APPARATUS FOR QUANTITATIVE ESTIMATION OF ORGANIC CARBON AND NITROGEN MINERALIZATION IN SOIL

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ABSTRACT

The paper presents the sketch, description and operation procedures of the apparatus Bio-MCN (Bio-mineralization of organic carbon and nitrogen) for determining the soil biological potentials, which are very important for characterizing the soil fertility level.

Key words: soil respiration potential, soil ammonification potential, apparatus for quantitative soil analyses.

INTRODUCTION

Biological cycles of carbon and nitrogen transformations in soil, from mineral elements to organic compounds and remineralization of these, are the work of autotrophic organisms, of those which fix molecular nitrogen and of the heterotrophic ones. From atmospheric CO_2, autotrophic microflora and macroflora, fix the carbon by metabolic assimilation in their own biomass, both as ternaries substances (containing carbon, oxygen and hydrogen) and quaternaries ones (including nitrogen too).

Molecular nitrogen enters, in most organic structures in soil, in two ways:

- an initial, mineral way, by oxidation of molecular nitrogen (N_2), transforming it in nitrate ion (NO_3), owing to electric energy from lightning and then, by precipitations, reaching the soil, in mineral form, as nitrate salts;
- an organic way, by the contribution of micro- and macrovegetal organisms, which, by enzymic processes (nitrogenase) reduce the atmospheric N_2 (mineral), transforming it in NH_3 and assimilating it, directly or indirectly, in organic substances (quaternaries).

Microbiologists discovered and studied, since the beginning of the 20 century, these processes, describing the cycle of the transformation from organic to mineral structures (Muntz, 1890 and Remy, 1902, cited from Waksman, 1932; Winogradsky, 1925, 1926, 1927, 1930; Stoklasa, 1922, 1929; Waksman, 1932; Fedorov, 1948; Pochon, 1954; Laszlo et al., 1956 etc.).

Limiting us to the aim of this communication, we mention that the first researches, regarding soil microbial processes, aimed at estimating the soil respiration level, by application of a bell jar on the soil surface, which collected the respired CO_2 from soil, in a vessel with NaOH, placed inside (Stoklasa, 1922, 1929). Beginning with the third decade of the 20th century, various apparatus and devices for measuring the soil respiration potential were created, among these, Ştefanic's respirometer (1989, 1991, 1994), by oxygen autogeneration, simple, easy feasible and ecological. Measuring soil respiration allows, in fact, an estimation of the organic carbon mineralization.

The Ştefanic's respirometer suggested to us the possibility to estimate also the other biological process of organic matter recycling, the process of organic nitrogen mineralization in soil, named ammonification. Waksman (1932) gave a special importance to this process, as precursor of the process of ammonia oxidation to nitrate, named nitrification. Waksman considered the level of ammonification as an index of soil fertility, taking over inexactly Remy's conclusion (1902), who observed a correlation between the quantity of ammonia formed in soil and the productivity of certain soils.

MATERIAL AND METHODS

The principle of the method, the method, the apparatus Bio-MCN (Bio-Mineralization of carbon and nitrogen) and the technology for estimation of soil fertility potential are especially adequate for soil cultivation management. Thus, for calculating the Indicator of Vital Activity Potential (IVAP%) of the soil,

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proposed by us, we consider necessary to measure, at the same time, the ammonification activity potential in soil (A), which is missing from the present calculus formula. Therefore the formula can become:

IVAP% = R% + C% + A%) / 3,

in which: R = respiration, C = cellulolyse and A = ammonification.

The ammonification potential depends on: efficiency of heterotrophic microflora activity; quantity of nitrogen in organic matter and ecological conditions where this process, which is part of the nitrogen biological cycle in the soil, develops.

The principle of the method was formulated by Remy (1902), by Stevens and Withers (1909) and Pochon (1954) and adapted by us to Ştefanic's respirometer (1989), which became now, the apparatus Bio-MCN (Figure 1).

