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Soil Ammonification Activity in the Conditions of Mineral and Organic Fertilizer Use

Pesakovic, M., Mandic, L., Djukic, D.

Faculty of Agronomy, Cacak, Serbia and Montenegro

Abstract: Two-year investigations (1996-1997) were carried out to examine the effect of different N rates (N₁-30 kg/ha, N₂-60 kg/ha and N₃-90 kg/ha), standard P and K amounts (75 kg/ha) and organic fertilizers (solid manure–45 t/ha and liquid manure–80 t/ha) on the microbiological activity of the smonitza soil under spring oats. The trial was set up on the Faculty of Agronomy Experimental Field in Cacak in a randomized block design with three replications.

The effect of the applied fertilizers on soil microorganisms had been determined twice during the growing season and observed by establishing the number of ammonifiers and the soil proteinase activity using standard microbiological and biochemical methods.

The investigations showed that the applied fertilization variants, sampling periods and study years had affected the number of microorganisms and the soil proteinase activity.

Mineral fertilizers had caused a reduction in the number of ammonifiers. The N_3 variant had the highest inhibitory effect of all the variants of the mineral fertilizers studied. The inhibitory effect of the fertilizers used was more pronounced in the second sampling period. As opposed to that, the same fertilizers increased the soil proteinase activity, particularly in the first sampling period.

Furthermore, the organic fertilizers used caused a reduction in the number of ammonifiers and proteinase activity.

The number of ammonifiers was bigger in 1996 and the soil proteinase activity was reported to be higher during 1997.

Key words: soil, microorganism, enzyme, fertilizer, oats.

Introduction

50-60 % of basic volume of agricultural production in developed countries is based on the use of mineral fertilizers (*Hajnis et al.*, 1979). Mineral

fertilizers often enter agroecosystems at much higher rates than necessary for the primary plant production, not only causing economic damage but reducing soil productivity, crop yield quality and increasing water eutrophication, thus negatively affecting human health and disturbing established nature's equilibrium (*Djukic, Mandic,* 1997).

Nitrogen fertilizers are the biggest danger, due to high nitrate-nitrogen mobility, resulting in surface and underground water pollution (*Byerly*, 1975, *Commoner*, 1975, *Schaessler*, 1986). As a result of agricultural production, at least half of the bound nitrogen enters waters (*Kling*, 1985; *Robier*, 1986), causing an excessive growth of algae, that are, after dying out, subjected to anaerobic degradation, which owing to an oxygen deficiency leads to death of fish and other aquatic animals. In addition, nitrates above the acceptable levels accumulate also in plants (*Govedarica et al.*, 1991 a, b) – both in those used in human nutrition and in those used as feed for livestock consumption (*Seiz*, 1986; *Marinkovic and Grcic*, 1993). The danger associated with nitrates is that they are easily converted into nitrites and nitrosamines, which are potent carcinogens (*Commoner*, 1975, *Hoffman*, 1986). Longer-term application of high mineral fertilizer rates does not always have an expected effect. Longer-term use of nitrogen and potassium fertilizers results in an agricultural crop yield reduction (*Avdonin and Lebedev*, 1970).

The effects or organic fertilizers on the soil biological component are reflected through direct inhibition or stimulation of major cellular processes or indirectly, through encouraging certain mineralization processes, thus leading to the improvement of both potential soil fertility and plant nutrition.

Bearing in mind the fact that microorganisms are the most active part of biogeocenosis, there is a possibility of an efficient protection from various dangers such as: accumulation of products of plant, animal and microorganism metabolism, as well as of those of the anthropogenic activity.

Having in mind the importance of microorganisms as bioindicators in the soil, the aim of these investigations was to examine the effect of different rates and combinations of mineral and organic fertilizers on the number and activity of different ecological-trophic groups of microorganisms.

Material and Method

The investigations were carried out over the 1996-1997 period on the Experimental Field and in the Microbiological Laboratory of the Faculty of Agronomy in Cacak. Field, laboratory and mathematical-statistical methods of conducting scientific research were used. The experiment was set up on the smonitza soil under spring oats in a randomized block design with three replications.

The soil the trial was set up on was characterized by very acid reaction (pH - 5.01) and good potassium supply (26.38 mg/100 g soil) and it was poor in easily available phosphorus (1.78 mg/100 g soil).

