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AEROBIC AND ANAEROBIC INCUBATION — BIOLOGICAL INDEXES OF SOIL NITROGEN AVAILABILITY

ABSTRACT: Our researches have been made on brown forest soil that had been used in long-term experiments set up according to specified fertilization system for over 30 years. We have chosen those experiment variants in which quantities of nitrogen fertilizers were gradually increased. The soil samples taken from 0 cm to 30 cm depth were used to determine biological indexes of nitrogen availability (aerobic and anaerobic incubation). The same samples were also used for pot experiments with oat. Plant and soil parameters obtained in controlled conditions were used for determination of biological indexes reliability in measuring the soil nitrogen availability. On the grounds of correlation analysis, it can be concluded that biological index of nitrogen availability achieved by the anaerobic incubation (without subtraction of the initial content of available nitrogen) of the investigated brown forest soil is the reliable indicator of soil nitrogen availability. That is not the case with the aerobic incubation in which reliability has not been established.

KEY WORDS: aerobic incubation, anaerobic incubation, biological index, nitrogen, plant and soil parameters, availability

INTRODUCTION

Biological methods used to determine the soil nitrogen availability index have been researched and considered to be quite reliable in assessing the soil nitrogen availability by a number of authors (Keeney and Bremner, 1966; Ozus and Hanway, 1966; Robinson, 1968; Stevanović, 1978; Confort and Walmsley, 1971; Gasser and Kalembara, 1976).

Aerobic incubation is a satisfactory method in assessment of the plant nitrogen availability, considering the fact that nitrogen mineralization during incubation is being caused by the same organisms that mineralize nitrogen in the field. Although this is a good argument, we cannot neglect the fact that the en-

vironment conditions (humidity, temperature, aeration) that are being monitored during mineralization in laboratory do significantly differ from the ones in the field. Despite the limitations that we mentioned, many researchers have established that the aerobic incubation procedure can give suitable results (Allison and Sterling, 1949; Fits et al., 1953; Munson and Stanford, 1955; Robinson, 1968 a, b; Stanford and Lagg, 1968; Fox and Piekielek, 1978; Power, 1980; Stajković, 1990).

In 1964, taking into consideration the mentioned limitations of the aerobic incubation, Waring and Bremner suggested a new method called anaerobic incubation to determine the nitrogen availability index.

This method, in comparison with aerobic method, has the following advantages:

Only $\text{NH}_4\text{-N}$ content is being estimated, the initial quantity of $\text{NO}_3\text{-N}$ is lost by denitrification, while further nitrification is completely stopped, bigger quantity of nitrogen is being developed in a shorter period of time (7 days) than in an aerobic incubation. That means that this is a faster method, which is important in routine analyses.

Anaerobic incubation, together with the aerobic one, has been the most commonly applied procedure among the biological methods used to determine the nitrogen availability index. The values obtained through these procedures ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in aerobic and $\text{NH}_4\text{-N}$ in anaerobic) are most frequently compared to the plant parameters (yield, N intake or N% in the cultivated plants), whether the plants have been cultivated in control conditions (pots) or in the field.

The aim of our researches was to verify the value of aerobic and anaerobic methods, as the most significant biological methods in assessment of soil nitrogen availability. The researches have been done on brown forest soil that had been used in long-term experiments set up according to specified fertilization system for over 30 years.

MATERIAL AND METHODS

The researches have been done on brown forest soil (Mladenovac) that had been used in long-term experiments carried out by the Institute for soils in Belgrade. The long-term experiments are set up according to specified fertilization system with mineral fertilizers for over 30 years.

Experiment variants with increasing doses of nitrogen fertilizer were selected and applied in our investigations: control (\emptyset), $\text{N}_1\text{P}_2\text{K}_2$ ($\text{N}_1\text{-60}$, $\text{P}_2\text{-120}$, $\text{K}_2\text{-120}$ kg P_2O_5 and $\text{K}_2\text{O/ha}$), $\text{N}_2\text{P}_2\text{K}_2$ ($\text{N}_2\text{-90}$ kg/ha) $\text{N}_3\text{P}_2\text{K}_2$ ($\text{N}_3\text{-120}$ kg/ha) and $\text{N}_4\text{P}_2\text{K}_2$ ($\text{N}_4\text{-150}$ kg/ha). The samples from the mentioned experiment variants were taken in March for both aerobic and anaerobic incubation, as well as for the pot experiments.

