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MEETING POINT OF THE SCIENCE AND PRACTICE IN THE FIELDS OF CORROSION, MATERIALS AND ENVIRONMENTAL PROTECTION

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Determination of micronutrient accumulation in livestock fodder and soil in three Belgrade municipalities

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Abstract

Mineral nutrients (micronutrients) are essential for plant growth and develpment. In low concentrations they have a stimulating effect on plant functioning, but in high concentrations they can be toxic. These micronutrients are naturally present in the environment, but can also originate from industrial plants or from contaminated water used to irrigate agricultural fields. These elements are taken up by plants used for human consumption or grown to feed domestic animals (fodder). In order to evaluate and reduce the risk of growing potentially contaminated plants for livestock feed on soils in the immediate vicinity of coal mines and thermal power plants, the presence of Mn, Se and Zn in alfalfa (Medicago sativa L.) samples was determined. Alfalfa samples and associated soils were collected from the territory of municipalities of Lazarevac (village Sokolovo) and Obrenovac (village Krtinka), while the territory of the municipality of Surčin (village Jakovo) was chosen as the control site. The bioconcentration factor (BCF), as well as Spearman correlations were calculated, which can provide information about the potential efficiency of the removal of elements from the soil by the plant. The results of the content of the studied elements in fodder were within the usual concentrations for conventional production, except in the case of Se. The Se content in the studied Medicago sativa samples was in a range that can cause chronic or acute poisoning in livestock if consumed, so special attention is needed if these plants are used in the diet of livestock. However, alfalfa was found not to be a significant accumulator of Mn, Se and Zn, as the values of the bioconcentration factor were below 1. Examined element concentrations in soil were within MAC values for soils according to the regulations of the Republic of Serbia. These results urge caution in the cultivation of fodder at investigated sampling sites.

Keywords: *micronutrients; Medicago sativa; livestock nutrition; bioconcentration factor; Spearman correlations*

Introduction

Mineral nutrients (micronutrients) are essential for plant growth and development, and it is considered that there are 14 of them in total. There are also elements that have some beneficial effect on plants, although plants can survive without them, and are called beneficial elements. In low concentrations they have a stimulating effect on plants, but in high concentrations they can be toxic. Essential elements have a significant physiological and biochemical role in plants, being involved in the biosynthesis of chlorophyll and DNA, in the process of photosynthesis, in redox reactions in chloroplasts and mitochondria, in sugar metabolism and in nitrogen fixation, etc. [1].

All micronutrients are naturally present in the environment in higher or lower concentrations. However, a large proportion of them, as well as other pollutants in soil and water originate from industrial plants, as a result of the use of pesticides and mineral fertilizers or from contaminated water used to irrigate agricultural fields. These micronutrients are taken up actively or passively by plants that can be used for human consumption, or feed for domestic animals. Depending on their total abundance in the soil, these concentrations can sometimes rise to levels that are toxic to plants, animals and, consequently, to humans who consume food produced on such soils, as they are incorporated into the food chain [2-4]. The uptake of micronutrients by plants depends on numerous parameters in the soil and the species-specific characteristics of the plant itself [5,6].

In order to determine the presence of essential micronutrients - Mn, Se and Zn in the food chain, to assess and reduce the risk of growing plants for animal feed on soils in the immediate vicinity of coal mines and thermal power plants, samples of alfalfa (*Medicago sativa* L.) and soils were collected on the territory of the municipalities of Lazarevac (village Krtinka) and Obrenovac (village Sokolovo). The area of Surčin municipality (village Jakovo) was chosen as the control site without direct industrial activity. In addition, the bioconcentration factor (BCF) and Spearman correlations were calculated, which can provide information about the potential efficiency of the removal of chemical elements from the soil by the plant.

Materials and methods

Species description

Medicago sativa is a perennial herb that is one of the most important fodder crops (Figure 1). It is important for improving the physical, chemical and microbiological properties of the soil, as its harvest leaves significant amounts of organic matter in the soil, which is further decomposed. Owing to its deep root system, it helps improve the nitrogen fertility of the soil and protect it from soil erosion. The depth of root system makes it very resistant, especially to droughts. It is one of the most important commercial plants in livestock nutrition [7].

