ABSTRACT

„CONTRIBUTIONS TO THE UNDERSTANDING OF THE MECHANISM OF NITROGEN NUTRITION PLANTS, OBTAINED THROUNG A NON-SYMBIOTIC WAY, AS AN ELEMENT OF SOIL FERTILITY”

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The premises of this thesis’ approach rise from the idea that the quantitative evaluation of the nitrogen in the soil, as one of the four elements generating amino acids, the building blocks of life in the vegetal and animal world, occurred out of the necessity to fix an error in another approach – error discovered by Gh. Ștefanic – which, beginning from the sixth decade of the previous century, underestimated the non-symbiotic biological activity of the microorganisms in the soil. Erroneous statements supporting only the symbiotic activity generating nitrogen, like those published by N.E.R. Campbell & assoc. (1967) founded and encouraged this idea which dominated the scientific world. They supported the misconception that in order to non-symbiotically fix dinitrogen to the level that leguminous plants do, the soil must be provided with 20 t of carbohydrates (dry matter) / hectare / year in order to fix 80 kg of N_2 / ha / year. However, these researchers do not take into account that the *Azobacter chroococcum* consume various energetic materials and most of the time substances which were not consumed by the heterotrophic microflora in the soil. Furthermore, the non-symbiotic fixation of dinitrogen happens at a global rate, in water as well as in soil, as long as the climatic conditions allow it.

G. Ștefanic and Georgeta Oprea (2010; 2011) discovered the fault in the analysis proposed by Waksman S.and Karunacker (1924 – quoted by S. Waksman, 1932), which states that before and after the incubation of a sample of 100 g of soil, the amounts of total nitrogen (N_t%) must be measured, while that of nitrates should not. The difference is represented by the amount of (non-symbiotically) fixed dinitrogen, produced for 100 g of soil. The authors of this method did not know that through the Kjeldahl method (of mineralizing the sample of soil) the nitrates in the soil are lost in the atmosphere and therefore all the nitrates produced by ammonium nitrification during

the incubation of the soil sample are lost as well. Not taking into account the nitrates formed during the incubation of the soil sample, the more favourable the conditions for nitrifications, the more it will reduce the amount of N_t % caused by the difference between the original and final values and, consequently, it would indicate that dinitrogen fixation is insignificant.

On account of these premises, the PhD thesis aims to develop the research about the facts of the cultivated soil as follows:

- By establishing the influence that different types of cultivated plants have on the process of non-symbiotic atmospheric dinitrogen fixation and by determining the dynamics of the fixation of atmospheric dinitrogen during the vegetation cycle;
- By investigating the influence of wheat and corn cultures in rotation and monoculture on the biological features of the chromic luvisol under the effect of chemical infertilization correlated with atmospheric dinitrogen synthesis;
- By examining the acidic reaction of the luvisol from the Didactic and Scientific Research Station Moara Domnească – Ilfov, belonging to the University of Agronomical Sciences and Veterinary Medicine – Bucharest, on the atmospheric dinitrogen.

The results of this research attempt to lead to the integration of the existent literature information on the topic of non-symbiotic nitrogen fixation.

The PhD thesis is divided into 7 chapters corresponding to the aimed purpose, as follows:

1. The stage of knowing the process of free atmospheric nitrogen fixation;
2. The natural environment in which the research took place;
3. The material and research method;
4. The influence of the wheat and corn crops in rotation and monoculture on the biological features of the chemically unfertilized chromic luvisol;
5. The influence of the soil's chemical reaction on the chromic luvisol's ability of freely fixing the non-symbiotic nitrogen;
6. The dynamics of the unrestricted atmospheric nitrogen fixation in the unfertilized soil under cultures of alfalfa, soy, wheat, corn and sunflower, during the warm seasons of the 2014;
7. General conclusions.

The first chapter is the stage of getting to know the process of free atmospheric nitrogen fixation and reviews the worldwide research of the unrestricted fixation of the atmospheric dinitrogen. The end of the chapter presents the importance of this research and outlines its purpose.

The second chapter is called **the natural environment in which the research took place** and it contains information regarding the geographic location, hydrography and hydrogeology, the climatic conditions (if it corresponds to Köppen's climatic formula, temperature, precipitation, relative humidity of air, aeolian regime), soil fertility and the vegetation at the Didactic Station Moara Domnească. The conclusion is that the chromic luvisol used for these experiments offers proper conditions for the growth of agricultural cultures, as well as for the atmospheric dinitrogen biosynthesis under the cultures.

