Modification of some pedo-enzymic indices of the reddish preluvosol during the action of fertilizers, after nineteen years of experimentation

Received for publication, January 15, 2013
Accepted, March 20, 2013

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Abstract
Natural productivity of a soil, under favourable climatic conditions, depends on its fertility level. By agro-phytotechnical measures, soil productivity may increase independently of its fertility, and that can be ameliorated or degraded by agro-technologies. Soil Biology, that appeared in the 20th century, has elaborated the theory of soil fertility maintenance and even of amelioration by some measures which could stimulate the life and biochemical processes in soil, as well as quantitative and qualitative increase in humus. The pedo-enzymic part in the circuit of chemical elements in soil is also known, in theory, but in agronomical practice even it is still neglected. Both the theory of plant mineral nutrition and the application of chemical fertilizers are maintained, therefore organic fertilization is rarely used (with stable compost or vegetal composted remains). The present paper deals with the study of some modification of pedo-amidase and pedo-phosphatase indices and of soil’s chemical reaction in a reddish preluvosol, in a stationary experiment of nineteen years.

Key words: total amidase, total phosphatase, catalase, Indicator of Enzymic Activity Potential (IEAP %), soil fertility and productivity

Introduction
Numerous studies suggested that the pedo-enzymic activities can be utilized as indicators of soil fertility (GIL-SOTRES & al. [1], TRASAR-CEPEDA & al. [2], MASIANDRO & al. [3]; SAVIOZZI & al. [4]; UZUN & UYANOS [5]; ŞTEFANIC & al.[6]) The specialized literature allowed KOZLOV [7], BOGUSLAWSKI [8], DURAND [9], MÜLLER [10], HERA & al. [11][12], ŞTEFANIC & al. [13], CERVELLI & al. [14], CHIRI Â & al. [15], BOLTON & al. [16], DINCĂ & al. [17][18] KISS & al.[19], Dick [20], etc. to establish that the effect of chemical fertilizers on soil enzymatic activity is interpreted in different ways: some researchers observed stimulating influences, others, inhibitor and others, the absence of any influence. ŞTEFANIC & al. [21] noted that chemical fertilizers, alone applied, determined the reduction of catalazic activity, but combined application of these fertilizers with stable manure or vegetable remains led to an increase of that activity. The Phosphorus content , in arable horizon, is of 20-40 mg P / 100 g of soil, but out of this quantity, 40-60 % is found in organic combination (MC LAREN & PETERSON [22]).

In the present paper, we are proposing to show and interpret the modifications produced in arable horizon of reddish preluvosol from, in a long-term stationary experiment conducted at the Experimental Didactic Station of Moara Domnească, Ștînga de Cârlige.
Material and Methods

The field experiment was set up in 1992, in a reddish preluvosol with loamy-clayey texture and 2.17-2.64 humus (1.26-1.53 C t%). The atmospheric temperature varied between 10.6 and 12.6 °C in 2010 and the precipitations between 444.9 mm (2002), 823 mm (2005) and 305 mm (2010). This experiment was in a crop rotation with annual crops: sugar-beet – winter wheat – winter barley, having in view 2 factors of influence: factor A – organic manures: a1 – without organic manures; a2 – stable manure (spontaneously composted), 30 t / ha / every third year, for sugar-beet; a3 – vegetable remains of sugar beet (crown and leaves), 40 t/ha every 3 years for winter wheat and factor B – ammonium nitrate: b1 – N0; b2 – N60; b3 – N100; b4 – N150; b5 – N200. Soil samples were sampled at 11 April 2011, by agrochemical core, from 0-20 cm horizon, from all variant-repetitions. The analyses were carried out on average samples, after their being sifted through a sieve of 2-3 mm and after removing the visible vegetal remains. During the analyses carried out in the laboratory, the soil samples were kept in well closed plastic bags at 4-7 °C, and maintained at the same humidity level at the one from from sampling (60-70 % s.u.). Pedo-enzymic analyses were carried out by Ştefanic methodology (ŞTEFANIC AND GHEORGHIŢĂ,[13]), according to which soil contains both the researched pedo-enzymes and the specific enzymic substrates. For this reason, the analyse without adding artificial substrates, accurately show the total-amidazic and total-phosphatasic potentials of soil, from which could result specific nutrients, necessary for micro- and macro-population of soil and vegetal cover. The ratios between the quantity and variety of these pedo-enzymes and the specific substrates existent at a certain point in arable soil, could determine the potential level of pedo-enzymes under the influence of the conditions in the soil, especially those anthropically determined. For a synthetic appreciation of how fertilizers influenced enzymic analyses from soil we have used a variant of the Numeric Taxonomy Method, transforming the results of each enzymic analysis in percents from the Maximum Empirical Value (MEV), typical of the analysis, considered 100 and then, calculating (in percentages) the average of the repetitions, we obtained the Indicator of Enzymic Activity Potential (IEAP %) by which the level of pedo-enzymic processes from each experimental variant can be indentified (ŞTEFANIC & al. [6]). The statistical interpretation was realized by variance analysis of with 2 factors and sequential (multiple) test after SNEDECOR [23], processed and expounded by ŞTEFANIC [24].

