Biotechnology in Animal Husbandry 24 (5-6), p 95-104 , 2008
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 633.331

NITRATES IN PLANTS AND SOIL AFTER FERTILIZATION OF GRASS-LEGUME MIXTURES **

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**Original scientific paper-Originalni naučni rad
Research was financed by the Ministry of Science of Republic of Serbia within project TR 20005/08 - Rad je finansiran od strane Ministarstva za nauku Republike Srbije u okviru projekta TR 20005/08

Abstract: Nitrates are one of the most frequent utilized forms of N from soil. Through root they reach stem and leaf where in photosynthetic processes they convert into proteins. Due to exposure to stress situations, and excessive nitrogen fertilization, nitrate accumulation in plant tissues and organs occurs. Also, nitrates can accumulate in harmful concentrations in soil. Nitrates accumulated in plants have harmful effect on animals which consume plants since they cause various health disorders. In variance analysis in both investigation years it was established that fertilization as well as interaction of factors mixture/fertilization had very significant effect on investigated quality parameter, contrary to mixture as single factor. In both investigation years, treatments with 210 kgN ha^{-1} had the highest nitrate concentration. Content of nitrate nitrogen in soil is increased by fertilization. Objective of the research was to determine nitrate quantities which are accumulated in plants subsequent to n fertilization and growing in mixtures with other species, as well as to determine nitrate quantities which remain in the soil unutilized, which are potential causes of environment pollution.

Key words: grass-legume mixture, soil, nitrate, fertilizer.

Introduction and literature review

Agricultural producers want to realize high and stable production of good quality. Therefore mineral fertilizers are applied, primarily N fertilizers which increase the quantity of N forms of minerals necessary for plant growth and development. Plants have different needs/requirements in regard to N.

Especially high quantities of N are accumulated by plants which are characterized by large vegetative mass (*Vojin et al., 2003*).

Nitrates are one of the most frequent utilized forms of N from soil. Through root they reach stem and leaf where in photosynthetic processes they convert into proteins. Due to exposure of plants to stressful situations (drought, warm and dry wind, cloudy and cold weather, frost, plant injuries, etc.) nitrates accumulate in plant tissues and organs, mainly in the bottom third of the stem (*Stanton, 2001*).

Introduction of high quantities of N fertilizer can also lead to accumulation of NO_3^- in plants, but also in soil, which can have negative consequences. From the aspect of soil, accumulated nitrates are being transported from rizosphere by precipitation to underground waters and surface waters and cause pollution. According to EU legislation, allowed quantity of nitrates in underground waters is up to 50 mg/l (Nitrate Directive- 91/676/EEC). Nitrates accumulated in soil have unfavourable effect on nitrogen fixation in leguminous plants, since they reduce the root surface, as well as number of root nodules (*Hannaway and Shuler 1993*).

According to *Wright and Davidson (1964),* nitrates accumulated in plants have harmful effect on animals which consume plants since they cause various health disorders, even deaths (*quote Shiel et al., 1999*). Agricultural Research Council (1980) states that nitrate concentrations over 3000-5000 ppm are considered as potentially dangerous and must be avoided in certain groups of animals – livestock in gestation, due to incidence of abortions (*quote Shiel et al., 1999*). However, *Stanton (2001)* states that forage containing less than 5000 ppm of nitrates is considered as harmless and can be used in nutrition, forage containing from 5000-10000 ppm is considered potentially toxic, and forage containing over 10000 ppm of nitrates is considered as toxic and can not be used in nutrition.

Content of NO_3^- in plants is in direct relation to content of nitrates in soil (*Ilinef, 2000*). Introduction of N to the soil through fertilizers increases the concentration of NO_3^- in soil solution, which leads to increased intake and accumulation of NO_3^- in plants, (*Petrović, 2003*).

Objective of the research was to determine nitrate quantities which are accumulated in plants subsequent to n fertilization and growing in mixtures with other species, as well as to determine nitrate quantities which remain in the soil unutilized, which are potential causes of environment pollution.

Material and methods

A two-factor trial was set at the Institute for Animal Husbandry, Belgrade-Zemun during 2003-2004. The first factor in the investigations was

sward type: pure lucerne and its three mixtures (mixture I, with cocksfoot; mixture II, with cocksfoot and tall fescue; and mixture III with cocksfoot, tall fescue and sainfoin. The second factor was fertilization with four different quantities of N (0, 70, 140 and 210 kg N ha^{-1}). Fertilization was carried out on two occasions during a single year, in 2003 after sowing and after the first cut, and in 2004 at the beginning of vegetation and after the first cut. The nitrate content in the herbage DM of mixtures was investigated as a quality parameter and is presented in this paper as an average value of all cuts in the investigation year. The data were analyzed by analysis of variance for a 4 x 4 randomized block design with four blocks, mean values tested with LSD test

The soil on which the trial was set was poor carbonate chernozem, of favourable water, air and thermal regime, and very good granular structure. Chemical characteristics of the soil are presented in Table 1.