Figure 1. *Medicago sativa* L. *(alfalfa)*

Sample collection and preparation

Sampling was conducted in three Belgrade municipalities – Surčin (village Jakovo), Lazarevac (village Sokolovo) and Obrenovac (village Krtinka). At each of the three villages, three sampling sites (gardens) were randomly selected for plant and associated soil sampling. All of the chosen gardens are located in the vicinity of the fly ash disposal site of the 'Nikola Tesla-A' thermal power plant. *Medicago sativa* was sampled in the form of hay in the amount of approximately 2 kg. Samples of plant material were first dried at room temperature for 10 days, then in drying chamber (Binder, Tuttlingen, Germany**)** to a constant weight. The associated soil was also sampled from three individual sampling points and then mixed into a composite sample at the depth of 0-20 cm following a harmonised sampling regime (approximately 2 kg per sample). The surface layer of soil was chosen for analysis because element deposition in soil mostly occurs in top soil [8]. Soil samples were dried to a constant mass and homogenized.

Micronutrients analysis

The concentrations of selected micronutrients in *Medicago sativa* material were measured after wet digestion following USEPA 3052 protocol. Concentrations were measured by the method of optical emission spectrometry for simultaneous multielemental analysis (ICP - OES, Spectro Genesis). Beech leaves (BCR - 100) were used as the reference material for validation of the analytical procedure and quality control of the laboratory protocol. The analysis was performed in six replicates (n=6). The detection limits (mg kg^{-1}) are as follows: Mn- 0.000157, Se- 0.0102 and Zn- 0.00348. The element content in the soil was determined in the same manner as for the plant material, using the USEPA method (3052) with the standard reference material (Loam soil - ERM - CC141) to validate the analytical procedure. Detection limits were identical to those of the plant material.

Statistical analysis and bioconcentration factor (BCF)

The data from this study was analysed using statistical analysis (ANOVA) and means were separated with a Bonferroni test at a level of significance of $p<0.05$, using the Statistica software package (StatSoft In., Tulsa, USA, 2007). Correlations between the levels of the tested elements in soil and associated fodder were obtained using the non-parametric Spearman rank-order correlation coefficient at a level of significance of $P<0.05$.

Parameter which indicates the potential efficiency of removal of chemical element from soil by plants - the bioconcentration factor (BCF) was also determined. This factor defines the ratio between the available amount of a chemical element in the soil and the amount in the plant material ($[Element]_{leaf}/[Element]_{soil}$) [9,10]. A value of BCF > 1 indicates the potential of plant for phytostabilization of certain soil element.

Results and discussion

The concentrations of micronutrients in plant material were compared with reference values for the leaves of most herbaceous plants [11], and the maximum allowable concentration of certain elements in fodder that do not adversely affect the diet of domestic animals [12,13], Table 1.

Table. 1 Limit values of micronutrients in plants and fodder (mg kg^{-1})

	Mn	Se	Zn
TL[a]	300-500	5-30	100-400
MAC	100-250[b]	2-5[c]	2000[b]

[a] Critical or toxic levels in leaf tissue for various species [11]; [b] maximum allowable concentration in fodder [12]; [c] Edmondson et al. [13]

The concentrations of micronutrients in fodder and associated soil at the investigated sampling sites are shown in Table 2.

Table 2. Difference in micronutrients content between sampling sites in fodder and associated soil

	Fodder				Soil			
Mn (mg kg^{-1})	av ± st dev	Surčin	Lazarevac	Obrenovac	av ± st dev	Surčin	Lazarevac	Obrenovac
Surčin	26.89±2.96	/	***	***	777.57±28.68	/	***	ns
Lazarevac	37.46±0.71	***	/	*	613.57±18.39	***	/	***
Obrenovac	33.30±2.31	***	*	/	792.26±17.75	ns	***	/
Se (mg kg^{-1})								
Surčin	2.48±0.86	/	ns	ns	7.99±1.38	/	ns	ns
Lazarevac	3.31±1.30	ns	/	ns	9.49±1.60	ns	/	ns
Obrenovac	2.39±0.66	ns	ns	/	8.86±1.47	ns	ns	/
Zn (mg kg^{-1})								
Surčin	39.16±3.37	/	***	ns	107.89±5.58	/	***	***
Lazarevac	55.63±4.11	***	/	***	89.98±7.58	***	/	ns
Obrenovac	37.69±2.25	ns	***	/	84.80±1.75	***	ns	/

