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CONTNET OF TRACE ELEMENTS AND SOME RADIONUCLIDES IN LUCERNE *(Medicago sativa)*

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Invited paper

Abstract: The samples of lucerne were collected from twelve different locations in Vojvodina during the summer period July– September 2004. Raw lucerne trees were cut on the height of 4–5 cm under the ground in the amount of 2–3 kg. The samples were air-dried, ground, powdered and mineralized by the method of dry burning. The content of minerals, toxic elements and radionuclides in the samples of lucerne was determined. Due to low concentrations of detected toxic elements and radionuclides and contents of micro- and macro-elements, the obtained results indicate that alfalfa from the examined localities is a healthy and ecology sound feed component. However, with the aim of protecting the environment and preservation of human health, continuous surveillance of the content of toxic elements and radionuclides in lucerne, particularly in localities with increased risk of contamination, is necessary. Special attention should be given to the content and origin of mercury in soil and a possibility of contaminating plants with this toxic element. The obtained results could serve as a base for further investigations, regarding radiation-hygienic control of herbal feedstuff for animals.

Key words: alfalfa, essential elements, toxic elements, radioactive pollution

Introduction

Being an excellent source of iron, calcium and beta-carotene lucerne is nowadays considered the most important fodder crop worldwide. Lucerne (*Medicago sativa*) is a perennial forage plant belonging to the pea family *Leguminosae*. It has a deep root system, sometimes stretching to even 4.5 m, with the ability to fix nitrogen. Lucerne has the potential to be the most prolific of all leaf vegetable crops, processed by drying and grinding into powder, or by pulping to extract leaf concentrate.

The food and feedstuff mainly include plants and their products; hence nutritive value of such food is highly determined by the content and ratio of the mineral matter in the plants *(Živkov-Baloš et al., 1999)*. Having in mind that also certain toxic elements are introduced into the food chain via the plants, they may also present a source of potential contamination of the raw material used in food processing industry *(Živkov-Baloš et al., 2000, 2007; Ćupić et al., 2006; Mihaljev et al. 2003; Šarić et al., 2002; Mašić et al., 2001)*.

The first global estimate of the fertility status and level of hazardous and harmful substances in the soils of Vojvodina was made in 1992-1993 *(Kastori, 1997)*. This, as well as some later studies pointed out that Vojvodina could be a suitable area for the production of safe food *(Mihaljev et al., 1995; Čuvardić et al., 2003)* .

The soil was contaminated by discharges from the Chernobyl accident in 1986 in numerous regions of Central and Eastern Europe. Radioactive elements build in plants by foil deposition and resorption through the root system. Animals and indirectly human population can be exposed to radioactivity by ingesting forage of plant origin. Radiation-hygienic monitoring requires determination of activity level for biologically significant radionuclides (BSR), which can be natural and produced. Among natural radionuclides significant is potassium-40, that in natural potassium is present with mass fraction of 0.0119% and its contribution of the total beta activity in all biological community is over 90%. Among the produced radionuclides, first of all ^{137}Cs and ^{90}Sr, that are, due to their physicochemical properties very toxic, and contributes radiation loading *(IAEA, 1989)*.

Considering the constant hazard of soil contamination and accumulation of harmful elements in plants at localities close to industrial plants, the need for continuous control of harmful substances in plants presents an issue of paramount importance.

Materials and Methods

The samples of lucerne were collected from twelve different locations in Vojvodina during the summer period July – September 2004. Raw lucerne trees were cut on the height of 4-5 cm under the ground in the amount of 2-3 kg. The samples were air-dried, ground, powdered and mineralized by the method of dry burning. In the air-dried samples the humidity content was determined at the temperature of 105ºC.

Determining macro- and microelements (Ca, Na, K, P, Mg, Mn, Fe, Zn, Cu). Samples were air-dried, ground, powdered and mineralized by the method of dry-burning at temperature 550±10°C. Contents of calcium, sodium and potassium was determined by the method of emission flame spectometry. The contents of magnesium, iron, copper, manganese, zinc and cobalt were determined by the atomic absorption spectrometry applying Varian Spectr AA-10, while phosphorus concentration was determined by spectophotometry using Spekol 221MA 9524.

Determining the lead, cadmium and arsenic contents. Samples were powdered and mineralized by the method of dry-burning at temperature 450±10^{0}C. Concentration of Pb and Cd was determined by using atomic absorption spectrometer Varian Spektra-10 with background corrector (D_2-lamp). The contents of As were determined using distillation procedure by the method of Kingsley-Schaffert. The developed color, the “molybdenum blue” complex, is read on an appropriate spectrophotometer at 840 nm.

Determining the mercury content. Samples were prepared for assaying using the method of wet digestion with automatic temperature regulation (max 55^{0}C) and an aluminium thermoblock. Decomposition of samples was performed using concentrated sulphuric acid and potassium poermanganate solution. Content of Hg was measured using the flameless atomic absorption method, (“Cold Vapour”).

