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THE DETERMINATION OF POTENTIAL AMMONIFICATION IN SOIL BY ARGININE METHOD

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Abstract: In this paper investigations were carried out on two soil types (vertisol and brown forest soil) with different doses of applied N-fertilizer: ∅, N_{60}; N_{90}; N_{120} and N_{250}. The potential ammonification in soil was obtained by arginine method. The following properties of soil were determined: pH value, organic C, available NH_4-N and mobile-Al.

The pH value in vertisol was 3.75-4.07; mobile-Al was 0.67-4.90 mg/100g; % organic C 1.38-1.46 and the content of available nitrogen was 4.4-11.2 ppm. The amount of released NH_4-N by arginine ammonification in this soil type was very low [(-0.12)-0.27μg/$g^{-1}h^{-1}$]. Correlation coefficients between released NH_4-N from arginine and soil pH were (-0.96*), mobile Al – (-0.99**), applied fertilizer doses – (-0.95*).

In brown forest soil the amount of released NH_4-N by arginine ammonification was greater than in vertisol, ranging from 3.16 to 7.11μg/$g^{-1}h^{-1}$. Correlation coefficients between soil properties and released NH_4-N from arginine were not statistically significant.

Key words: N-fertilizer, potential ammonification, arginine ammonification.

Introduction

Alef and Kleiner (1986) have suggested a new method of determination of potential microbial activity, by using arginine ammonification.

It is well known that arginine can be decomposed by microorganisms in several reactions. Many details connected with the structure and function of enzyme engaged in these reactions are explained in last decades (Abdelal, 1979).

Alef and Kleiner have used observation of Zobell and Upham (1944) and Saper (1981) that the most, if not all heterotrophic bacteria release NH_4-N, when substrate rich with nitrogen is used as carbon source.

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This method is simple, cheap, relatively fast. If we take into consideration that plants can not use arginine and animals, first of all protozoa, use it very slowly, the obtained data reflect most probably only activities of microorganisms.

Alef and Kleiner (1987) showed that the application of this method has two limitations. First, this method can not be applied in soils which have high initial lavel of NH_4-N. It is clear, considering the fact that content of the final product (NH_4-N) inhibits the activity of enzyme arginase which transforms initial substrate (arginine). Second, this method can not be applied on samples where source of energy is not limited.

The aim of this investigation was to consider the application of proposed method on two important Serbian soil types, as possible alternative to incubation method. The replacement of this last method would be of great importance, because of its complexity in application, specially in needed time to be conducted, and, moreover, as a most important fact, the incubation method induces changes of physiological conditions and number of microorganisms, which can give a wrong picture of soil condition.

Material and Methods

The investigations were carried out on two soil types as a part of the long-term experiments: one was on brown forest soil (Mladenovac-Institute of Soil Science - Belgrade) and the second one vertisol soil (Kragujevac-Center for Small Grains). The treatments with different nitrogen doses were selected: ∅, N_{60}, N_{90}, N_{120} and N_{250} kg/ha. The samples were collected from 0-30 cm depth in spring, before vegetation starts up.

Analytical methods

In investigated soils standard soil analyses were applied:

-Soil reaction (pH) was estimated in soil suspension with 1M KCl potentiometrically with glass electrode.

- Organic C was estimated by Tjurin `s method, modification by Simakova (1957).

- In soil available, an initial content of NH_4-N was estimated by the distillation process, where 1M KCl and MgO and $CaCl_2$ was added to the soil suspension. Released NH_4^+ was captured in boric acid and determined with 0.00025 M H_2SO_4 by titration.

- Titration method of Sokolova was used for the determination of mobile aluminium.