ANOVA, n=5, *p<0,05, **p<0,01, ***p<0,001, ns-no statistical significance

Manganese is an essential micronutrient that plants need for photosynthesis, respiration, synthesis of chlorophyll, synthesis of ATP, synthesis of acyl lipids, flavonoids, lignin and proteins. It is also an essential component of numerous enzymes [14, 15]. Its uptake is metabolically controlled, but under conditions of elevated Mn concentrations, passive absorption is also possible [16]. Manganese has an irreplaceable function in plants, but despite this, the lack of this element is often accompanied by the absence of visible symptoms of damage. Normal Mn concentrations were measured in the studied *Medicago sativa* samples (26-37 mg kg^{-1}, [11], Table 1), with statistically significant differences in accumulation capacity among all sampling sites (Table 2). All obtained results were well below the MAC for animal fodder (Table 1). Maximum allowable concentrations, limit and background values for Mn in soil are not defined in European and national legislation [17-20]. The highest average values for Mn in soil were measured in Obrenovac (792 mg kg^{-1}). Statistically significant differences in Mn content (<0.001***) were found between the control site in Surčin and Obrenovac (Table 2). The higher Mn content at these sites is probably due to local sources of pollution (thermal power plant), since Mn can enter the soil not only through the decomposition of the parent rock, but also through various anthropogenic activities [16]. To understand the pathways of element uptake, distribution and accumulation, the relationships between the content of micronutrients in fodder and the associated soil at all sampling sites were analyzed using the Pearson correlation coefficient. The correlation analysis showed a statistically significant positive correlation at all sites (Table 3), indicating the accumulation of Mn from the soil, except for the control site in Jakovo, where the lowest Mn content (26.89 mg kg^{-1}) was measured in the fodder. The presence of both positive and negative correlations indicates a double origin of Mn in the studied samples of *M. sativa*.

Selenium is an important micronutrient, necessary for the most basic physiological functions as a component of the amino acid selenocysteine. However, it is a relatively rare element. The uptake of selenium by plants and the total amount in plant tissues is influenced by many factors, including its content in the soil, its form, the pH and redox potential of the soil, and the mineral structure of the soil and fertilizers. From the plant physiological point of view, selenium belongs to the group of useful or beneficial elements. Selenium concentrations in animal feed from 0.1 do 0.5 mg kg^{-1} dry weight are considered safe with respect to possible selenium toxicity. Chronic and acute symptoms of poisoning are described at concentrations of 2 do 5 mg kg^{-1} [21-23]. From the obtained results (Table 2), it appears that the Se concentrations in the fodder are in a range that can be considered potentially risky, i.e., they can lead to poisoning or death of farm animals during consumption [23]. Namely, in 2012 in Saskatoon, Saskatchewan, Canada, there was a mass mortality of lambs given Se in the form of sodium selenite intramuscularly, resulting in pancreatic hemorrhage, subacute to acute heart failure and myocardial necrosis three days after treatment, suggesting acute selenium intoxication. These results urge caution in consumption of fodder at the studied sampling sites. From a statistical point of view, no significant differences in Se content were found between sampling sites, either in fodder nor in soil (Table 2). Most noncontaminated world soils contain 0.10 to 2 ppm of total Se [24]. In principle, soils that contain above 6 ppm of Se are considered selenium-rich. These soils are also known as seleniferous. The Se content in the studied soils varied between 8 and 9.5 mg kg^{-1}, indicating that these soils are enriched in Se, although the MAC regulations for harmful elements in agricultural soils do not specify values for this element [18]. In contrast, the National regulation of the Republic of Serbia [19] sets the upper limit for Se of 0.7 mg kg^{-1}, while concentrations of 100 mg kg^{-1} indicate significant contamination. Such inconsistencies in setting the MAC indicate the need to adopt new regulations, legislation, and harmonization of the MAC at the national level. The highest Se concentrations in fodder and soil were measured in Lazarevac. Correlation analysis revealed two significant negative correlations in Surčin and Obrenovac (Table 3), indicating that there is a different uptake of Se from soil and that this may be related to pollution from thermal power plants.