I. Methods applied to estimate nitrogen availability

a) *Aerobic incubation*

250 ml glass jars, into which 10 g of air-dried soil was weighed, were used. The soil humidity was brought at 30%, the jars were covered with plastic foil. Incubation lasted for 14 days at the temperature of 30°C (Bremner, 1965). After that, in an hour shaking, a 2 M KCl solution extraction was taken. Short lasting distillation in the presence of MgO and CaCl₂ and titration with 0.0025 H₂SO₄ were applied to determine the contents of NH₄-N and NO₃-N.

b) *Anaerobic incubation*

Five grams of air-dried soil were measured, put into tube and 12.5 ml of distilled water was added. Completely anaerobic conditions were achieved that way. The period of incubation lasted for 7 days at the temperature of 40°C (Waring and Bremner, 1964). After that, content from the tube was quantitatively transferred to distillation flask by multiple rinsing with 12.5 ml of 2 M KCl. Distillation was carried out with MgO and CaCl₂ and distillate libated was held in boric acid. Titration was done with 0.0025 M H₂SO₄ and the quantity of obtained NH₄-N was determined.

II. Methods used to establish chemical properties of the soil

Basic agochemical analysis of soil samples were taken from the long-term experiment were carried out using the following methods:

— Soil reaction: pH value in suspension with water and 1 M KCl was determined potentiometrically with glass electrode;

— Organic carbon and humus were determined using dichromatic method by Tjurin, modification of Simakov;

— Total nitrogen quantity was determined using semi-micro Kjeldahl method where the soil sample was digested with concentrated H₂SO₄ with the addition of catalyst mixture K₂SO₄: CuSO₄: Sn-1-1:10:100 (Bremner, 1965)

— Content of available nitrogen was determined by steam distillation method from soil salt extract obtained after one-hour shaking with 2 M KCl. NH₄-N content was established through a short distillation in the presence of small quantities of MgO and CaCl₂. Distillate was caught in boric acid and titrated with 0.0025 M H₂SO₄. When NH₄-N had been determined, Devarda's alloy was added and new distillation was performed. This time NH₄-N was held in a new quantity of boric acid and titration with 0.0025 M H₂SO₄ was used to establish the contents of NO₃-N.

— Available phosphorus and potassium were established using Al-method by Egner-Riehem (1960).

III. Experiment in pots

Plastic pots, each containing 2 kg of soil from the layer 0 — 30 cm of soil, were used in the experiment. The soil was taken out in spring (March) from the labeled experiment variants in the field. Before the beginning of the experiment, soil was brought up to the air-dried conditions, ground and the desired quantity was measured. The vegetation experiment was performed with two fertilizing variants, the PK and NPK. Prior to sowing, the soil was mixed with the fertilizers that had previously been dissolved in water, i.e. with: NH_4NO_3 , KH_2PO_4 and KCl. The used NH_4NO_3 was labeled with a stable isotope ^{15}N (11.8%). In the PK variant 50 mg of P_2O_5 and K_2O /kg of soil was used, while in the NPK variant 50 mg of N, P_2O_5 and K_2O /kg of soil was used. Ten plants of oats per pot were used in the experiment. The plants were grown to the phase when plants blade. During the experiment, the humidity was maintained at level 60 to 80% of water retention capacity.

IV. Parameters of plants and soil in pots

Having in mind the most commonly used parameters for plants in pots, the following parameters were used in our researches to assess the values of the applied methods (Keeney and Bremner, 1966; Sirota, 1973; Peterson et al. 1960 and Sapošnjikov's suggestions, 1973): plant parameters (yield of oats in NPK and in PK experiment variants, relative increase in yield (PK = 100), relative increase in yield in NPK variant ($\emptyset = 100$), relative increase in yield in PK variant ($\emptyset = 100$), difference in yield (NPK-PK), difference in yield (NPK- \emptyset), content of nitrogen in cultivated oats (in NPK and PK)) and soil parameters (total uptake of nitrogen (NPK); uptake of soil nitrogen (NPK), uptake of fertilizer nitrogen (NPK), ratio of soil and fertilizer nitrogen uptake and uptake of soil nitrogen (PK)).