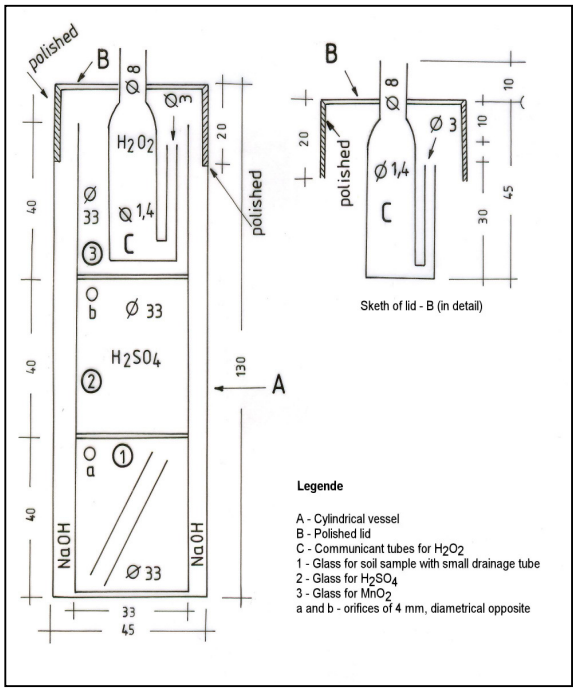

Figure 1. Sketch of the Apparatus Bio-MCN

Working method. Twenty g of fresh soil, with a known humidity, are well mixed with 0.2 g of straw, finely hacked (for maintaining the soil aerated). The mixture is introduced around a small drainage tube in the glass (1) – having 2 orifices (a) for exchanging of gases and for being manipulated by a special nipper. The glass has to be knocked slightly to the table, for compacting the soil. Over the soil, one pours 2 ml 1% peptone solution in distilled water. One pours distilled water in addition, to achieve a humidity of 20% (in ratio with soil desiccated at 105°C). In the 200 ml cylindrical vessel (A) – with polished lid (B) and communicating tubes (C) – 20 ml of NaOH 0.2n are introduced (for combining it with CO_2 released from soil) then, with a special nipper, the glass (1), with 20 g soil is introduced in NaOH solution, in cylindrical vessel (A). Then, the glass (2) with H_2SO_4 0.2n and 2 orifices (b) is introduced over the glass (1). Sulphuric acid 0.2n has the role to combine it with NH_3, released from soil. The glass (3), with 0.2 g MnO_2 powder, is introduced over the glass (2)**.** In this moment, the cylindrical vessel is closed with the polished lid (B) and the communicating tubes (C) with 2-3 ml H_2O_2 are introduced in vessel (3) with 0.2 g MnO_2.

The soil activity develops in standardized aerobic conditions (28°C, 7 days). The communicating tubes with H_2O_2 20% v/v (C) and MnO_2 are the providers of oxygen instead of that consumed by respiration. When the oxygen is consumed in the cylindrical vessel, a small depression is produced, which attracts 1, 2, 3.... drops of H_2O_2. These falls over MnO_2, in vessel (3), release oxygen and the normal pressure is remade in the cylindrical vessel.

After the soil incubation period, the excess of H_2O_2 is extracted by absorption. The result of the processes, which develop in the soil sample, is the release of CO_2 (by soil respiration) and NH_3 (by ammonification); CO_2 combines with NaOH, and NH_3, with H_2SO_4. Then, the apparatus Bio-MCN is taken to pieces in inverse order of assembling.

Measuring the quantity of released CO_2. In the vessel (A), a magnetic rod is introduced, then 2 ml of $BaCl_2$ solution 20% and a drop of thymolphtalein 1% in alcohol, which will colour the NaOH in blue. Then, placing the cylindrical vessel on a magnetic stirrer, the excess of NaOH is titrated by a solution of HCl 0.1n, until the blue colour will disappear. The same operations are also made in control variant (with boiled distilled water instead of soil sample).The difference between the two titrations gives the CO_2 quantity released by soil respiration.

Calculation of respiration potential of the soil sample. 1 ml HCl 0.1n corresponds with 0.1 m.eq. of CO_2, that is, with 2.2 mg CO_2. Formula of calculation for estimating the level of soil respiration is:

C-CO_2 mg/100 g of soil d.s.= (A-B) x f x 2.2 x 5 x KU x 0.273,

in which: A = number of ml of HCl 0.1n by which the titrations were effectuated (average of repetitions) of the control vessels; B = number ml of HCl 0.1n by which the titrations were effectuated in the vessels with soil samples; f = the solution factor of HCl 0.1n; 2.2 = equivalent of CO_2; 5 = coefficient of reference of the 20 g soil to 100 g soil; KU = coefficient of correction for soil humidity; 0.273 = coefficient of reference of CO_2 to carbon (C). Performing the simplifications, the calculation formula becomes:

C-CO_2 mg/100 g soil d.s. = (A-B) x f x 3.003 x KU.

Measuring the quantity of released NH_3. The glass (1) is emptied of soil sample using a large funnel, in a bottle of 100 ml. Both the glass (1) and the funnel will be washed by exactly 40 ml solution of potash alum 0.3%. Then, the bottle will be tightly closed and stirred for 15 minutes. All the bottle content will be well filtered and the filtrate will be recovered in a glass. Over this filtrate sulphuric acid will be added, together with the glass (2), in which NH_3 released from the soil sample was recovered, reaching the 50 ml/bottle liquid of reference, which will enter in the calculation formula for quantifying the total NH_3 released by ammonification process. From the filtration glass, 1-10 ml liquid will be taken, and introduced in a 50 ml calibrated bottle. 2 ml of Seignette salt 25% are added, the liquid is stirred for homogenizing, distilled water is added till 2/3 of the calibrated bottle, then 1 ml of Nessler reagent is added, one stirs and after 30 minutes, NH_3 is spectrophotometrically quantified at 425 nm. In parallel, an etalon solution, containing 0.1 mg/ml NH_4 will be coloured by Nessler reagent, in the same way.

Calculation of ammonification potential of the soil sample. The level of ammonification potential is expressed by N-NH_3 quantity produced in 7 days of soil incubation at 28°C, by formula:

A mg N-NH_3/ 100 g soil d.s. = Ep x C x F x 100 x KU / Eet x 20 x 0.994,

in which: A = ammonification potential; Ep = extinction from soil sample; Eet = extinction from etalon; C = concentration of etalon (NH_4^+ = 0.1 mg/ml); F = volume of extracting liquid-filtrate (50 ml + number ml of water added to soil for bringing it to the humidity of 20%; 100 = g of fresh soil; KU = coefficient for transformation of NH_4^+ in N.

By introducing the appropriate values, the formula becomes:

A mg N-NH_3 = Ep x 0.1 x F x 100 x KU / Eet x 20 x 0.776

and by simplification: A mg N-NH_3 = Ep x F x KU x 0.776 / Eet x 0.5.

CONCLUSIONS

The apparatus Bio-MCN (Bio-mineralization of organic carbon and nitrogen) is intended for estimating the soil potentials for respiration and ammonification.

Soil potential of nitrogen mineralization (ammonification) can be utilized in formula of calculation for a synthetic estimation of soil vitality, by the Indicator of Vital Activity Potential – IVAP% (Ştefanic, 1994).

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