Zinc is an essential element that represents a building component of enzyme structure, is involved in the metabolism of carbohydrates and proteins, affects the permeability of membranes and the resistance of plants to pathogens. It also affects membrane permeability and stabilization of cellular components [16,25,26] and has a particular importance for chloroplasts, i.e., enzymes involved in the process of photosynthesis [27]. The normal Zn content in plants is in the range of 15-150 mg kg^{-1} [11], so it is evident from the results obtained (Table 2) that the fodder absorbed Zn in sufficient quantity for normal function. The accumulation of Zn depends on the amount available in the soil solution, from which the plant takes it up either as Zn^{2+} or in hydrated form, although it can also be absorbed in the form of complexes and organic chelates [11,28]. Statistically significant differences in Zn content (<0.001***) were found between the control site in Surčin and Lazarevac, while there were no differences between Surčin and Obrenovac (Table 2). All obtained results were well below the MAC for animal fodder (Table 1). Soil Zn content depends on the nature of the parent rock, organic matter content, soil texture, and pH [16,24] and did not exceed the MAC in soil and limit values proposed by European and national legislation (300 mg kg^{-1} ,[18]; 50 - 300 mg kg^{-1}, [17]). The highest Zn content in soil was measured in Surčin, the control sampling site (108 mg kg^{-1}), which was significantly different from the other two sampling sites (< 0.001***, Table 2). Correlation analysis revealed only one significant correlation in Obrenovac (Table 3), indicating that Zn is taken up from the soil at this site.

Table 3. Spearman's correlation coefficient for the tested micronutrients between fodder and associated soil

			Medicago sativa	
Sampling site		Mn_{soil}	Se_{soil}	Zn_{soil}
Surčin Jakovo	Mn	-.975**		
	Se		-.850*	
	Zn			-0.067
Lazarevac Sokolovo	Mn	0.992**		
	Se		0.211	
	Zn			-0.615
Obrenovac Krtinska	Mn	0.885*		
	Se		-0.891*	
	Zn			0.855*

Based on the obtained element concentrations in soil and plant material, the bioconcentration factor (BCF) was also determined. This parameter indicates the potential efficiency of removal of chemical element from soil by plant, so called phytostabilization. From the results obtained it was found that *Medicago sativa* is not effective in immobilizing the examined micronutrients, as all obtained values for BCF were below 1 (Figure 2).

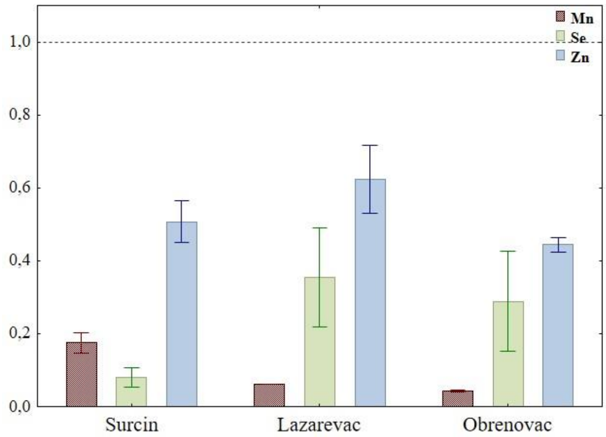

Figure 2. *Affinity of fodder to accumulate the tested micronutrients at the sampling sites based on the bioconcentration factor (BCF)*

Conclusion

The concentration of the examined micronutients measured in livestock fodder from the selected sampling sites was within the usual concentrations for conventional production, except in case of Se. According to the available literature data, the Se concentration in the studied samples of *Medicago sativa* was in a range that can cause chronic or acute poisoning of livestock when consumed, so special caution should be exercised when it is used as livestock feed. Nevertheless, the fodder was found not to be a significant accumulator of Mn, Se and Zn, as the bioconcentration factor values were less than 1, indicating that the fodder is not suitable for phytostabilization of these elements in the studied soils. It was found that the highest content of micronutrients in fodder was measured in Sokolovo (Lazarevac). On the other hand, no clear pattern was observed in the soils. The investigated micronutrient concentrations in soil were within the MAC values for soils according to the regulations of the Republic of Serbia and the limit values proposed by the Council Directive of the European Community. However, the results for Se in soil also show that the regulations and laws of the Republic of Serbia need to be revised, as there are clear contradictions between the maximum allowable concentrations, limit and remediation values.

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