The simple linear correlation analysis was used to establish all the mentioned parameters of the plants and soil in pots, as well as for the biological methods applied (aerobic and anaerobic).

Absolute values of the nitrogen developed after both aerobic and anaerobic incubation procedures, with and without taking into account the initial content of available nitrogen (NH_4 and $\text{NH}_3\text{-N}$ in aerobic and $\text{NH}_4\text{-N}$ in anaerobic incubation) were used as biological indexes of nitrogen availability.

RESULTS AND DISCUSSION

Brown forest soil (Mladenovac) was used in these researches. Basic chemical properties of the investigated soil are presented in Table 1.

Table 1 — Chemical properties of the investigated soil

Variants	pH		Total nitrogen (%)	Humus %	C:N	Available		
	H ₂ O	1 MKCl				P ₂ O ₅ (mg/100 gr)	K ₂ O (gr)	NH ₄ +NO ₃ (ppm)
Control	5.40	4.60	0.10	1.43	8.5	6.4	16.2	4.9
N ₁ P ₂ K ₂	5.10	4.30	0.10	1.67	8.7	18.0	21.8	8.4
N ₁ P ₂ K ₂	5.00	4.20	0.11	1.81	9.3	14.4	21.8	15.4
N ₃ P ₂ K ₂	4.90	4.15	0.10	1.85	9.5	16.0	25.0	12.2
N ₄ P ₂ K ₂	5.00	4.05	0.12	1.88	9.4	12.5	21.8	51.3

On the grounds of the obtained soil pH values (M KCl) the conclusion can be drawn that the investigated brown forest soil belongs to the acid or very acid soil category.

According to the humus content, the brown forest soil comes to the category of soils poor in humus, while its total nitrogen content puts it at the limits of poor content. The C/N ratio is somewhat lower (less than 10) than in (standard) arable soils due to the fact that drop in humus exceeded the drop of the total nitrogen content.

The content of available phosphorous varied in the field experiment variants. The lowest was in the control variant (6.4 mg/100 gr) and the highest was in the variant N₁P₂K₂ (18 mg/100 gr), which had put the soil into the poor to medium soil category.

Quantities of available nitrogen varied in the experiment variants in the regular pattern starting with the control experiment variant (4.9 ppm) to N₄P₂K₂ variant (51.3 ppm).

The aerobic incubation procedure used in these researches was introduced by Bremner (1965) as modification of some previous procedures (Allison and Starling, 1949; Fitts at al., 1953; Hanway and Dumenil, 1953).

It was Bremner's suggestion to calculate the quantity of the mineral nitrogen obtained during aerobic incubation by subtracting the established quantities of available nitrogen after and before incubation.

However, quite frequently quantities of the established mineralized nitrogen prove to be lower after incubation than before the incubation, particularly with the poorly fertile soils or with the soils with high content of residual mineral nitrogen. Hence, the quantity of mineralized and nitrified nitrogen may have negative values. One can suppose that such results are caused by immobilization. When soils of poor fertility are being used for the laboratory experiments, such conditions are favourable for mineralization processes. However, the obtained mineral nitrogen is being used by microorganisms so that the final count gives negative results. On the other hand, in the soils with higher contents of available nitrogen which, as a rule, originates from the previous fertilization (Herron at al., 1977), in conditions of optimum humidity and temperature, microorganisms will also develop and take up the available nitrogen in a more intensive way, so that the obtained results will have negative values in this case, too.

The conclusion may be drawn that negative results, when initial content of available nitrogen is subtracted, are the results of: disturbed balance between the process of mineralization and immobilization and too short incubation period (two weeks) for reestablishing balance between those two processes.

Due to all mentioned facts and suspicions connected to the origin of aerobic incubation (Stanford and Smith, 1972) on the one side and very precise Bremner's instructions (1965) regarding keeping and preparation of the soil samples on the other side, dilemma remains whether or not to subtract the initial content of the available nitrogen.

Because of the above-mentioned suspicions and dilemmas regarding calculation of the results, both procedures, i.e. nitrogen availability indexes were used in our investigations — initial condition was both subtracted and not subtracted.

Table 2 presents the quantities of mineralized and nitrified nitrogen in aerobic procedures, both with the initial content subtracted and not subtracted.