Method of arginin ammonification

The determination of arginin ammonification was obtained in wet soil samples (40% WHC), where 0.2% arginin solution was added, and then the

samples were kept for 3 hours in dark at 25° C. Then, the samples were frozen at –15° C during 24h, in order to stop ammonification process. After that period, samples were de-frozen, 40 ml of 2 KCl was added and they were shaked for 30 minutes. After filtration, in filtrate NH_4-N was estimated by distillation method with MgO and $CaCl_2$ addition. The arginin ammonification ($\mu g/g^{-1}h^{-1}$) in soil samples was determined by the NH_4-N difference between the samples treated and non-treated with arginin. All these estimations have been conducted in three repetitions.

Results and Disscusion

The experiment was conducted on two different soils types: brown forest soil (Mladenovac) and vertisol soil (Kragujevac). The results of standard soil analyses are presented in Tab.1.

Tab.1. - The standard soil analyses of investigated soils

	pH (in KCl)	% C	NH_4-N μg/g (initial content)	mobile Al
Vertisol				
∅	4.07	1.38	8.5	0.63
N_{60}	3.88	1.54	5.0	3.04
N_{120}	3.88	1.49	4.4	2.62
N_{250}	3.75	1.46	11.2	4.90
Brown forest soil				
∅	4.60	1.12	4.5	0.18
N_{60}	4.30	1.20	5.2	0.54
N_{120}	4.15	1.25	7.3	2.25
N_{250}	4.05	1.19	17.8	0.99

The range of pH in brown forest soil was between 4.05 to 4.60, where the decrease of pH value followed an increase of added nitrogen with fertilizers. The same results were obtained for vertisol soil, where pH was much lower (3.75-4,07), mostly coming bellow 4.0.

According to the low pH, in the investigated soil, the content of mobile Al was estimated. In vertisol, this mobile Al fraction (0.63-4.9 mg/100 g) was much higher than in brown forest soil (0.18-2.25 mg/100 g), generally well associated with pH decrease of the soil.

The content of organic C ranged in brown forest soil between 1.12 and 1.25%, while in vertisol this range was from 1.38 to 1.54%.

In the spring, an initial content of available NH_4-N was determined in 0-30 cm soil layer. This content in brown forest soil was closely related with applied dose of N from fertilizers, giving 4.5-17.8 μg/g. Also, the same observation was present in a vertisol soil, where the available NH_4-N was 4.4-11.2 μg/g. In both

cases, the origin of this nitrogen form was closely related with former N fertilizer application (H e r r o n et al. 1971., H a y n e s,1981).

As presented in Tab.2, very small amounts of released NH_4-N in all treatments were estimated during the arginin ammonification in vertisol soil, specially compared with the results repoted by the authors of this method (A l e f and K l e i n e r, 1987_a, 1987_b, A l e f et al. 1988).

T a b .2. - The content of NH_4-N produced by arginin ammonification

Treatments	NH_4-N ($\mu g/g^{-1}h^{-1}$)
Vertisol	
∅	0.27
N_{60}	0.10
N_{120}	0.13
N_{250}	-0.12
Brown forest soil	
∅	4.52
N_{60}	6.33
N_{90}	7.11
N_{120}	5.33
N_{250}	3.16

Negative correlation was found between soil pH and produced NH_4-N by arginin ammonification in vertisol soil (r=-0.96*). These results are in disagreement with the results of A l e f and K l e i n e r (1986, 1987_a, 1987_b), where this relationship was not found. The discrepancy between these results could be explained by the fact that used soils were not with such low pH value. In presented papers (1987_a, 1987_b,) the soils were within the 4.2-7.9 pH range, while in the other paper (1986) the soils were with 4.2-6.6 pH.

