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METHOD FOR ESTIMATING THE SOIL CAPACITY OF ATMOSPHERIC DINITROGEN FIXATION

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ABSTRACT

Importance of nitrogen in the nutrition of all living on earth, made the scientists to study the extent of the atmospheric nitrogen fixation by unsymbiotic microflora in soil. The methods of quantitative estimation, used in the 20th century, have not been entirely satisfactory, because they overlooked the nitrification processes, testing fixed nitrogen by the Kjeldahl method only. This paper suggests a more complete method for determining the natural contribution of atmospheric nitrogen fixation to the soil. This method takes into account both the N-organic and N-NH_4^+ as quantified by the Kjeldahl method and the nitric N produced by mineralization of the N compounds resulting from dinitrogen fixation during the soil incubation period.

Key words: unsymbiotic fixation of atmospheric dinitrogen, method for quantitative estimation, interdependence fixation - nitrification of nitrogen.

INTRODUCTION

Soil supply with nitrogen has always been increased in 3 natural ways:

1. oxidation of atmospheric dinitrogen by electric discharges in high zones of the atmosphere and its bringing by rainfall to the soil in nitric form, about 3-5 kg/ha/year (after Schmalfuss, 1963, cited by Müller,1965);

2. reduction of atmospheric dinitrogen to ammonia by microflora, containing nitrogenase enzyme, *Azotobacter chroococcum* being the most wide spread bacteria which freely fixes the dinitrogen with the highest efficiency;

3. symbiotic fixation of atmospheric dinitrogen (between 50-100 kg/ha/year), the most wide spread being the bacteria of the genera *Rhizobium* and *Bradyrhizobium,* which activate in the nodules of leguminous plants roots.

Nitrogen importance in the nutrition of all terrestrial living awakened the interest of scientists, who wanted to estimate the non-symbiotic microflora contribution with nitrogen to the soil supply. About the importance of soil organisms, fixing molecular atmospheric dinitrogen, Vaillant (1901) wrote: *"the higher the humus content is, the more fertile is the soil and this fertility seems to be due, especially, to a high number of dinitrogen fixing organisms, which live here".*

Waksman and Karunaker (1924, cited by Waksman, 1932) were among the first who tried to estimate the quantity of atmospherical dinitrogen that a soil can fix by the population of *Azotobacter.* With that end in view, they mixed 1-2 g of mannitol in 100 g of fresh soil, sieved, and they fitted this mixture to optimum moisture. After 28 days of incubation at 28°C, they determined the nitrogen quantity. For control, they determined the nitrogen quantity in the same soil, without moistening and incubation. The additional nitrogen, determined by the Kjeldahl method, was considered the measure of soil capacity of fixing the atmospheric dinitrogen.

Winogradsky (1925) mentioned that many other methods, for measuring the quantity of nitrogen fixed by the soil bacteria, were suggested, but none could give satisfaction, though the bacteria *Clostridium pasteurianum* (anaerobic) and *Azotobacter chroococcum* (aerobic) had been discovered in soil and tested their ability to assimilate atmospheric dinitrogen, for a long time. Referring to the mixture of mannitol with the soil, Winogradsky wrote that the methods which

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use 10% mannitol, mixed in soil, are totally faulty, because the addition of manitol causes the multiplication of heterotrophic microorganisms, which during the incubation time of 15-20 days, compete with the molecular nitrogen fixing bacteria, which multiply slower and endure of oxygen deficiency.

The second method, rejected by Winogradsky, used only 1-2% mannitol as against the soil quantity, moistened in optimum and incubated for 3-4 weeks. After incubation, the quantity of additional nitrogen, as compared with the unincubated soil, was determined and referred to the mannitol quantity used.

The third method, also rejected by Winogradsky, took as measure of dinitrogen fixation by the soil, the capacity of mannitol decomposition in soil.

Finally, Winogradsky (1925, 1930) and Winogradsky and Ziemseka (1928, cited by Winogradsky, 1949) proposed 2 methods, with different aims:

1. 1 g of soil is sieved on the surface of the silica-gel (impregnated with nutritive solution with 2 g mannitol, without nitrogen) in a 20 cm diameter Petri dish of. After 2-3 days of incubation, at 30°C, *Azotobacter chroococcum* colonies are counted and after 8 days of carrying on the incubation bacterial colonies become brown (specific color for these bacteria) the dishes are dessicated about 10 hours, at a suitable low temperature. The dessicated silica-gel is curetted, integrally introduced in a Kjeldahl balloon and nitrogen is measured. This represents atmospheric dinitrogen fixed by specific bacteria.

2. spontaneous cultures are obtained in small portions of soil for proving the activity of *Azotobacter chroococcum* in fixation of the atmospheric dinitrogen, in case of limiting factors: calcareous stone, phosphoric acid and nitrate regime.