The quantity of mineralized and nitrified nitrogen in aerobic procedure obtained without subtraction of the initial content of available nitrogen points to the conclusion that there are no significant regularities in either increase or decrease mineralized and nitrified nitrogen in experiment variants.

Table 2 — Quantities of mineralized and nitrified nitrogen in aerobic procedure, initial content of the available nitrogen in the brown forest soil both subtracted and not subtracted

Experiment variants	Initial content of available nitrogen not subtracted	Initial content of available nitrogen subtracted		
	Quantities of mineralized and nitrified $\text{NH}_4+\text{NO}_3\text{-N}$ (ppm)	Before $\text{NH}_4+\text{NO}_3\text{-N}$ (ppm) incubation	After $\text{NH}_4+\text{NO}_3\text{-N}$ (ppm) incubation	Quantities of mineralized and nitrified $\text{NH}_4+\text{NO}_3\text{-N}$ (ppm)
Control	24.7	4.9	24.7	19.8
$\text{N}_1\text{P}_2\text{K}_2$	24.2	8.4	24.2	15.8
$\text{N}_1\text{P}_2\text{K}_2$	32.2	15.4	32.2	16.8
$\text{N}_3\text{P}_2\text{K}_2$	30.6	12.2	30.6	18.4
$\text{N}_4\text{P}_2\text{K}_2$	27.8	51.3	27.8	-23.5

Similar situation was also obtained with the quantities of mineralized and nitrified nitrogen when we subtracted the initial quantity. The only difference was that $\text{N}_4\text{P}_2\text{K}_2$ experiment variant gave negative value (-23.5 ppm), i.e. the quantity of mineralized nitrogen was smaller after incubation than before it. Therefore, one may suppose that during the incubation a part of available nitrogen gets immobilized by microorganisms.

An experiment in controlled conditions was carried out with the aim to assess the value and reliability of nitrogen availability biological indexes (obtained by aerobic and anaerobic incubation) through the plants' and soil's parameters.

Table 3 presents the results of the plants' and soil's parameters obtained in the controlled conditions.

Table 3 — Plants' and soil's parameters in the controlled conditions (in pots).

Plants and soil parameters	Experiment variants				
	Control	N ₁ P ₂ K ₂	N ₂ P ₂ K ₂	N ₃ P ₂ K ₂	N ₄ P ₂ K ₂
Yield (NPK) (g/pot)	10.26	10.84	10.86	11.22	11.10
Yield (PK) (g/pot)	2.25	3.58	4.18	4.69	6.36
Relative increase in yield (PK = 100)	456	303	260	239	174
Relative increase in yield (NPK) (Ø = 100)	100	106	106	109	108
Relative increase in yield (PK) (Ø = 100)	100	159	186	208	283
Difference in yield (g/pot) (NPK-PK)	8.01	7.26	6.68	6.53	4.74
Difference in yield (g/pot) (NPK-Ø)	—	0.58	0.60	0.96	0.84
Difference in yield (g/pot) (PK-Ø)	—	1.33	1.93	2.44	4.11
Total uptake of nitrogen (NPK)	90.0	105.8	118.0	110.1	115.1
Uptake of soil nitrogen (NPK)	58.1	72.4	83.8	78.0	84.4
Uptake of fertilizer nitrogen (NPK)	31.9	33.4	34.2	32.1	30.7
Ratio of soil and fertilizer nitrogen uptake	1.8	2.2	2.4	2.4	2.7
Uptake of nitrogen (PK)	18.5	22.9	30.3	30.1	48.5

The majority of authors have used absolute values, i.e. quantities of mineralized and nitrified nitrogen when applying aerobic incubation, i.e. biological indexes of nitrogen availability.

In our researches we have also compared absolute values with plants' and soil's parameters. In order to assess the value, i.e. reliability of aerobic incubation in estimation of the soil nitrogen availability, we have calculated the correlation coefficients.

Table 4 presents the values of correlation coefficients between mineralized and nitrified nitrogen in aerobic procedure.