Gamma-spectrometry analysis. The samples were air-dried, and then ground, powdered and mineralized by the method of dry burning at temperature of 450ºC ± 10ºC. In the air dried samples the humidity content was determined at 105ºC. Gamma spectrometry measurements of lucerne ash were performed by means of ultra low-level background germanium detector using both active and passive shielding. Time of measurement was 80 ks. Extended range (10 keV-3 MeV) GMX type HPGe detector, nominal efficiency of 32% in 12 cm thick cylindrical lead shield, lined with 3.5 mm Sn + 0.5 mm Cu is surrounded by five 5 cm x50 cm x50 cm plastic veto shields. Veto plastic scintillators and Ge detector operate in anticoincidence mode, and on that way all events that are simultaneously detected in any veto and Ge detector will be rejected. The active shield reduces the integral background by factor 3 in the energy range from 50 to 2800 keV. Gamma spectrum of lucerne sample measured by active shielded HPGe detector Samples of Lucerne ash were measured in cylindrical geometry (67 mm x 62 mm) with 80 ks time of measurement. The example of gamma spectrum of lucerne sample is shown in Figure 1. The background is significantly suppressed by the developed active shielding method devices. For cesium ^{137}Cs 10 mBq/kg order of magnitude detection limits were achieved.

Statistical analysis. Statistical analysis of the obtained data (mean, standard deviation, variation range) was performed using the software package Statistica 7.0

Results and Discussion

The results of measurements of macro and microelements (Table 1) indicate the variability of the content of mineral matter in lucerne samples originating from different locations, which may be due to the pedological properties of the soil and carrying out the agrotechnical measures. Higher variability was noticed for microelements, particularly iron (20.00 – 271.01 mg/kg). The lowest variability was observed for the contents of phosphorus, magnesium and sodium, and partly also for calcium. Samples from certain localities exhibited strong deficiency of microelement contents that could be subject of further investigation. We must emphasize that lucerne belongs to rare feedstuff with measurable cobalt concentrations, and lack of this component in a diet may cause problems, particularly in ruminants that are readily susceptible to the cobalt deficiency.

Table 1. Concentration of essential elements in lucerne samples taken from different locations in Vojvodina

Elements	Ca	P	K	Na	Mg	Fe	Cu	Mn	Zn	Co
	($g\ kg^{-1}$)					($mg\ kg^{-1}$)				
Mean	9.97	2.36	17.92	0.87	2.57	104.7	4.3	23.5	13.5	0.06
Sd	2.05	0.41	6.58	0.43	0.64	103.1	2.8	12.7	7.5	0.04
Min	7.83	1.76	10.01	0.36	1.84	20.0	0.1	0.1	0.08	0.01
Max	14.03	3.09	32.69	2.12	3.74	271.0	8.3	46.0	26.3	0.15

Results on lead, cadmium, arsenic and mercury concentration in lucerne samples originating from different localities in Vojvodina are summarized in Table 2.

Table 2. Concentration of toxic elements in lucerne samples taken from different locations in Vojvodina

	Pb	Cd	As	Hg
($\mu g\ kg^{-1}$)				
Mean	509	33.8	220	127.1
Sd	301	11.1	85	120.0
Min	200	20.0	141	20.0
Max	1130	60.0	442	370.7

As obvious from the displayed data, the content of lead in the analyzed lucerne samples is not significant (200 – 1130 µg/kg) ranging within the maximum allowed concentration. The same applies to arsenic content, which ranged from 141 to 442 µg/kg. The cadmium concentrations measured in the investigated samples ranged within an interval of 20.0 - 60.0 µg/kg. The results given in Table 2 indicate that the concentrations of selected toxic elements (Pb, Cd and As) are lower than the maximum allowed concentrations *(Pravilnik, 2010)*. Our analysis revealed that the contents of Hg in alfalfa samples from some of the locations were higher than maximum allowed concentration. The cause of this phenomenon still remains to be elucidated *(Čuvardić et al., 2006)*.

Results of gamma spectrometry analysis of lucerne samples are shown in Table 3. Activity concentrations of radionuclides: ^{134}Cs, ^{137}Cs, ^{226}Ra, ^{232}Th and ^{40}K were expressed in units of mBq/kg. In some of samples cesium ^{137}Cs was detected in traces. These concentrations are 200 times lower as compared with the results obtained in 1988, two years after Chernobyl accident *(Bikit et al., 1990)*, when the average ^{137}Cs activity concentration was (9.0±1.0) Bq/kg. The sophisticated sample preparation and counting techniques enabled the detection of ^{226}Ra and ^{232}Th in almost all samples. The dominant ^{40}K activity concentration is expected for plants *(Forkapić et al., 2006)*.