Following the basic cultivation of the soil, the oats sowing was performed and appropriate mineral and organic fertilizer rates were applied.

As regards the mineral fertilizers, nitrogen fertilizers were applied at three rates ($N_1 = 30$ kg/ha, $N_2 = 60$ kg/ha and $N_3 = 90$ kg/ha), whereas phosphorus and potassium fertilizers were used at the rate of 75 kg/ha each. Nitrogen, phosphorus and potassium rates were applied in the form of urea (46 % N), superphosphate (18 %) and 60 % KCl, respectively.

As far as the organic fertilizers are concerned, burnt (45 t/ha) and liquid (80 t/ha) manures were used.

The size of the experimental plot was 21.25 m^2 . During the growing season, the usual cultural operations were used. The soil that had not been treated with the mentioned fertilizers was used as the control.

Results and Discussion

Based upon the study results, it could be concluded that the number of ammonifiers in the soil depended on the fertilization variants used, sampling period and study year.

The fertilizers used had an inhibitory effect on the development of ammonifiers in the soil (tab.1).

Tab. 1. Ave	rage number of an	monifiers (10°/1	.0 g absolutely	dry soil) in the soil
depending	g on the fertilizers	used (A), samplii	ng period (B) a	ind study year (C)

Fertilizer (A)						ø	ľ	N ₁	N	N_2		N_3		Liquid manure	X
r (C)	96	od (B)		I	109.11		102	93.5		50	49.50		85.00	42.25	93.22
	19	Perio		П	16	66.68	128	8.00	102.	83	68.17	7	118.83	52.33)3.22
Yea	97	iod 3)	Period (B)		6	0.50	52	.75	38.0	00	29.25	5	58.67	26.50	26.47
	19	Per (]			2	5.69	2.	16	1.8	1.83			20.33	0.66	20.47
V			I	84.81		77.63		65.75		39.38	8	71.84	34.38	62.30	
	А			П	96.19		65.08		52.33		34.74		69.58	26.50	57.40
$\overline{\mathbf{X}}$			9	0.49	9 71.3		59.04		37.05		70.71	30.44	59.85		
lsd															
lsd A		A		В	В		С		AxB		AxC	BxC	AxBxC		
	0.05	24.17		13.9	95 13		.95 3		4.18		34.18	19.73	48.34		
	0.01 32.28		18.3	37	18.37		4	5.65		45.65	26.36	64.56			

Through comparison of the average numbers of ammonifying microorganisms between the fertilization variants, it can be concluded that the highest number was obtained in the control variants (84.805 x 10^6 and 96.167 x 10^6 in the first and second sampling periods, respectively). The use of mineral and organic fertilizers caused a decrease in the number of this physiological

group of microorganisms and the most depressive effect was produced by the liquid-manure variant. The variant in question caused a statistically highly significant decrease in the number of ammonifiers in both periods investigated $(34.375 \times 10^6 \text{ and } 26.495 \times 10^6 \text{ in the first and second sampling periods},$ respectively), being probably the result of Al compound entering into the soil solution that as such becomes toxic for many microorganisms (*Emtsev, Djukic*, 2000). A somewhat smaller depressive effect was produced by the N₃ variant, which had statistically highly significantly influenced the reduction of the number of ammonifiers (34.733 x 10⁶) only in the second sampling period, whereas in the first period, the decrease had been less pronounced and therefore reported to be statistically significant (39.375×10^6) . Of all the fertilizers applied, the variant with solid manure $(71.833 \times 10^6 \text{ and } 69.58 \times 10^6 \text{ in the first and})$ second sampling periods, respectively) had exerted the smallest effect on the development of ammonifiers in the soil. The use of manure reduces soil acidity, Al and Mn mobility, increases nutrient availability, due to which the depressive nitrogen effect is less pronounced, which indirectly affects the development of soil microorganisms as well.

By the analysis of the obtained data it can be concluded that the number of ammonifiers was higher in the first than in the second sampling period (62.30 x 10^6 and 57.40 x 10^6 , respectively). The difference could be the result of the differences in soil moisture and temperature in these two periods. In their investigations on the effect of moisture on various processes, *Misustin et al.* (1978) established that ammonification processes are most undisturbed in the soil saturated with 60 % MWC.