Table 4 — Correlation coefficient between the plants' and soil's parameters and mineralized and nitrified nitrogen in aerobic procedure, the initial content of available nitrogen in the brown forest soil both subtracted and not subtracted

Plants and soil parameters	Quantities of mineralized and nitrified N, initial content of N subtracted Biological ind. I	Quantities of mineralized and nitrified N, initial content of N not subtracted Biological ind. II
Yield (NPK)	NS	NS
Yield (PK)	NS	NS
Relative increase in yield (PK = 100)	NS	-0.54*
Relative increase in yield (NPK) (Ø = 100)	NS	NS
Relative increase in yield (PK) (Ø = 100)	0.81**	NS

Difference in yield (g/pot) (NPK-PK)	NS	NS
Difference in yield (g/pot) (NPK-Ø)	NS	NS
Difference in yield (g/pot) (PK-Ø)	0.81**	NS
N(%) in plants (NPK)	NS	0.56*
N(%) in plants (PK)	NS	NS
Total uptake of nitrogen (NPK)	NS	0.54*
Uptake of soil nitrogen (NPK)	NS	0.57
Uptake of fertilizer nitrogen (NPK)	-0.62*	NS
Ratio of soil and fertilizer nitrogen uptake	NS	NS
Uptake of nitrogen (PK)	NS	NS

** significant at probability level 0.01

* significant at probability level 0.05

NS not statistically significant

Statistically significant correlation dependence between plants' and soil's parameters and aerobic incubation of available nitrogen was established in just a few cases and it was closer to low than to medium correlation dependence of minor statistical significance ($r(-0.54^*)$, 0.56^* , 0.54 and 0.57).

Statistically significant correlation between an aerobic incubation with initial content of available nitrogen subtracted and the plants' and soil parameters was also established in just a few cases. A high correlation dependence was established only in the relative increase of yield in the PK experiment variant ($\text{Ø} = 100$) and regarding the difference in yield (P-Ø). The value of coefficient was $r = 0.81^{**}$. Medium negative correlation dependence was established in the uptake of fertilizer nitrogen ($r = -0.62^*$).

As we have already mentioned, anaerobic method, together with aerobic one is the most commonly applied procedure used to establish biological indexes of the soil nitrogen availability.

In the research process, the quantity of the obtained $\text{NH}_4\text{-N}$ in both anaerobic and aerobic procedures was calculated without subtracting the initial content of $\text{NH}_4\text{-N}$. However, the calculation procedure suggested by *Waring and Bremner* (1964) was also used, i.e. the initial $\text{NH}_4\text{-N}$ content before the incubation was subtracted from the $\text{NH}_4\text{-N}$ content established after the incubation.

Table 5 presents quantities of mineralized nitrogen that were established in anaerobic procedure, with the initial content both subtracted and not subtracted.

The quantities of mineralized nitrogen obtained without subtraction of the initial content of available nitrogen can be used as grounds for the following conclusion: there is a regular increase in the quantity of mineralized nitrogen, starting from the control variant and going towards the variant with the highest dosage of fertilizer nitrogen.

Table 5 — Quantities of nitrogen mineralized in anaerobic procedure, the initial content of $\text{NH}_4\text{-N}$ available in brown forest soil subtracted and not subtracted

Experiment variants	Initial content of available $\text{NH}_4\text{-N}$ not subtracted	Initial content of available $\text{NH}_4\text{-N}$ subtracted		
	Quantities of mineralized nitrogen ($\text{NH}_4\text{-N}$, ppm)	Before $\text{NH}_4\text{-N}$ incubation (ppm)	After $\text{NH}_4\text{-N}$ incubation (ppm)	Quantities of mineralized nitrogen $\text{NH}_4\text{-N}$ (ppm)
Control	14.8	3.8	14.8	11.0
$\text{N}_1\text{P}_2\text{K}_2$	18.0	6.6	18.0	11.4
$\text{N}_2\text{P}_2\text{K}_2$	17.7	12.6	17.7	5.1
$\text{N}_3\text{P}_2\text{K}_2$	19.7	7.5	19.7	12.2
$\text{N}_4\text{P}_2\text{K}_2$	20.1	45.5	20.1	-25.4

Quantities of nitrogen mineralized in anaerobic procedure, with the initial content of available $\text{NH}_4\text{-N}$ counted out, considerably varied with the field experiment variants. No regularity was observed regarding the increase or decrease of mineralized nitrogen quantities going from the control variants towards the highest dosage of the applied fertilizer nitrogen. Negative values for the mineralized nitrogen quantities were established as in the aerobic incubation in the $\text{N}_4\text{P}_2\text{K}_2$ experiment variant.