Results and discussions

1. The long-term influence of organic and mineral fertilization, on the chemical reaction (pH-H2O) of soil.

From the variants of the factor A, the fertilization with stable manure, spontaneously composted, (given every 3 years, 30 t / ha for sugar-beet) showed the highest chemical reaction (a 5.07), and the variants of factor B, showed a diminution of the pH indicator simultaneously with the increase of ammonium nitrate doses. During the interaction of A*B factors, one observes an increase in soil acidity, together with the increase of ammonium nitrate doses. When we are comparing the interaction of B*A, we are finding that stable manure have improved soil chemical reaction, and mineral fertilizer have depreciated it. In the Table 1, the results of the analyses are presented, regarding the soil chemical reaction (the letters which accompany the numbers, whether they are of the same manner, signify that they are statistically undifferentiated).
Table 1. The influence of organic and mineral fertilization on actual chemical reaction (pH-H₂O) of soil (after SĂNDIU & al., [25]).

<table>
<thead>
<tr>
<th>A</th>
<th>B1 – N₀</th>
<th>B2 – N₆₀</th>
<th>B3 – N₁₀₀</th>
<th>B4 – N₁₅₀</th>
<th>B5 – N₂₀₀</th>
<th>Average A</th>
</tr>
</thead>
<tbody>
<tr>
<td>a₁ – unfertilized</td>
<td>b 5.01 a</td>
<td>b 4.98 a</td>
<td>b 4.76 b</td>
<td>a 4.69 b</td>
<td>a 4.64 b</td>
<td>b 4.82</td>
</tr>
<tr>
<td>a₂ – remanence manure 30 t/ha</td>
<td>a 5.47 a</td>
<td>a 5.27 b</td>
<td>a 5.01 c</td>
<td>a 4.83 d</td>
<td>a 4.78 d</td>
<td>a 5.07</td>
</tr>
<tr>
<td>a₃ – applying of 40 t/ha sugar beet</td>
<td>b 5.16 a</td>
<td>b 4.99 b</td>
<td>b 4.84 b</td>
<td>a 4.70 c</td>
<td>a 4.66 c</td>
<td>b 4.87</td>
</tr>
</tbody>
</table>

Average B

5.22 a 5.08 b 4.87 c 4.74 d 4.69 d

Factors

A B B* A A*B

DL P 5%

0.12 0.08 0.16 0.15

Figures in the same column, preceded by different letters are significantly different at P ≤ 0.05
Figures in the same row, followed by different letters are significantly different at P ≤ 0.05

2. The influence of organic and mineral fertilization on total-amidasic activity (mg NH₄⁺/100 g soil s.u.), in 24 hours of incubation at 28°C.