The literature of the period 1924-1960 is poor in papers, repeating, without success, the methods described by Waksman (1932) or Winogradsky (Pochon and Tchan, 1948; Pochon, 1954; Laszlo et al., 1956).

The special importance of the soil potentiality to fix atmospheric molecular nitrogen determined us to renew research in this domain, in order to establish an adequate method for estimating correctly this potential, for utilizing it, together with respiration and cellulolytic potentials of the soils in the calculation of the Indicator of the Vital Activity Potential (IVAP%), partial indicator for calculation of Biological Synthetic Indicator (BSI%), suggested by Ştefanic for estimating the level of soil fertility (Ştefanic, 1994a and 1994b; Ştefanic et al., 1997; 2001).

The main defficiency of earlier presented methods consists in ignoring the reality that by Kjeldahl analysis, N-organic + N-NH_4^+ retained by soil, can be quantified, but N-NO_3^-, is not. Or, it is known that, in the soil sample (like in nature) a succession and a simultaneousness of organic nitrogen mineralization, of N-NH_4^+ accumulation and nitrification processes (in aerobic conditions) exist.

For this purpose, a correct method must assure the determination of initial content (in soil sample, before incubation) and final content (after incubation at 28°C), of both the organic nitrogen + ammoniacal nitrogen content and of the nitric one. A positive difference (if it exists) between the final and initial contents represents the soil capacity for fixing the atmospheric dinitrogen (N_2), that is reported to 100 g of desiccated soil.

MATERIAL AND METHODS

Soil samples were sampled in summer from:

V1 - a permanent lawn, lasting from 50 years, from the park of National Agricultural Research and Development Institute Fundulea, soil type is cambic chernozem.

V2 - a permanent lawn, under trees, lasting from 100 years, from the park of University of Agronomical Sciences and Veterinary Medicine – Bucharest, soil type is reddish preluvosol;

V3 - a permanent lawn in the forest skirt beside of fruit-growing farm from Mioveni, district Argeş, soil type is albic luvosol.

After sampling, the samples were sieved by a sieve of 2.5 mm, rough vegetal remains were simultaneously moved away, the soils

were optimum moistened, introduced in plastic bags (which permit the gas diffusion, but not the diffusion of water vapours) and kept at room temperature, for continuing the rotting process of fine root remains. After a few months, the experiment for estimation of soil capacity for atmospheric dinitrogen fixation was initiated.

Utilized method: 20 g of soil were introduced in a 12 cm diameter Petri dish and remoistened at 18% with distilled water. Then, the Petri dish was covered and weighted with a view maintain the optimal moisture during to incubation. Three replicates of Petri dishes were prepared for each soil type. The dishes with soil were put to incubation for 30 days, at 28°C.

Then, the contents in organic and ammoniacal nitrogen – by Kjeldahl method – and also in nitric nitrogen – by phenyl disulfonic acid or Peter-Griss method, modified by Marinescu (1973) – were determined for the incubated soil samples and for the control.

The differences between the content of nitrogen (Kjeldahl, on one side, or nitric, on the other one), also the sums of differences, between the results of final analyses and of the initial ones, formed the quantity of dinitrogen fixed by soil microflora.

By the data analysis we obtain:

a**.** the additional organic + ammoniacal nitrogen, representing the dinitrogen quantity fixed by the microflora;

b**.** the additional quantity of nitrates, produced by mineralization (nitrification) of organic nitrogen compounds resulted from vital and parallel processes developed during the incubation.

A mention: Nitrogen from the nitrates, resulted, by nitrification processes from humus and unhumified remains, does not influence the sum of fixed nitrogen: what it changes is only the form in which the nitrogen is combined.

RESULTS AND DISCUSSION

The data from the table 1 show that in cambic chernozem samples, 13.442 mg N/100 g of soil were fixed; in reddish preluvosol samples, 17.712 mg and in albic luvosol only 7.145 mg N/100 g of soil were fixed in 30 days of incubation, at 28°C. Statistical analysis of these data point out that differences between the results from chernozem and reddish preluvosol are not significant. Only the results from albic luvosol are statistically different. Referred to one hectar (Table 2) and to 90 favorable days (usually present in the months April - October), that means: 606, 798 and 324 kg free N_2 fixed in soil.

Scientific literature (Feher, 1954; Rippel-Balddes, 1955; Müller, 1965) mentions that the free dinitrogen fixation (non symbiotic) is at the level of 5-15 kg/ha/year. By our method (that includes both the fixed and nitrified nitrogen), we estimated in permanent lawns higher quantities, which reach values between 300 and 700 kg/ha/year. These values are much superior to those specified in literature for atmospheric dinitrogen symbiotic fixation, usually evaluated between 50 and 100 kg/ha/year.