The third chapter reflects the **material and research method** regarding the quantification of the non-symbiotic activity of atmospheric dinitrogen fixation into the soil. In this matter, there were collected samples of soil, out of class N_0P_{70}, from under diverse rotations and cultures in the Experimental Field of crop rotation from the Agricultural Technology discipline, which was founded in 1981 by C. Pintilie, D.I. Săndoiu and Gh. Ștefan at the Didactic Farm Moara Domnească – Ilfov belonging to the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The experience was difactorial, type 6x4, organised after the technique of parcels divided in 4 repetitions, on a kind of chromic luvisol called Elrș.

The methodology of the study consists of a set of actual analysis, necessary for the identification of the non-symbiotic nitrogen in the soil, as well as for identifying and explaining its dynamics in relation with variant types of biological and enzymatic tests conducted under different cultures during the vegetation period.

Based on the field from which the samples were collected, there have been organized 4 practices for the identification of the amounts of non-symbiotic nitrogen.

The first practice aimed to identify the amounts of non-symbiotic nitrogen under diverse cultures during the vegetation period. The factors of production were factor A – the culture under which the gathering took place and factor B – the interval (month) when the samples were collected.

The second practice unfolded in the Laboratory of Soil Biology from the Agriculture Factory. Its purpose was to determine the influence of the soil's pH on the formation of non-symbiotic nitrogen. The experience was conducted because the chromic luvisol is actually defined by the fertilization with ammonium nitrate fertilizers for more than 50 years through an acidic reaction (pH=5), unfavourable for non-symbiotic nitrogen fixation.

The third practice intended to identify the influence that the pH value and the plants had over the non-symbiotic nitrogen production, in conditions of soil amendment.

The fourth practice attempted to analyse the influence that corn and wheat cultures planted in rotation and monoculture had on the biological features of the chromic luvisol in conditions of chemical non-fertilization. The sample gathering took place in April 2012 and the samples were kept cold until the physiological and enzymatic indicators in the soil were analysed.

Chapter four outlines **the influence of wheat and corn crops in rotation and monoculture on the biological features of the chemically unfertilized chromic luvisol**. The research intended to widen the knowledge of the influence of wheat and corn cultures in rotation and monoculture on the physiological and enzymatic components of the soil, the Indicator of Vital Activity Potential (IVAP%), the Indicator of Enzymatic Activity Potential (IEAP%) and the Synthetic Biologic Indicator (SBI%) of the chemically unfertilized chromic luvisol from Moara Domnească.

With regard to the **physiological components of the soil**, the research confirmed the following:

- the highest registered rate of 2.44 mg of biodegradable cellulose / 100 g soil was registered on the soil planted with corn in rotation after a wheat crop, belonging to value group **a**, while the lowest rate of 0.9 mg of biodegradable cellulose / 100 g soil was registered on the soil planted with wheat in rotation after soybean crop, belonging to value group **c**;
- the highest rate of soil respiration potential of 25.83 mg CO_2 /100 g soil was recorded on the corn crop planted after a wheat crop belonging to value group **a**, in contrast with the respiration activity potential of the monoculture of corn of 21.98 mg CO_2 / 100 g soil, which statistically occupies a place in group **b**;
- the highest rates in the process of ingraining non-symbiotic atmospheric dinitrogen of 17.88 mg N/100 g soil were registered under wheat monoculture, the result being significantly different from the average experience considered and classified in group **a**.

With regard to the **enzymatic components of the soil**, the research confirmed the following:

- the complete pedo-phosphatasic activity of the soil planted with corn after wheat and the soil planted with wheat in rotation after soy was superior, with rates of 5.13 and 4,26 mg/100 g soil and very significant increases of 1.71 and significant increases of 0.84 mg/100 g soil, belonging to value group **a**, in comparison with the complete pedo-phosphoric activity of the soil under wheat and corn monocultures, where much lower rates, belonging to value group **b**, were registered.

- the complete pedo-amidasic activity of the soil planted with corn following a wheat crop was over 1.08 mg / 100 g soil, the result being classified in value group **a**. The results of the complete pedo-amidasic activity of the corn monoculture (0.58 mg/100 g soil) were classified in group **b**, as well as those of the soil planted with wheat in rotation after a soy crop (0.46 mg/100 g soil), while the wheat monoculture was distributed to group **c**, with a value of 0.23 mg/100 g soil.