Table 1. Chemical characteristics of the soil on which the trial was conducted
Tabela 1. hemijske karakteristike zemljišta na kojem je izveden ogled

Depth/ Dubina	**pH**	**pH**	**Humus**	**$CaCO_3$**	**N Total**	**N-NO_3**	**P_2O_5**	**K_2O**
cm	H_2O	KCl	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
0-20	7.29	7.08	43.5	3.3	1975	52	909	162
20-40	7.23	7.09	44.3	4.7	1938	57	918	165

Average air temperatures during 2003 and 2004 were higher than the long-term average and were recorded as 12,7 °C and 12,4 °C, respectively. Based on the data on total annual precipitation it can be concluded that 2003 was dry with 551,9 mm of precipitation, which was 93,3 mm lower than the average value determined for several years. In 2004, however, the precipitation was as high as 831,6 mm which had a positive effect on the productive characteristics of plants.

Results and discussion

Analysis of DM yield and nitrates in plants

In variance analysis in both investigation years it was established that fertilization as well as interaction of factors mixture/fertilization had very significant effect on investigated quality parameter, contrary to mixture as single factor.

In the first year, higher nitrate concentrations in plants by 21,3% or 459 ppm was determined compared to the second research year (table 2).

Mixtures had no statistically significant effect on content of nitrates in plants, but it was registered that by combining grasses and legumes in mixture, content of nitrate increases compared to single lucerne crop. Same results were obtained by *Shiel et al. (1999),* in their investigation of content of nitrate in mixtures where by increase of share of grasses also the nitrate concentration increased. This is interpreted in a way that grass species in mixtures have greater physiological ability for accumulation of higher concentrations of nitrate ions in relation to leguminous plants *(Nešić et al., 2007, 2008).*

In both years, fertilization had very significant effect on content of NO_3^- in plants. With the increase of the quantity of added N, also the content of NO_3^- in mixtures increased. Treatments with 210 kgN ha^{-1} resulted in significantly higher concentration of NO_3^- compared to other treatments, as well as treatments with 140 and 70 kgN ha^{-1} compared to treatment without N. For instance, in year 2003, maximum content of NO_3^- was established in treatment with the highest added quantity of N - 3401 ppm, which was by 185,7% more compared to treatment without N. In the next research year, treatments with 210 kg N ha^{-1} had maximum concentration of 2847 ppm, and treatments without N minimal of 732 ppm which represents difference of 288,9%. Obtained differences between treatments were at the level of probability of 99%. *Totev et al. (1997)* and *Shiel et al. (1999),* also state that content of NO_3^- in mixtures increases with increase of amount of added nitrogen by fertilization, and that it differs in various cuts.

Interaction of factors in both investigation years showed very significant effect on content of nitrate ion in forage mixtures, and established differences at the level of significance of $p<0,01$ are present within individual mixtures for certain fertilization treatments, and between mixtures.

Based on these results it can be concluded that fertilization, in general, did not induce the increase of nitrate content above the harmful level (Agricultural Research Council, 1980). Exceeding of this limit occurred in some cases depending on the year, cut and treatment.

Table 2. Content of NO_3^- (ppm) in DM of sowed grassland depending on the share of lucerne in mixture and N fertilization in years 2003 and 2004
Tabela 2. Sadržaj NO_3^- (ppm) u suvoj materiji sejanog travnjaka u zavisnosti od udela lucerke u smeši i đubrenja N u 2003. i 2004. godini