The number of ammonifiers depended highly on the study year as well. The results obtained indicate the statistically highly significant reduction in the number of ammonifiers during 1997 (93.22 x 10⁶ during 1996 and 26.47 x 10⁶ in the year 1997). The effect of the established depressive effect can be associated with a high sensibility of this group of microorganisms in long-term fertilized soils (*Suhovickaja, Miljto*, 1982).

The analysis of the effect of the fertilization variants applied during 1997 has shown that the variants statistically insignificantly influenced the reduction of the number of this group of microorganisms. During 1996, the most depressive effect was registered with the variant with liquid manure (42.29×10^6) and the N₃ variant (58.8333×10^6). The depressive effect of mineral fertilizers on the development of ammonifiers in soil was recorded by a number of authors (*Duljgerov*, 1982; *Pavlenko*, 1982; *Miljto*, 1982; *Djukic*, 1997).

Based upon the results obtained, it can be concluded that the number of ammonifiers in all the periods investigated was statistically highly significantly greater in 1996, being the result of an increase in soil biological productivity occurring due to applying growing mineral fertilizer rates during the first year. Two-three years upon the use of mineral fertilizers, however, quality changes during the humification and dehumification processes occur, negatively affecting the presence of ammonifiers in soil as well.

Furthermore, a higher number of ammonifiers during 1996 was recorded in the second sampling period (106.137×10^6). As opposed to that, in the second sampling period, during 1997, there was a statistically highly significant reduction in the number of this group of microorganisms (8.658×10^6) .

Judging by the results obtained, the soil proteinase activity was affected by the fertilizers used, the sampling period and the study years.

The fertilizers used had a stimulating effect on the soil proteinase activity. The mineral fertilizer stimulating effect differed, varying from being statistically insignificant ($N_3 - 49.603$ and 44.375 in the first and second sampling periods, respectively; the N_2 variant – 47.252 in the second sampling period), significant (the N_2 variant – 51.608 in the first sampling period) to being statistically highly significant (the N_1 variant in both periods investigated – 53.933 and 52.252 in the first and second periods, respectively). Organic fertilizers proportionally at the same level had a stimulating effect on the proteinase activity in both sampling periods. The highest proteinase activity was determined in the variant with liquid manure (62.70 gelatinolytic units per 1.0 g air-dry soil). Higher proteinase activity due to the effect of fertilizers was reported by other investigators as well (*Rankov et al.*, 1997; *Djukic*, 1984, 1991) and explained to be the result of soil improvement with mineral elements necessary for the development of this group of microorganisms.

Tab. 2. Average	proteinase activ	vity (number	of gelatinolyt	ic units/1.0	g air-dry so	il) in the
soil depending of	on the fertilizers	s used (A), th	e sampling pe	riod (B) and	d the study	year (C)

Fertilizer (A)					Ø	N_1		N	2 N ₃			Solid manure	Liquid manure	X	
	96	od (B)	I	4	44.05	53	.04	48.0	67	47.2	1	57.50	64.63	47.98	
r (C)	15	Peri	п	4	40.38	43	.71	33.8	38	32.50	0	53.59	56.67		
Yea	97	iod (8	I	4	48.40	54	.83	54.5	55	52.00	0	57.18	67.63	56.67	
	19	Der (]	LII 47.5		47.50	60	60.80		53	56.25		58.45	61.88	56.07	
T		I	4	46.23		53.94		51.61		1	57.34	66.13	54.14		
	А		п	4	43.94		52.26		47.26		8	56.02	59.28	50.52	
$\overline{\mathbf{X}}$		4	45.08	53.09		49.43		46.99	9	56.68	62.70	52.33			
	lsd														
	lsd A			В	(c .		AxB		AxC	BxC	AxBxC		
	0.05	5	3.50		2.0	2 2.0		02 4		4.95		4.95	2.86	7.00	
	0.01		4.68		2.7	0	2.70		(6.61		6.61	3.82	9.35	

Through comparison of average proteinase activity over study years, it can be observed that the activity was lower in 1996 (47.98 GU/1.0 g air-dry soil) than in 1997 (56.67 GU/1.0 g air-dry soil). During the year 1996, a statistically highly significant effect of the variants with organic fertilizers (55.543 and 60.647 GU/1.0 g air-dry soil in the variant with solid manure and the liquid-

manure variant, respectively) was recorded. A high level of the soil proteinase activity affected by the liquid manure is the result of direct incorporation of this group of microorganisms into the soil, since there are about 3×10^8 microorganisms per cm³ liquid manure (*Misic*, 1978). Furthermore, a statistically significant effect of the N₁ variant (48.373 GU/1.0 g air-dry soil) was registered. In the second study year, all the variants used highly significantly affected the proteinase activity increase, with the exception of the N₃ variant (54.125 GU/1.0 g air-dry soil), whose effect was evaluated as significant. *Leontjev* (1969) also pointed out to the stimulative effect of the NPK fertilizers on proteinase activity. In addition, according to *Polesko* (1970), in different fertilization variants used, proteinase activity increases in the grown-plant root system.