Regarding the synthetic indexes of the chromic luvisol from Moara Domnească:

- the highest rate of the Indicator of Vital Activity Potential (IVAP %) of 106.99 % was recorded under wheat monoculture, with a very significant increase of 22.1% from the average experience, which classified it in group **a**. The soil under the corn monoculture and that under wheat culture planted in rotation after soy, as well as the one under corn crops planted after wheat are part of group **b**. The Indicator of Vital Activity Potential of the soil planted with wheat after soy (value 68.13 %) showed significantly lower rates of 16.26% that the average results;
- the highest value of the Indicator of Enzymatic Activity Potential (IEAP %) of 12.97 % presented significantly increased values of 4.65% in comparison with the average experience, being included in group **a**. It was catalogued in the soil under cultures of corn planted after wheat;
- the highest rate (55.41 %) of the Synthetic Biological Indicator (SBI %) was registered under wheat monoculture. It was situated in value group **a**, with a significant increase of 9.04%. With respect to the Synthetic Biological Index of the soil under wheat cultures planted after soy crops (value 38.91 %), there was an important negative difference of 7.45%, which situated the result in value group **c**. Also, we acknowledge that the rates of the Synthetic Biological Index under corn monoculture and corn culture planted in rotation did not exhibit significant differences from the average, the results being situated in value group **b**.

Chapter five illustrates **the influence that the chemical reaction of the soil has on the chromic luvisol's ability of freely fixing non-symbiotic nitrogen.** After research in vegetation pots, it showed that soil amendment, followed by a pH increase of 0.55 units at a value of 6.21 determined, in the absence of vegetation, the increase of atmospheric Nitrogen's non-symbiotic biosynthesis of almost thrice the non-amended soil value. During this process, the share of the two participants in the organic Nitrogen's biosynthesis NH_4^+ remained the same.

The highest non-symbiotic biosynthesis of atmospheric Nitrogen under amendment conditions was registered under sunflower crops at the level of 74.09 mg N_2/100 g soil corresponding to 1111.35 kg/ha with an organic Nitrogen (NH_4^+) share of 99.32 %.

Chapter six - The dynamics of the unrestricted atmospheric nitrogen fixation in the unfertilized soil under cultures of alfalfa, soy, wheat, corn and sunflower, during the warm seasons shows differences between cultures, as well as between the plant's phenophases, these differences being the result of the rhizosphere's zone as well. Depending on the cultures, the conclusions are the following:

- the highest non-symbiotic activity of the atmospheric nitrogen's fixation under the alfalfa culture of 34.09 mg/100 g soil was recorded in July and represented a quantity of 511.35 kg N_2/ha out of the whole biosynthesis of 1521.3 kg N_2/ha.
- the highest non-symbiotic activity of the atmospheric nitrogen's fixation under peas was of 31.15 mg N_2/100 g soil was reported in May and represented a quantity of 467.25 kg N_2 / ha out of the total biosynthesis of 1278.3 kg N_2 /ha and out of the total activity of 982.5 kg / ha of non-symbiotic nitrogen fixation during the vegetation period of 982.5 kg N_2/ha.
- regarding the soy crop, there is a fluctuation between the average rates with both organic Nitrogen NH_4^+ and nitric nitrogen. The highest non-symbiotic activity of nitrogen ingraining (value 21.65 mg/100 g soil) was registered in August and it represented a quantity of 324.75 kg / ha out of the whole biosynthesis of 1305.45 kg/ha.
- with reference to the wheat, the highest non-symbiotic activity of nitrogen fixation (value 29.28 mg /100 g soil) was registered in May and it measured 439.2 kg/ha out of the total biosynthesis of 1367.7 kg/ha and out of the total activity of 945.15 kg/ha of non-symbiotic nitrogen ingraining during the vegetation period.
- under corn cultures there was registered a fluctuation between 6.2 mg/100 g soil (93 kg N_2/ha) in September and a maximum of 23.28 mg/100 g soil (349.2 kg N_2/ha) in June with both organic nitrogen NH_4^+ and nitric nitrogen. The highest biosynthesis rate from June corresponds with the complete development of the radicular system with a maximum of rhizospheric activity out of the whole value of 1157.7 kg/ha.
- under sunflower cultures there was registered a fluctuation between 5.64 mg N_2 /100 g soil (84.6 kg N_2/ha) in August and a maximum of 30.37 mg/100 g soil (455.55 kg N_2 /ha) in July not only of organic nitrogen NH_4^+, but of nitric nitrogen as well. The maximum biosynthesis value from July corresponds with the complete development of radicular system with a maximum of rhizospheric activity out of the whole value of 1250.55 kg N_2/ha.

- the highest quantity of non-symbiotic free ingrained nitrogen of 34.080 mg/100 g soil and 31.52 mg/100 g soil was registered in July and August under alfalfa crops, followed by pea crops in May with a value of 31.14 mg N_2/100 g soil, by sunflower crops in July with a value of 30.86 mg N_2/100 g soil, and then by wheat in May, with 29.28 mg N_2/100 g soil.

The research has greatly contributed to a better understanding of the quantification method of the non-symbiotic biosynthesis of atmospheric nitrogen and of its dynamics under wheat and corn monocultures or planted in different rotations, as well as of its interaction with the physiological, enzymatic components of the soil and with the biological components of the soil's fertility.