Year/Godina			**2003**	**2004**
Mixtures/Smeše (A_n)	**N (B_n)**		**Nitrates/ Nitrati**	**Nitrates/ Nitrati**
Lucerne/ Lucerka	0		1054	836
	70		1694	1271
	140		1899	1875
	210		3055	2550
Average/Prosek			**1926**	**1633**
Mix./ Smeša I	0		1175	593
	70		1350	1441
	140		2807	2167
	210		3227	2972
Average/Prosek			**2140**	**1793**
Mix./ Smeša II	0		1317	652
	70		1939	839
	140		2366	1698
	210		3812	2386
Average/Prosek			**2358**	**1394**
Mix./ Smeša III	0		1215	846
	70		1820	1300
	140		2227	2215
	210		3508	3479
Average/Prosek			**2192**	**1960**
Average/Prosek	0		**1190**	**732**
	70		**1701**	**1213**
	140		**2325**	**1989**
	210		**3401**	**2847**
	0,01	A	2022,3568	1198,0148
		B	886,4897**	954,0737**
		AB	2585,1746**	2073,4136**
	0,05	A	1334,9628	790,8126
		B	654,1704	704,0429
		AB	1774,3776	1469,8517
P<0.05 *; P<0.01 **				

Analysis of nitrates in soil

Content of nitrates in soil subsequent to fertilization at the end of trial is presented in table 3. Content of ammonia and nitrate forms of nitrogen increased in relation to the beginning of trial in average by 52,1% NH_4^+- N and 40,1% NO_3^- - N.

Content of ammonia nitrogen was the highest in soil on which the mixture II was investigated - 35 ppm, and the lowest in mixture III of 19 ppm, which is difference of 16 ppm. In relation to pure/single lucerne crop, where content of ammonia nitrogen in soil was 26 ppm, content of ammonia nitrogen in mixture II was higher by 9 ppm, and lower in mixture III by 7 ppm.

In treatments without fertilization the highest concentration of $NH4^+$- N in soil of 30 ppm was realized, and treatments with 70 kgN ha^{-1} resulted in the lowest concentration of 26 ppm, which is lower by 4 ppm.

The highest amount of nitrate nitrogen was determined in mixture I - 94 ppm, and the lowest in mixture III - 61 ppm, which makes the difference of 33 ppm. In comparison to pure lucerne crop, where nitrate concentration of 71 ppm in soil was established, in mixture I concentration higher by 23 ppm, and in mixture III lower by 10 ppm were established. Content of NO_3^- - N in soil increases with fertilization. The lowest NO_3^- - N was established in treatments without fertilization - 61 ppm, and the highest in treatments with 210 kgN ha^{-1} of 89 ppm, which is by 46 % higher compared to the minimum.

According to results of *Petrović et al. (1985)* that content of nitrate and ammonia N, in soil type chernozem with intensive agricultural production, is 9,4 and 3,8 ppm, respectively. *Andraski and Bundy (2002)* obtained results on content of nitrate nitrogen in soil after growing of lucerne on depth of 30 cm of 10,0 - 43,0 ppm, and *Ferguson et al. (2002)* of 2,7-14 ppm in soil on depth of 90 cm. Results in stated research indicate that content of this nutrient is high which can represent potential threat and danger of pollution of soil, water and plants with nitrates. It is considered that such high concentrations of NO_3^- in soil are consequence of application of high quantities of N mineral fertilizers (*Elgersma and Hassink, 1997; Eghball, 2002*), as well as accumulation of N in soil from nitrogen fixation which plants have not utilized.

Table 3. Chemical analysis of soil depending on the type of mixture and amount of added N fertilizer subsequent to trial in 2004
Tabela 3. Hemijska analiza zemljišta u zavisnosti od vrste smeše i količine dodatog N đubriva nakon izvođenja ogleda u 2004. godini

MIXTURE/SMEŠA	N	NH_4^+	NO_3^-	Content of NH_4^+ and NO_3^- In trial design/Sadržaj NH_4^+ i NO_3^- u postavci ogleda
		N mg/1000g(ppm)		
Lucerne/ Lucerka	0	33	62	
	70	25	69	
	140	22	75	
	210	25	78	
Mixture/ Smeša I	0	36	70	
	70	23	75	
	140	30	96	**18,3 ppm NH_4^+**
	210	35	136	
Mixture/ Smeša II	0	34	58	**51,9 ppm NO_3^-**
	70	38	62	
	140	32	61	
	210	36	79	
Mixture/ Smeša III	0	16	53	
	70	16	57	
	140	32	69	
	210	13	64	
$\overline{X}$		28	73	

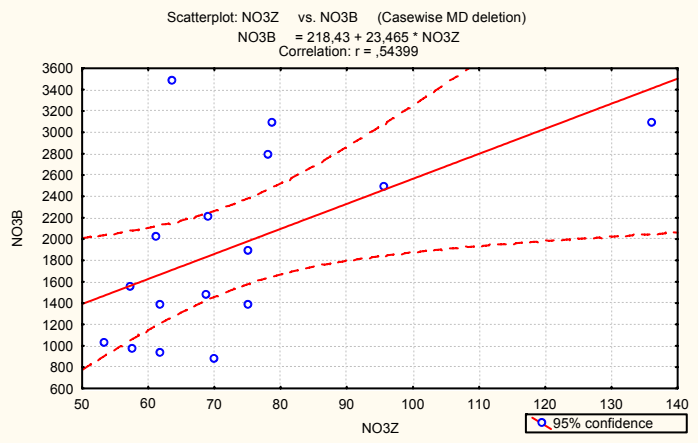
By correlation analysis of variable traits dependence between investigated parameters was established (graph 1).