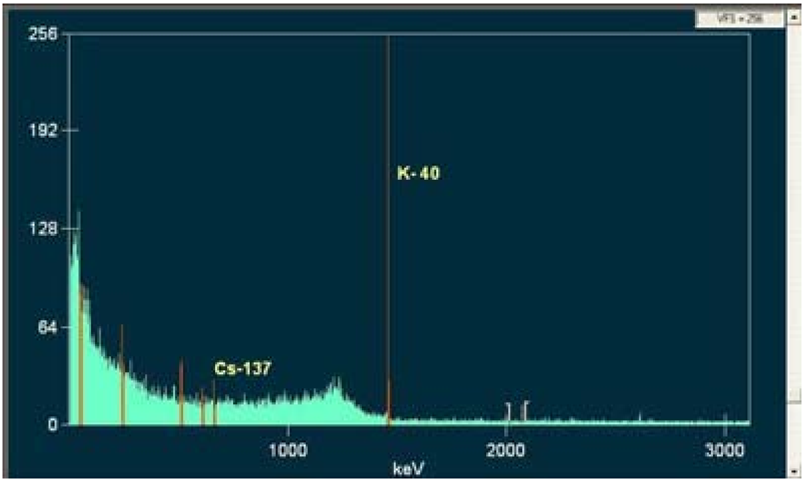

Figure 1. Gamma spectrum of Lucerne sample measured by active shielded HPGe detector

Table 3. Activity concentrations of ^{134}Cs, ^{137}Cs, ^{226}Ra, ^{232}Th and ^{40}K in lucerne samples taken from different locations in Vojvodina

Isotope	^{134}Cs	^{137}Cs	^{226}Ra	^{232}Th	^{40}K
Location	A [mBq/kg fresh mass]				
1	<75	122±22	175±100	90±62	$(121\pm4)x10^3$
2	<100	<100	302±70	218±25	$(140\pm4)x10^3$
3	<80	70±15	<300	80±15	$(92\pm3)x10^3$
4	<75	<100	<150	<38	$(103\pm3)x10^3$
5	<100	<100	<1500	112±28	$(141\pm4)x10^3$
6	<100	<125	275±225	132±30	$(191\pm6)x10^3$
7	<75	<75	450±150	335±22	$(88\pm3)x10^3$
8	<75	<100	175±150	65±20	$(144\pm5)x10^3$
9	<125	<150	198±53	125±75	$(272\pm8)x10^3$
10	<68	<75	200±150	75±58	$(133\pm4)x10^3$
11	<125	<150	<375	188±55	$(158\pm7)x10^3$
12	<1125	125±32	<350	105±38	$(115\pm6)x10^3$

Obvious variations in potassium content lead to the conclusion that incorporation of radionuclides from the soil into the plants largely depend on pedological properties, soil exploitation and agrotechnical treatments. It is known that phosphoric manure contains significant quantities of heavy elements – products of uranium decay and so contributes the increase of environment radioactivity *(Ćupić et al., 2005)*.

Conclusion

Due to low concentrations of detected toxic elements and radionuclides and contents of micro- and macro-elements, the obtained results indicate that alfalfa from the examined localities is a healthy and ecology sound feed component. However, with the aim of protecting the environment and preservation of human health, continuous surveillance of the content of toxic elements and radionuclides in lucerne, particularly in localities with increased risk of contamination, is necessary. Special attention should be given to the content and origin of mercury in soil and a possibility of contaminating plants with this toxic element. The obtained results could serve as a base for further investigations, regarding radiation-hygienic control of herbal feedstuff for animals.

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Sadržaj minerala, toksičnih elemenata i radionuklida u uzorcima lucerke *(Medicago sativa)*

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Rezime

Uzorci lucerke sakupljeni su sa 12 lokacija u Vojvodini, tokom perioda jul-septembar 2004. godine. Stabljike lucerke sečene su na visinu od 4-5 cm iznad površine tla, u količini od 2-3 kg uzorka. Uzorci su prirodno sušeni, mleveni i mineralizovani metodom suvog spaljivanja. U uzorcima je određen sadržaj minerala, toksičnih elemenata i radionuklida. Na osnovu niskih koncentracija toksičnih elemenata i radionuklida, a povoljnog sadržaja makro i mikroelemenata može se zaključiti da je lucerka sa ispitivanih lokaliteta zdravo i ekološki bezbedno hranivo. U cilju zaštite okoline i zdravlja ljudi i životinja, neophodan je monitoring prisustva toksičnih elemenata i radionuklida, posebno na lokacijama sa povećanim rizikom zagađenja. Posebnu pažnju treba posvetiti istpitivanjima sadržaja i porekla žive u zemljištu i mogućnostima kontaminacije biljaka ovim elementom.

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