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THE POTENTIAL OF SOILS FOR FREE-FIXING (ASYMBIOTICALLY) ATMOSPHERIC DINITROGEN, AN ESSENTIAL INDICATOR IN THE COMPOSITION OF THE SYNTHETIC INDICATOR OF SOIL FERTILITY

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ABSTRACT

The accumulation in soil of the atmospheric dinitrogen is as important as that of organic carbon. The accumulation in soil of the atmospheric dinitrogen is the result of both aerobic and anaerobic microbial processes. That very important feature of soil: free-fixation of the atmospheric dinitrogen, has been missing from the technology for estimating the soil vital activity. For remedying that methodological deficiency, we have improved the classical method proposed by Waksman and Karunacker in 1924 (cited after Waksman, 1932) and forgoten in science archives and we have applied our method, for exemplifying it in the present communication, and recommending the introduction of that in the calculation of the Indicator of the Vital Activity Potential (IVAP %) of soil. By improving the calculation mode of IVAP %, the sensibility in differentiation of soil quality, as consequence of crop management interventions, increased.

Key words: chernozem, free-fixation of the atmospheric dinitrogen, IVAP %, soil fertility.

INTRODUCTION

A Synthetic Indicator of Soil Fertility was first proposed by Ştefanic (1994) based on the correct and comprehensive defining of soil fertility: *„Fertility is the fundamental feature of the soil, that results from the vital activity of micropopulation, of plant roots, of accumulated enzymes and chemical processes, generators of biomass, humus, mineral salts and active biologic substances. The fertility level is related with the potential level of bio-accumulation and mineralization processes, these depending on the programme and conditions of the ecological subsystem evolution and on anthropic influences"*.

From this definition results that soil fertility is a synthesis of soil's own characteristic features, which cannot include the size of agricultural production, because that is dependent not only on soil, but especially on crop management and on climate. At that time, for an objective estimation of soil fertility level, an approach to synthesize the following main partial indicators of soil fertility was elaborated:

1. The partial synthetic indicator – IVAP % (Indicator of Vital Activity Potential), uniting as average of the respiration and cellulolytic potentials, average of data expressed as the percentage from MEV = Maximal Empiric Value (established by the author).

2. The partial synthetic indicator – IEAP % (Indicator of Enzymic Activity Potential), uniting the catalasic, saccharasic, ureasic and phosphatasic potentials, as average of data expressed as percentage from MEV = Maximal Empiric Value, established by the author.

3. The partial chemical synthetic indicator – CSI %, as average of the soil contents in: C_t – organic (humus), percentage data (by ratio with MEV = Maximal Empiric Value, established by the author) of the soil contents in: C_t – organic (humus), extractable humus, huminic acids, fulvic acids, N_t, V% and pH (H_2O) data, expressed as percentage from MEV = Maximal Empiric Value, (established by the author);

4. Partial pedo-genetical indicator (IPG %), having in view the humus repartition in soil profile.

The accumulation in soil of the atmospheric dinitrogen is very important and results from microbic processes, both aerobic

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and anaerobic, but this very important feature – free-fixing of the atmospheric has been missing from our technology for estimating the soil vital activity. For remedying that methodological deficiency, we have improved the classical method (cited by Ştefanic and Oprea, 2010), forgoten in science archives and we have applied it, in the present communication, for exemplification, recommending it for this purpose.

MATERIAL AND METHODS

The principle of the method is based on that of Waksman and Karunacker (1924, cited after Waksman, 1932). The experiment was modelled in conformity with the method improved by Ştefanic and Oprea (2010): in a Petri dish, with 10 cm diameter, one introduces 30 g of fresh soil, sieved and brought with distilled water to optimum moisture of 18%.

The Petri dish is closed with its lid and is weighed, in order to maintain the same humidity by adding small quantities of distilled water, from time to time. Soil incubation is done at 28^{o}C, for 30 days. Initially, from the same soil sample, a sufficient quantity of soil, is put to dry at air, to be useful for control sample, in chemical analyses for estimating the dinitrogen free-fixed from the atmosphere.

When the incubation is finished, the soil from the Petri dishes is dried in air, mixed and analysed in parallel with control samples. For establishing what quantity of dinitrogen was fixed, during 30 days of incubation, from the nitrogen quantified at the end of the incubation (ammoniacal and protein Nitrogen, as well as Nitrogen from nitrates), one substracts the Nitrogen quantities found in soil control samples.

The improvement of the classical method consists in totalising (both for initial analysis and also for the final one) the quantities of Kjeldahl Nitrogen with quantities of nitric Nitrogen, and then making the difference between final and initial quantities. The differences represent the quantity of dinitrogen free-fixed in soil (mg N/100 g soil d.w.), which can be reported to hectare, for 0-15 cm horizon, ecologically comparable with the Petri dish, relating to the atmosphere accessibility in the process of free-fixing dinitrogen in soil.