Average value of nitrates in plants in both investigation years and content of nitrate soil in different treatments at the end of trial are in positive correlation r_{xy}=0,54399 (graph 1), i.e. with the increase of content of nitrate in soil also the content of nitrate in plant increases, which is in accordance with results obtained by *Ilinef (2000).*

From results relating to nitrate in soil we can see that values are not negligible and if by precipitation they would be completely washed off, which is highly probable, and considering that analysis was done in autumn, in underground waters higher concentrations above allowed limits would occur

(Nitrate Directive- 91/676/EEC). Therefore it is necessary to reduce the amount of N applied with fertilization, and in this way not only the quantity of nitrate in soil would be reduced but also in plant, which is good from the aspect of quality.

Graph 1. Dispersion diagram of dependence of nitrates in plants and soil
Grafikon 1. Dijagram raspršenosti zavisnosti nitrata u biljkama i nitrata u zemljištu



Conclusion

Content of NO_3^- in grass-leguminous mixtures in both investigation years is highly dependant on fertilization and interaction of investigated factors.

With the increase of quantity of N also the content of $NO3^-$ increases. The highest content of nitrates was established in treatments with 210 kgN ha^{-1} of 3401 ppm in year 2003 and 2847 ppm in year 2004, whereas the lowest content was established in treatments without N in both investigation years - 1190 ppm and 732 ppm, respectively.

In interaction of investigated factors, the highest content was established in mixture of lucerne, cocks foot and tall fescue (II) with 210 kgN ha^{-1} of 3812 ppm in first investigation year and in the second investigation year mixture with sainfoin (III) with 210 kgN ha^{-1} of 3479 ppm.

Fertilization with N caused increase of content of NO_3^- in soil. Content increased in average by 40% compared to content prior to beginning of trial and varied from 53 ppm in mixture III 0N to 136 ppm in mixture I 210N.

Between nitrates in plant and nitrates in soil there is positive correlation r_{xy}=0,54399, i.e. with the increase of content of nitrates in soil also the content of nitrate in plant increases.

Fertilization of grass-leguminous mixtures with high doses of nitrogen, in general, does not induce accumulation of nitrates in plant above allowed limits, which would cause harmful effect on health condition of livestock, however, these amounts of nutrients added to fertilizer have harmful effect on environment since they cause accumulation of nitrates in soil and pose potential threat/danger of underground and surface water pollution.

Nitrati u biljci i zemljištu nakon đubrenja travno-leguminoznih smeša

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Rezime

Nitrati su jedan od najčešće usvajanih oblika N iz zemljišta. Preko korena oni dospevaju do stable i lista gde se fotosintetičkim procesima konvertuju u proteine. Usled izloženosti stresnim situacijama, kao i preteranim đubrenjem azotnim đubrivima, dolazi do nagomilavanja nitrata u tkivima i organima biljaka. Pored toga oni se mogu nakupljati u štetnim koncentracijama i u zemljištu. U biljkama nagomilani nitrati imaju štetnog uticaja na životinje, jer ishranom, izazivaju različite zdravstvene poremećaje. Analizom varijanse u obe godine ispitivanja utvrđeno je da su đubrenje kao i interakcija faktora smeša/đubrenje imali vrlo značajnog uticaja na ispitivani parametar kvaliteta, za razliku od smeše kao pojedinačnog faktora. U obe ispitivane godine tretmani sa 210 kgN ha^{-1} imali su najveću koncentraciju nitrata. Sadržaj nitratnog azota u zemljištu povećava se đubrenjem.Cilj istraživanja je da se utvrdi koje su to količine nitrata koje biljka akumulira nakon đubrenja N đubrivim i gajenjem u smeši sa drugim vrstama, kao i koje su to količine nitrata koje ostaju u zemljištu ne iskorišćenje, a koje mogu biti potencionalni zagađivači životne sredine.

Ključne reči: travno-leguminozne smeše, zemljište, nitrat, đubrivo.

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