T a b .3.- Correlation coefficient between soil properties and arginin ammonification

Soil properties	Arginin ammonification	
	Vertisol	Brown forest soil
% C	-0.39^{NS}	0.41^{NS}
pH	-0.96*	0.09^{NS}
NH_4-N	-0.48^{NS}	-0.70^{NS}
Fertilizer doses	-0.95*	0.25^{NS}
Mobile Al	-0.99**	-0.56^{NS}

It is well known that microorganisms population and its activity depend greatly on soil pH, where its low values significantly decrease its activity (H a y n e s, 1986), So, it is absolutely clear that vertisol arginin ammonification was depressed with low pH. Another important influence on NH_4-N production

by arginin ammonification in vertisol soil depends on the content of mobile aluminium, where the highest negative correlation was achieved (r = - 0.95). This comes from the decrease of pH, which can induce toxic effects of many elements, specially aluminium. The obtained results represent this negative effect of mobile aluminium on the content of produced NH_4-N by arginin ammonification. Some other relations were also observed, such as the relation between applied amounts of N fertilizers and produced NH_4-N (-0.95*), where the relation between available NH_4-N and organic C was not found.

The production of NH_4-N by arginin ammonification in the investigated brown forest soil was considerably higher than in vertisol. The correlative dependence of produced NH_4-N by arginin ammonification with soil pH value was not found, what is in a accordance with the results of A l e f and K l e i n e r (1986, 1987_a, 1987_b). This indicates that soil microbial activity on this soil type was not affected by soil pH value. If we related two investigated soils according to the effects of mobile aluminium, similar results woul be obtained. This correlative dependence between initial NH_4-N content in soil and the produced NH_4-N by arginin ammonification was not found, except that the produced NH_4-N by arginin ammonification in treatment with highest initial NH_4-N content (17.8 μg/g) was at the lowest level (3.16 μg/g). It should be also noted that the authors of this method did not give precise data about the amount of available NH_4-N which inhibited the activity of arginase, but it is a fact that the amount of produced NH_4-N by arginin ammonification was lowered with the increase of available NH_4-N. Considering all results, it would be useful, if possible, to compare this applied arginin method with the incubation methods (aerobic and anaerobic) on our predominant soils. This would be the right approach to the evaluation of methods, specially because of its short term of implementation.

C o n c l u s i o n

According to the obtained results of potential ammonification in vertisol soil by using arginin method, this method could not be recommended for soils whose characteristics have negative effects on microbial activity (low pH value, high content of mobile aluminium).

It could be also concluded, upon our results for brown forest soil, that the closest pH value which limits its application is around 4.00 (nKCl). Also, it is certainly of great concern a level of available NH_4-N when this method is applied.

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ODREDJIVANJE POTENCIJALNE AMONIFIKACIJE U ZEMLJIŠTU METODOM SA ARGININOM

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Rezime

Cilj ovog rada je bio da se na dva značajna tipa zemljišta Srbije primeni i proveri predložena metoda, kako bi se sagledala kao moguća alternativa za inkubacione metode.

U tu svrhu istraživanja su obavljena na dva tipa zemljišta, gajnjača (Mladenovac) i smonica (Kragujevac) u okviru stacionarnih ogleda. Korišćene su varijante ogleda sa rastućim dozama azota djubriva. Odabrane su sledeće varijante

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ogleda: ∅, N_{60}, N_{90}, N_{120} i N_{250}. Uzorci su uzimani sa dubine 0-30 cm u proleće, pre kretanja vegetacije. Na uzetim uzorcima zemljišta primenjena je metoda amonifikacije arginina, kako su predložili Alef i Kleiner (1986). Takodje je u uzorcima zemljišta utvrdjena pH vrednost, sadržaj mobilnog Al, sadržaj organskog ugljenika i sadržaj pristupačnog NH_4-N.

Na osnovu dobijenih rezultata (smonica) ne može se preporučiti korišćenje metode sa argininom kod zemljišta čija svojstva imaju direktan negativan uticaj na aktivnost mikroorganizama (niska pH vrednost, visok sadržaj mobilnog Al). Mogli bi da zaključimo na osnovu naših rezultata (za gajnjaču) da je približna granica pH vrednosti zemljišta ispod koje ovu metodu ne treba primenjivati 4,00 (nKCl). Na kraju kod primene ove metode obavezno se mora voditi računa o sadržaju pristupačnog NH_4-N u zemljištu.

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