The crop size and quality of plant nutrition with nitrogen is mentioned, in general lines, by agronomic scientific literature. At the same time, the soil acidification by long-term application of ammonium nitrate is well known. It is less known how the soil acidification influences the total-amidasic activity, by which the level of ammoniacal nitrogen can increase, as a consequence of enzymatic hydrolysis of amides resulted in soil by proteolytic processes. Ammonium, from ammonium nitrate, given in soil as fertilizer, can inhibit (by “feed-back” process) amidasic activity.

Examining the results presented in table 2, one observes that the factor A (organic fertilization) shows that application of 30 t / ha stable manure, spontaneously composted, stimulated amidasic activity (enzymes from hydrolysis group), even after 2 years since its application (a 0.284 comparatively with c 0.181 and b 0.236). The factor B (fertilization with ammonium nitrate and superphosphate) inhibited this activity (0.356 a comparatively with 0.102 c, 0.201 b and 0.218 b, the effect of N₂₀₀ can’t be explained). The combined influence of the 2 factor of anthropic influence, though obvious doesn’t conclusively show the variation cause.

Table 2. The influence of organic and mineral fertilization on total-amidasic activity (mg NH₄⁺/100 g)

<table>
<thead>
<tr>
<th>A</th>
<th>B1 – N₀</th>
<th>B2 – N₆₀</th>
<th>B3 – N₁₀₀</th>
<th>B4 – N₁₅₀</th>
<th>B5 – N₂₀₀</th>
<th>Average A</th>
</tr>
</thead>
<tbody>
<tr>
<td>a₁ – unfertilized</td>
<td>b 0.323 b</td>
<td>a 0.170 c</td>
<td>b 0.200 c</td>
<td>c 0.030 d</td>
<td>b 0.460 a</td>
<td>b 0.237</td>
</tr>
<tr>
<td>a₂ – remanence manure 30 t/ha</td>
<td>a 0.543 a</td>
<td>c 0.033 d</td>
<td>c 0.137 c</td>
<td>a 0.423 b</td>
<td>a 0.577 a</td>
<td>a 0.343</td>
</tr>
<tr>
<td>a₃ – applying of 40 t/ha sugar beet</td>
<td>c 0.200 b</td>
<td>b 0.103 c</td>
<td>a 0.267 b</td>
<td>b 0.200 b</td>
<td>c 0.410 a</td>
<td>b 0.236</td>
</tr>
</tbody>
</table>

Average B

0.356 b 0.102 d 0.201 c 0.218 c 0.482 a

Factors

A B B* A A*B

DL P 5%

0.0274 0.0466 0.0755 0.0808

Figures in the same column, preceded by different letters are significantly different at P ≤ 0.05
Figures in the same row, followed by different letters are significantly different at P ≤ 0.05
An assumed comparison. As we have seen in table 1, the soil sample from the control parcel (without: fertilization with organic matter and without ammonium nitrate), total-amidase delivered from specific substrates in soil, a quantity of 0.323 mg NH₄ / 100 g soil d.s./ 24 hours. A simple calculation, referring to 1500 t of soil, on the depth 0 – 10 cm, points out that this quantity, evidently very small, delivered in soil by total-amidasic process, represents 680 kg N / ha in 180 days, in the active vegetation period of agricultural crops. If the crops are fertilized by a dose of N₁₅₀ kg / ha, this represents 450 kg of commercial fertilizer NH₄NO₃, with N 33%, that is 680 – 150 = 530 kg more than it would be necessary. Certainly, the natural process in soil is not linear and permanent. This comparison is given with the aim of to understanding that the natural soil, unsymbiotic fixing atmospheric nitrogen also, may help the adapted vegetation to grow naturally. It must take into account that at an artificial addition of ammoniacal nitrogen, over the limits which are necessary, the total-amidasic activity could be diminished and even stopped, because of the “feed-back” reaction.

3. The influence of organic and chemical fertilization on total phosphatasic activity (mg P / 100 g soil d.s.), in 24 hours of incubation at 28°C.