The established absolute values regarding mineralized nitrogen, with the initial content of available $\text{NH}_4\text{-N}$ both subtracted and not subtracted, were compared to the plants' and soil's parameters. The obtained correlation dependences were used to assess how reliable this method is in estimation of soil nitrogen availability.

Table 6 presents the values of correlation coefficients between the plants' and soil's parameters and nitrogen mineralized in anaerobic procedure, with the initial content of $\text{NH}_4\text{-N}$ in the brown forest soil both subtracted and not subtracted.

Table 6 — Correlation coefficients between the plants' and soil's parameters and mineralized and nitrified nitrogen in anaerobic procedure, the initial content of available nitrogen in the brown forest soil both subtracted and not subtracted

Plants' and soil parameters	Quantities of mineralized nitrogen, initial $\text{NH}_4\text{-N}$ content subtracted	Quantities of mineralized nitrogen, initial $\text{NH}_4\text{-N}$ quantity not subtracted, biological ind. II
Yield (NPK)	NS	0.70**
Yield (PK)	-0.79**	0.90**
Relative increase in yield (PK = 100)	0.61*	-0.92**
Relative increase in yield (NPK) ($\emptyset = 100$)	NS	NS
Relative increase in yield (PK) ($\emptyset = 100$)	NS	0.81**
Difference in yield (NPK-PK)	0.80**	-0.74**
Difference in yield (NPK- \emptyset)	NS	NS
Difference in yield (PK- \emptyset)	NS	0.80**

N(%) in plants (NPK)	NS	NS
N(%) in plants (PK)	NS	-0.54
Total uptake of nitrogen (NPK)	NS	0.67**
Uptake of soil nitrogen (NPK)	-0.53**	0.75**
Uptake of fertilizer nitrogen (NPK)	NS	NS
Ratio of soil and fertilizer nitrogen uptake	-0.76**	0.88**
Uptake of nitrogen (PK)	-0.91**	0.77**

** significant at probability level 0.01

* significant at probability level 0.05

NS not statistically significant

Statistically significant correlation dependence between the plants' parameters and nitrogen mineralized in anaerobic procedure, with the initial content of $\text{NH}_4\text{-N}$ subtracted, was not found in major number of cases, except for the yield obtained in the PK variant ($r = 0.79^{**}$) and regarding the difference in obtained yield (NPK-PK) ($r = 0.80^{**}$), where it was high. The relative yield increase (PK-100), using the method mentioned, gave medium correlative dependence ($r = 0.61^*$). However, the remaining plants-in-pots-parameters in anaerobic incubation did not give statistically significant correlation dependences. The uptake of soil nitrogen in the NPK experiment variant in pots, with the nitrogen mineralized in anaerobic procedure, gave the medium negative correlation dependence ($r = -0.53^*$). Nevertheless, a considerable negative correlation dependence was established ($r = -0.91^{**}$) between the uptake of nitrogen in the PK variant and the method mentioned.

The values of correlation coefficients established for the obtained yields in the PK and NPK variants corresponded to the results obtained by G a s s e r and K a l e m b a s a (1976). It is also the case with the uptake of nitrogen in those experiment variants. However, our researches resulted in negative correlation dependences as the increased available contents of ammoniacal nitrogen in soil have directly influenced the process of mineralization in anaerobic conditions. There was more nitrogen in the soil, mineralization was less intensive, while the yield and uptake of nitrogen were higher, because the plants have mainly used the available ammoniacal nitrogen, already present in the soil, to satisfy their nitrogen needs.

As it can be seen from the results presented in Table 6, a high and very high correlation dependence of major statistical significance was established between the nitrogen mineralized in anaerobic procedure (the initial content of $\text{NH}_4\text{-N}$ not subtracted) and the plants-in-pots-parameters. A medium negative correlation dependence ($r = -0.54^*$) was only established between the content of nitrogen in oat plant (PK) and the nitrogen mineralized in just mentioned procedure. Mainly high correlation dependence of major statistical significance was established between the soil in pots and the mineralized nitrogen.