During 1996, proteinase activity was higher in the first sampling period (52.514 gelatinolytic units per 1.0 g air-dry soil). By nitrogen incorporation into the soil, the hydrolytic processes occur much more rapidly and microorganisms start to use even those organic compounds that have been unavailable to them up to then. The level and speed of these processes depend on climatic factors. Hence, a decrease in the proteinase activity at the end of the growing season in 1996 (43.452 GU/1.0 g air-dry soil) is assumed to be affected by the unfavourable precipitation distribution. A lower proteinase activity was observed in the first sampling period the following (1997) year, as compared to the second sampling period (55.763 and 57.584 GU/1.0 g air-dry soil, respectively). During the mentioned year, a far better precipitation distribution was registered, which affected the increase of the total soil biological activity.

Conclusion

Based upon the study results on the effect of mineral and organic fertilizers on the number and activity of microorganisms in the soil, the following conclusions could be inferred:

- the number of ammonifiers, as well as the soil proteinase activity depended on the type and rate of fertilizers, the sampling period and the study year;
- by the use of mineral fertilizers, the number of ammonifiers decreased, which was not the case with the soil proteinase activity, except in the final growing stages;
- of all the mineral fertilizer variants studied, the variant with the highest nitrogen rate had exerted the most pronounced inhibitory effect;
- the organic fertilizers used, particularly liquid manure, caused a reduction in the number of ammonifiers and an increase in the soil proteinase activity;
- the fertilizers used had a pronounced stimulative effect at the onset of the growing season and a marked inhibitory effect at the end;
- the number of ammonifiers and the soil proteinase activity were higher in 1996 and 1997, respectively.

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AMONIFIKACIONA AKTIVNOST ZEMLJIŠTA U USLOVIMA PRIMENE MINERALNIH I ORGANSKIH ĐUBRIVA

- originalni naučni rad -

Pešaković, M., Mandic, L., Djukic, D.

Agronomski fakultet, Čačak

Rezime

Tokom dvogodišnjih proučavanja (1996-1997) praćen je uticaj različitih doza N (N₁-30 kg/ha, N₂-60 kg/ha i N₃-90 kg/ha), jednakih količina P i K (75 kg/ha) i organskih đubriva (čvrstog 45 t/ha i tečnog stajnjaka 80 t/ha) na mikrobiološku aktivnost smonice pod jarim ovsem. Ogled je izveden na Oglednom polju Agronomskog fakulteta u Čačku po sistemu slučajnog blok rasporeda u tri ponavljanja.

Efekat primenjenih dubriva na zemljišne mikroorganizme određivan je dva puta tokom vegetacije, a praćen je putem određivanja brojnosti amonifikatora i proteinazne aktivnosti zemljišta standardnim mikrobiološkim i biohemijskim metodama.

Istraživanja su pokazala da su na brojnost mikroorganizama i proteinaznu aktivnost zemljišta uticale primenjene varijante đubrenja, periodi uzimanja uzoraka i godine istraživanja.

Primena mineralnih dubriva je izazvala sniženje brojnosti amonifikatora. Od svih ispitivanih varijanti mineralnih dubriva najizraženiji inhibitorni uticaj pokazala je N₃ varijanta. Inhibitorni uticaj primenjenih dubriva je bio izraženiji u drugom periodu uzimanja uzoraka. Nasuprot tome ista dubriva uticala su na povećanje proteinazne aktivnosti, posebno u prvom periodu uzimanja uzoraka.

Primenjena organska đubriva su, takođe, delovala u pravcu sniženja brojnosti amonifikatora i proteinazne aktivnosti.

Brojnost amonifikatora bila je veća tokom 1996. godine, a proteinazna aktivnost zemljišta tokom 1997. godine.