Table 1. Soil potential of atmospheric dinitrogen (symbiotic) fixation in permanent lawn of V1. cambic cernozem, V2. reddish preluvosol and V3. albic luvosol, after 30 days incubation of, at 28°C (N, mg/100 g soil d.s.)

Soil types	Moment of analysis	N-org. + NH_4^+ (N, mg)	Differences F – I (N, mg)	$N\text{-}NO_3^-$ (mg)	Differences F – I (N, mg)	N total fixed (colons) 4 + 6 (mg)
1	*2*	*3*	*4*	*5*	*6*	*7*
V1. Cambic chernozem	Final Initial	270 263	7	7.066 0.624	6.442	a 13.442
V2. Reddish preluvosol	Final Initial	287 276	11	8.082 1.370	6.712	a 17.712
V3. Albic luvosol	Final Initial	225 222	3	4.736 0.541	4.195	b 7.145
LSD P 1% = 5.29						

Table 2. Estimated quantities of free dinitrogen fixed per hectare, in 30 days of favorable conditions of humidity and temperature, in the 0-10 cm horizon

Soil types	N-org. + NH_4^+ (mg/100 g soil d.s.)	N-NO_3^- (mg/100 g soil d.s.)	Colons 2 and 3 (x 15) = N_2 (kg/ha)		Total N_2 – fixed/ ha (colons 4 + 5)
			N-org. + NH_4^+ (mg/100 g soil d.s.)	N–NO_3^- (mg/100 g soil d.s.)	
1	2	3	4	5	6
V1. Cambic chernozem	7	6.442	105	96.63	a 202
V2. Reddish luvosol	11	6.712	165	100.68	a 266
V3. Albic luvosol	3	4.195	45	62.92	b 108
				LSD P 1 % = 79	

One can observe that in cambic chernozem, besides the 7 mg nitrogen, as organic N or NH_4^+ (determined by Kjeldahl), 6.442 mg N-NO_3^- were additionally produced by the oxidation (nitrification) of ammonia emitted in soil by the dinitrogen fixing microorganisms and/or by mineralization of organic nitrogen. The same phenomena were also produced, in reddish preluvosol samples (besides 11 mg of fixed N_2 , determined by Kjeldahl, other 6.712 mg in the form of N-NO_3^- were produced), as well as in albic luvisol samples (besides 3 mg fixed N_2, in the form of organic N or NH_4^+, other 4.195 mg in the form of N-NO_3^- were produced).

This nitric nitrogen, which the old methods did not take into account, is the result of autotrophic and heterotrophic bacteria activation (Kalinenko, 1948; Florenzano, 1984) which oxidize ammonia resulted from the N-molecular fixation. Undoubtedly, N-NH_4^+ is realized not only from N_2 fixed by *Azotobacter* and other microorganisms in soil, but also from ammonia produced by organic matter mineralization (ammonification process) in soil.

But this ammoniacal and nitric nitrogen does not appear in addition, having been already present in the soil resources, in one chemical combination or another. In nature, N-NO_3^- can also appear in soil, brought by precipitations, from the high zones of atmosphere, owing to electrical discharges, which oxidize the dinitrogen; but in laboratory analyses, this nitrogen cannot appear.

The data from table 1 also show that different potentials exist, both of N_2 – fixation and nitrification, determined in ecological conditions. We observe that the lawn from cambic chernozem, from the total fixation of nitrogen (13.442 mg/100 g of soil), the nitrification represents 47.9 %; in the lawn from reddish preluvosol, from a total fixation of nitrogen (17.712 mg/100 g of soil), the nitrification represents 37.9 %, and in the lawn on the albic luvosol, from a nitrogen total fixation of 7.145 mg/100 g of soil, nitrification is in proportion of 58.71%. However, statistical analysis of these data point out that these differences are not significant. The observation, that a correlation between the two forms of nitrogen (more correctly said, between the two microbial processes) does not exist, suggests that the determination of the two forms of nitrogen and totalizing them are necessary for quantifying the soil capacity of atmospheric dinitrogen fixation.

CONCLUSIONS

A laboratory method for quantitative estimation of atmospheric dinitrogen by soil microflora, was elaborated and presented.

It was observed that, the fixed dinitrogen is partially, oxidized to nitrates by microbial nitrification. This nitrification process is not quantitatively proportional with the dinitrogen fixation process.

For this reason, the determination of soil capacity for atmospheric dinitrogen fixation is possible only totalizing the nitrogen from both processes at the end of soil incubation period.

The deficiency of the old methods was that they evidenced only the nitrogen quantified by Kjeldahl method, which does not evidence the nitrates, produced during incubation, from the atmospheric dinitrogen microbial fixed.

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