The percentage of correlation dependences for $r = 0.50\text{--}0.90$, with significance of 0.05—0.01 probability level and for $r = 0.70$, with significance of 0.01 probability level was calculated on the grounds of the established correlation coefficients values for both methods used, i.e. two ways used to calculate the results.

This calculation was done with the aim to define clearly which of the nitrogen availability biological indexes that were used can be considered reliable to estimate nitrogen availability. The aim was also to determine which is the most suitable procedure to calculate results in aerobic and anaerobic incubation.

Table 7 presents the correlation coefficients expressed in percentages (in aerobic and anaerobic incubations), for both criteria.

Table 7 — Correlation dependences (in percents) between the plants and soil parameters and nitrogen availability biological indexes when $r = 0.50-0.99$ (**, *) and when $r = 0.70$ **.

Parameters	Percentage of correlation dependence for $r = 0.50-0.99$ (**, *)	Percentage of correlation dependence for $r \geq 0.70$ **
Aerobic incubation, the initial content of available nitrogen not subtracted		
Plants and soil in pots parameters	26.7	0.0
Aerobic incubation, the initial content of available nitrogen subtracted		
Plants and soil in pots parameters	20.0	13.3
Aerobic incubation, the initial content of available $\text{NH}_4\text{-N}$ not subtracted		
Plants and soil in pots parameters	73.3	60.0
Aerobic incubation, the initial content of available $\text{NH}_4\text{-N}$ subtracted		
Plants and soil in pots parameters	40.0	26.7

As presented in the Table 7, the highest percentages of correlative dependence, for both criteria, were established for anaerobic incubation when initial content of available $\text{NH}_4\text{-N}$ was not subtracted. However, considerably lower percentages were established for the same method when initial condition was not subtracted.

As opposed to anaerobic incubation, low percentages of correlative dependence were established in aerobic incubation for both calculation procedures.

CONCLUSIONS

The results presented here may be used to conclude the following:

Anaerobic incubation, i.e. the established biological index of availability in the investigated brown forest soil can be considered reliable in assessing the soil nitrogen availability.

Considering that in anaerobic incubation significantly higher percentages of correlation dependences of available $\text{NH}_4\text{-N}$ were determined when the initial content of available $\text{NH}_4\text{-N}$ was not subtracted than in the cases in which the initial content of available $\text{NH}_4\text{-N}$ was subtracted, nitrogen availability biological index determined in anaerobic procedure without subtraction of the initial content of available $\text{NH}_4\text{-N}$, can be recommended to be used.

Both plants' and soil's in parameters obtained by experiment in pots can be used on an equal level to assess the value of the investigated nitrogen availability biological index.

The established reliabilities of biological index are significant for estimation of the soil nitrogen availability because anaerobic procedure is simple and quick from an analytical view and it can be used in routine analyses.

Nitrogen availability biological index established in our researches after the aerobic incubation, with the initial content of available nitrogen both subtracted and not subtracted, cannot be considered reliable to estimate the soil nitrogen availability.

REFERENCE

- Allison, F. E. and Sterling, L. D. (1949): *Nitrate formation from soil organic matter in relation to total nitrogen and cropping practices*, Soil Sci. 67, p. 239—252.
- Bremner, J. M. (1965): *Nitrogen availability indexes*. In: *Methods of soil analysis*, Part. 2 (C. A. Black ed.). Agronomy 9, p. 1324—1345. Am. Soc. of Agron; Madison, Wisconsin.
- Conforth, I. S. and Walmsley, D. (1971): *Methods of measuring available nutrients in west Indian soils*. Nitrogen, Plant and Soil 35. p. 352—357.
- Fitts, J. W., Bartholomew, W. V. and Heidel, H. (1953): *Correlation between nitrofiabile nitrogen and yield response of corn to nitrogen fertilization on Iowa soils*, Soil Sci. Soc. Am. Proc. 17, p. 119—122.
- Fox, R. H. and Piekielek, W. P. (1978): *Field testing of several nitrogen availability indexes*, Soil Sci. Soc. Am. J. 42, p. 747—750.
- Engner, H., Riehm, H., Domingo, W. R. (1960): *Untersuchungen über die shemische Bodenanalyse als grundlage für die Beurteilung des Nährstoffzustandes der Böden*, vol. II. Shemische extraktionsmethoden zur phosanor und kaliumbestimmung. Kungl. Lant Brukshögskolans. Annaler 26.
- Gasser, J. K. R. and Kalembara, S. J. (1976): *The effects of lays and organic manures on the available-N in clay and sandy soils*, J. Soil Sci., Vol. 27, p. 237—249.
- Herron, G. M., Dreier, A. F., Flowerday, A. D., Coluille, W. L. and Olson, R. A. (1977): *Residual mineral N accumulation in soil and its utilization by irrigated corn*, Agron. J. 63, p. 322—327.
- Hanway, J. and Dumenil, L. (1953): *Predicting nitrogen fertilizer needs of Iowa soils: III use of nitrate production together with other information as a basis for making nitrogen fertilizer recommendations for corn in Iowa*, Soil. Sci. Soc. Am. Proc. 19, p. 77—80.
- Keeney, D. R. and Bremner, J. M. (1966): *Comparasion and evaluation of laboratory methods of obtaining an index of soul nitrogen availability*, Agron. J., Vol. 58, p. 498—503.
- Munson, R. D. and Stanford, G. (1955): *Predicting nitrogen fertilizer needs of Iowa soils. IV Evaluation of nitrate production as a criteriion of nitrogen availability*, Soil. Sci. Soc. Am. Proc. 19, p. 464—468.

