Quality tobacco is also produced as a result of the addition of peat. However, the introduction of 30 t/daa and 40 t/daa of vermicompost into the soil results in lower quality tobacco (Schmuck number below 0.5).

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