The largest part of phosphorus in soil occurs in organic combination and represents the most important source of supplying this nutrient for micro- and macropopulation of soil and its vegetal cover. PAVLOVSCHI & IONESCU [26] sustained that the variation of assimilable phosphorus in soil depends on the phosphatasic activity. It is known very little about why one develops the activity of phosphatasic hydrolases in soil under the influence of agronomic activity of applying the chemical fertilizers. From table 3, one observes that applying of vegetal remains, from sugar-beet, have stimulated the total-fosfatasic activity. The factor B (chemical fertilization) has influenced negatively total phosphatasic activity. The values take in column N₂₀₀ can’t be interpreted as veridical and we don’t know the cause. When factors (A*B) are combinated one notes, the negative influence of N doses, when stable dust is remanent and positive one of sugar-beet refuses, in the case of little doses of nitrogen. The combination of the factors (B*A) shows that without chemical fertilization, the stable dust stimulated phosphatasic activity, and also N₁₅₀. The remains of sugar-beet stimulated total-phosphatasic activity of N₁₀₀ and N₁₅₀.

Table 3. The influence of organic and mineral fertilization on total-phosphatasic activity of soil (mg P/100 g soil) 

<table>
<thead>
<tr>
<th></th>
<th>b₁–N₀</th>
<th>b₂–N₆₀</th>
<th>b₃–N₁₀₀</th>
<th>b₄–N₁₅₀</th>
<th>b₅–N₂₀₀</th>
<th>Average A</th>
</tr>
</thead>
<tbody>
<tr>
<td>a₁– unfertilized</td>
<td>b 13.873 a</td>
<td>b 10.990 a</td>
<td>b 6.530 b</td>
<td>a 6.470 b</td>
<td>a 12.687 a</td>
<td>b 10.110</td>
</tr>
<tr>
<td>a₂– remanence manure 30 t/ha</td>
<td>a 20.470 a</td>
<td>b 6.403 b</td>
<td>b 6.963 b</td>
<td>a 6.660 b</td>
<td>a 11.240 b</td>
<td>b 10.347</td>
</tr>
<tr>
<td>a₃– applying of 40 t/ha sugar beet</td>
<td>b 10.693 c</td>
<td>a 27.847 a</td>
<td>a 16.603 b</td>
<td>a 5.303 c</td>
<td>a 8.200 c</td>
<td>a 13.729</td>
</tr>
</tbody>
</table>

Average B: 15.012 a 15.080 a 10.032 b 6.144 c 10.709 b

Factors: A B B*A A*B

DL P 1%: 2.9770 3.3496 5.7185 5.8017

Figures in the same column, preceded by different letters are significantly different at P≤0.01
Figures in the same row, followed by different letters are significantly different at P≤0.01

An assumed comparison. As we have seen in table 3, in the soil sample from the parcels fertilized with stable manure and without nitrogen fertilizer, 20.47 mg P / 100 g d.s. were liberated by total-phosphatasic activity. This quantity of phosphorus, enzymically
released, though seems to be very little, calculated to hectar, on the depth 0 – 10 cm, represents 0.3075 kg in 1500 t of soil, in 24 hours. Whether this activity took place continually for 150 days (in agriculture active period) 55.269 kg / ha would be released. These, translated in to P2O5, would represent 248.7 kg / ha. We are mentioning that it is not known whether in soil the phosphatasic processes are continuous and if the effect of “feedback” process exists in the case of chemical fertilization with superphosphate.

4. The influence of organic and mineral fertilization on soil catalasic activity (cm³ O₂ / minute / 100 g soil d.s.)

Slow oxidation of organic matter in soil is realized by the free catase in the soil (PAVLOVSCHI & GROZA [27]). If the catalase accumulation in soil is passive (resulting from celllar contents of soil micro- and macro-population, in course of decomposition), the role of this catalase, in dead organic matter mineralization, is associated to the general processes of mineralization which provide the necessary energy for biotransformations and mineral nutrition of the vegetation. In Table 4, we are showing the results of catalazic activity on oxygenated water, which results in the respiration process of soil micro- and macro-population.