We applied this method to a field experiment, conducted by senior researcher Alexandru Cociu, on the cambic chernozem from NARDI Fundulea. The experiment included the folowing treatments:

V_1. Ploughing to 20 cm (permanently);
V_2. Loosening of the soil vith chisel, to 20 cm, three years;
V_3. Loosening of soil with vibromixt (similar to harrow), to 15 cm, three years;
V_4. Loosening of soil in bands, at 20 cm, three years;
V_5. No tillage, three years, direct sowing.

After three years of experimentation, in field, at the end of the March, soil samples from 0-20 cm layer were collected, before applying fertilizers. The soil samples were sieved, by a sieve of 2.5 mm, the vegetable remains were removed and the laboratory experimental model was achieved, using the above presented method.

Quantities of free-fixed Nitrogen in soil, during 30 days were transformed in percents in relation with MEV = 30 mg N/100 g soil d.w., value obtained in conformity with our method (Maximal Empirical Value, apreciated by authors, at the level of the fertile soils from Romania).

RESULTS AND DISCUSSION

Chemical analysis data, about soil samples from experimental model (proteic and ammoniacal Nitrogen), by Kjeldahl method, as well as nitric Nitrogen (by phenoldisulphonic acid reagent), initially and finally determinated, were submitted to arithmetical operations, necessary for obtaining the gain of Nitrogen in soil, free-fixed from the atmosphere (Tables 1 and 2).

The applied systems of soil working created different conditions of evolution in the process of free-fixation of the atmospheric Nitrogen (Table 1).

Other vital processes (soil respiration and cellulolyse), were not significantly influenced, and this suggests that the process of free-fixing atmospheric nitrogen is more sensitive to the variations of soil conditions (Table 2). The influence of soil aeration state, microflora taxonomic components, soil genetic type, the mode of fertilization etc. has been known from previous microbiological research. For example, chemical fertilizing with ammoniacal and nitric Nitrogen provokes a change of microflora metabolisme of dinitrogen free-fixing, changes the consumption of combined nitrogen, stopping the assimilation of dinitrogen from the atmosphere, without microflora disappearence.

The lack of oxygen in soil (anaerobiose) determines a multiplication of the anaerobical bacterium *Clostridium pasteurianum*, which is a bacterium which free-fixes dinitrogen, although less actively.

Table 1. Determination of dinitrogen contents, fry-fixing from atmosphere, in cambic chernozem from NARDI Fundulea, in laboratory model, in 30 days of incubation, under the influence of the basic loosening mode of soil (N, mg/100 g soil d.w.)

Basic work	Analysis	N_t		Nitrates		All N_2 (free-fixed) (Columns 4 + 6)
		Organic N + NH_4^+	F-I	$N-NO_3^-$	F-I	
1	*2*	*3*	*4*	*5*	*6*	
Plowing to 20 cm, permanently	Final (F) Initial (I)	154 151	3	2.70 1.38	1.32	d 4.32
Worked with chizel 20 cm, repeated 3 years	Final (F) Initial (I)	174 162	12	3.75 1.41	2.34	b 14.34
Worked with vibromixt, 15 cm, repeated 3 years	Final (F) Initial (I)	172 162	10	2.47 1.25	1.22	c 11.22
Plowing in bands, at 20 cm, repeated 3 years	Final (F) Initial (I)	178 159	19	2.63 1.11	1.52	a 20.52
No-tillage - permanently, direct sowing and drilling	Final (F) Initial (I)	171 156	15	2.11 1.48	0.63	b 15.63
						LSD P 0.1% = 1.59

Values in the same column followed by the same letter are not significantly different at P<0.1%)

Table 2. Influence of different ways of working the cambic chernozem (after maize), estimated by some biological indicators

Basic work	Respiration (R) (CO_2, mg/100 g soil d.w.)	Cellulolyse (C) (% of hydrolysed cellulose)	N_2-free-fixed from atmosphere (A) (mg/100 g soil)	IVAP % (R+C+A)	IVAP % (R+C)
Plowing to 20 cm, permanently	a 19.59	a 7.35	d 34.32	b 10.42	a 13.45
Worked with chisel to 20 cm, repeated 3 years	a 22.32	a 8.34	b 14.34	a 15.00	a 15.33
Worked with vibromixt, 15 cm, repeated 3 years	a 24.60	a 7.92	c 11.22	a 14.58	a 16.26
Plowing in bands, at 20 cm, repeated 3 years	a 20.17	a 1.45	a 20.52	a 14.05	b 10.81
No-tillage permanently, direct sowing and drilling	a 19.98	a 12.82	b 15.63	a 16.14	a 16.40
LSD	P 5% = 9.33	P 5% = 5.50	P 0.1% = 1.59	P5% = 2.5	P5% = 4.05

After 3 years, the largest quantity of dinitrogen was free-fixed in soil in the variant worked in bands (20.60 mg N/100 g soil d.w., in 30 days of incubation). Referred to hectare (0-15 cm), that means a gain of 309 kg and it is easy to understand, that during the long period of vegetation there are numerous favorable situation for free-fixing dinitrogen (asymbiotically), from the atmosphere.