- Oz us, T. and Han way, J. J. (1966): *Comparisons of laboratory and greenhouse tests for nitrogen and phosphorus availability in soils*, Soil Sci. Soc. Am. Proc., Vol. 30, p. 224—228.
- Power, R. F. (1980): *Mineralizable soil nitrogen as an index of nitrogen availability to forest trees*, Soil Sci. Soc. Am. J. 44, p. 1314—1320.
- Robinson, J. B. D. (1968a): *A sample available soil nitrogen index: I Laboratory and greenhouse studies*, J. Soil Sci. 19, p. 269—279.
- Robinson, J. B. D. (1968b): *A sample available soil nitrogen index: II Field crop evaluation*, J. Soil Sci. 19, p. 280—290.
- Stajković, M. (1990): *Režim azota u zemljištu tipa sleudoglej*, Magistarska teza, Beograd.
- Stanford, G. and Legg, J. O. (1968): *Correlation of soil nitrogen availability indexes with in uptake by plants*, J. Soil Sci. 105, p. 320—326.
- Stanford, G. and Smith, S. J. (1972): *Nitrogen mineralization potentials of soils*, Soil Sci. Soc. Am. Proc. 36, p. 465—472.
- Stevanović, D. (1978): *Sadržaj nekih oblika azota u gajnjačama i njihov uticaj na efikasnost azotnih đubriva*, Agrohemiја 3—4, p. 93—97.
- Waring, S. A. and Bremner, J. M. (1964): *Ammonium production in soil under waterlogged conditions as an index of N availability*, Nature 201, p. 951—952. London.

АЕРОБНА И АНАЕРОБНА ИНКУБАЦИЈА — БИОЛОШКИ ИНДЕКСИ ПРИСТУПАЧНОСТИ АЗОТА ЗЕМЉИШТА

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Резиме

Истраживања су обављена на гајњачи (Младеновац) која се користи у оквиру дугогодишњег стационарног огледа, са одређеним плодоредом и системом ђубрења већ више од тридесет година. За ова истраживања одабиране су варијанте огледа где је примењена растућа доза азота ђубрења. За утврђивање биолошких индекса приступачности азота примењене су аеробна и анаеробна метода са два различита начина обрачуна резултата (са одузимањем и без одузимања почетног садржаја приступачног азота). Ради одређивања параметара биљака и земљишта који су вредновали поузданост коришћених метода за оцену приступачности азота земљишта изведен је оглед у контролисаним условима уз примену изотопски обележеног азота (^{15}N). На основу урађене корелационе анализе односно утврђене корелативне зависности између биолошких индекса и параметара биљака и земљишта може се закључити да се биолошки индекс који је утврђен анаеробним поступком без одузимања почетног садржаја приступачног NH_4 може сматрати поузданим за оцену приступачности азота земљишта. Поузданост није утврђена за аеробну методу (са одузимањем и без одузимања почетног садржаја приступачног азота) као и за анаеробни поступак са одузимањем почетног садржаја приступачног $\text{NH}_4\text{-N}$.