The indicators (Ştefanic et al., 2001) of vital activity potential of the soil (IVAP %) calculated with and without the participation of the indicator of free-fixing dinitrogen from the atmosphere (IVAP % = R + C + A as compared with IVAP % = R + C), are presented in table 2. There are few significant differences among treatments. We consider as necessary to use our improved method of soil potential of free-fixing dinitrogen from the atmosphere, because is very important to have a new parameter to the calculation of soil vitality by IVAP %.

In the last half of the 20 century, many researchers ceased to be concerned with dinitrogen free-fixation (asymbiotical) productivity; or, they emitted theoretical arguments (by practice appearances), from which resulted that only the nitrogen symbiotical-fixation is important (Campbell and Liss, 1967). In this way they ignored biological and biochemical knowledge accepted at the end of the 19 century, when Vaillant (1901) wrote: *“.....both the humus content is higher and the soil is more fertile and this fertility seems to be due, specially, to a big number of nitrogen fixing organisms, which live here”.*

For sustaining the fact pointed by us (Ştefanic and Oprea, 2010), that the absence of nitrates, in the analyses for determining quantitatively the dinitrogen free-fixed from the atmosphere, led to unsatisfactory results, we present, in table 3, a recalculation of the results from a communication presented to the International Congress of Soil Science, in Paris by Laszlo et al. (1956).

The authors, commenting their own results regarding the weekly quantitative evolution of nitrates (obtained by laboratory model with soil, after Waksman, 1932) – presented as a graph –, considered (as did the predecessors: Berthelot, 1885; Löhnis, 1935; Feher, 1954; Rippel-Baldes, 1955, cited after Müller, 1966, Waksman and Karnauker, 1924, cited by Waksman, 1932; Winogradsky, 1925-1930 and 1949) as „*power of soil for free-fixing of atmospheric Nitrogen*” only the quantities obtained by Kjeldahl method (called, total Nitrogen – with the symbol – Nt), ignoring the transformation into nitrates of a part of the quantity of free-fixed Nitrogen.

That is the cause of too small quantities of free-fixing dinitrogen in soil from the atmosphere, recorded in soil biological litterature.

Table 3. Quantities of fry-fixed dinitrogen from the atmosphere in chernozem or podzol, in laboratory models (4 weeks incubation and stimulation with glucose)*

Soil	Analysis	N_t		Nitrates		All N_2 free-fixed	
		Organic Nitrogen + NH_4^+	F - I	$N-NO_3^-$	F - I	N/100 g soil (Columns 4 + 6)	kg/hectare (Column 7 x 15**)
Columns: 1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Chernozem	Final (F) Initial (I)	320 240	80	42 5	37 41	117	1755
Podzol	Final (F) Initial (I)	240 160	80	42 1	41	121	1815

*Recalculation of the results from graphics, after Laszlo et al., 1956 (N, mg /100 g soil d.w.).
**Coefficient of transformation: mg/100 g soil of all nitrogen free-fixed in soil, in kg/ha, in the layer of 0-15 cm.

From the data presented in table 3, one can observe that, totalising nitrogen from nitrates resulted from the nitrification of the free-fixed dinitrogen, with the Nitrogen (determined by Kjeldahl method – Nt), one obtains the quantities 117 and respectively 121 mg/100 g soil d.w. (in function of soil type) and not 80 mg of nitrogen Nt , free-fixed from the atmosphere). Cited authors ignored the complexity of the phenomenon, and this way, did not reach the correct conclusions, that would indicate incredible quantities of free-fixed atmospheric dinitrogen in the two soil types by reference to hectare (1755 and 1815 kg/ha, respectively). We specify however that their experimental model comprised the stimulation of microbiological process with a glucose solution 1%, for making the humidity of soil samples to the optimum for incubation.

CONCLUSIONS

Free asymbiotical fixation of the atmospheric dinitrogen, is present at planetary level with different intensities, and can be considered the most important natural source of accumulation of this vital, chemical element, in biosphere.

Considering that by Kjeldahl soil analysis one obtains the total contents of the nitrogen (Nt) – most authors have not taken in calculation the nitrates, which result in soil mostly from the nitrification of ammoniacal and proteic Nitrogen (resulting from the microbial free-fixing of dinitrogen).

Correction of classical method (in models with soil) for estimating the total Nitrogen quantity, free-fixed from the atmosphere, by totalising the nitrates, resulted by nitrification, with Nitrogen, resulted by Kjeldahl analysis, permits the understanding of the accumulation in soil, by natural way, of a colossal quantity of nitrogen in terrestrial and aquatic biosphere.

Including the soil potential for free-fixing the atmospheric dinitrogen, in IVAP % (Indicator of Vital Activity Potential), for estimating the soil fertility state, will increase the sensitiveness in soil analysis.

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