<table>
<thead>
<tr>
<th>A (Factor)</th>
<th>B</th>
<th>b₁ – N₀</th>
<th>b₂ – N₆₀</th>
<th>b₃ – N₁₀₀</th>
<th>b₄ – N₁₅₀</th>
<th>b₅ – N₂₀₀</th>
<th>Average A</th>
</tr>
</thead>
<tbody>
<tr>
<td>a₁ – unfertilized</td>
<td>a</td>
<td>134.5 b</td>
<td>a 179.09 a</td>
<td>a 61.98 c</td>
<td>b 72.77 c</td>
<td>a 68.82 c</td>
<td>a 103.43</td>
</tr>
<tr>
<td>a₂ – remanence manure 30 t/ha</td>
<td>b</td>
<td>59.06 b</td>
<td>b 112.37 a</td>
<td>a 66.08 b</td>
<td>c 8.89 c</td>
<td>a 49.24 b</td>
<td>b 59.12</td>
</tr>
<tr>
<td>a₃ – applying of 40 t/ha sugar beet</td>
<td>c</td>
<td>49.21 b</td>
<td>c 5.03 c</td>
<td>a 70.33 b</td>
<td>a 115.55 a</td>
<td>a 67.54 b</td>
<td>b 61.53</td>
</tr>
<tr>
<td><strong>Average B</strong></td>
<td></td>
<td>80.93 b</td>
<td>98.83 a</td>
<td>66.12 b</td>
<td>65.74 b</td>
<td>61.865 c</td>
<td></td>
</tr>
</tbody>
</table>

Figures in the same column, preceded by different letters are significantly different at P ≤ 0.01
Figures in the same row, followed by different letters are significantly different at P ≤ 0.01

From Table 4, one finds that the most intensive activity was produced, as average for factor A, in the case of control variant (without organic fertilization, but with chemical one). The influence of factor B (as average of the variant A) did not lead to the catalase activity differentiation. The presented data, if interpreted as high value of catalase activity, are the highest in the case of control variant (without organic and mineral fertilization), which correlates with the level of soil chemical reaction, which moved down as consequence of annual fertilization with ammonium nitrate and superphosphate.

5. Influence of organic and mineral fertilization on soil enzymic potential.

For the general appreciation of the influence that the soil method fertilization has had on pedo-enzymic activities the Indicator of Enzymic Activity Potential (IEAP %, realized especially to make comparisons between different experimental variants of fertilization) was used. This indicator was realized according to the method of Numerical Taxonomy of transformation in percentage values of all absolute values obtained at enzymic tests for appreciating the fertility of soils. In Table 5, the results of the analyses are presented and interpreted.
Modification of some pedo-enzymic indices of the reddish preluvosol during the action of fertilizers, after nineteen years of experimentation

Table 5. The influence of organic and mineral fertilization on enzymic potential of soil (IPAE %)

<table>
<thead>
<tr>
<th>A</th>
<th>b1– N0</th>
<th>b2–N60</th>
<th>b3–N100</th>
<th>b4–N150</th>
<th>b5–N200</th>
<th>Average A</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1– unfertilized</td>
<td>b 31,520 a</td>
<td>b 23,307 b</td>
<td>b 16,407 c</td>
<td>b 12,840 c</td>
<td>a 33,397 a</td>
<td>b 23,494</td>
</tr>
<tr>
<td>a2– remanence manure 30 t/ha</td>
<td>a 46,390 a</td>
<td>c 11,523 d</td>
<td>b 14,943 d</td>
<td>a 23,140 c</td>
<td>a 35,027 b</td>
<td>a 26,205</td>
</tr>
<tr>
<td>a3– applying of 40 t/ha sugar beet</td>
<td>c 21,747 c</td>
<td>a 40,660 a</td>
<td>a 32,197 b</td>
<td>b 15,663 d</td>
<td>b 25,727 c</td>
<td>a 27,199</td>
</tr>
<tr>
<td>Average B</td>
<td>33,219 a</td>
<td>25,163 b</td>
<td>21,182 c</td>
<td>17,214 d</td>
<td>31,383 a</td>
<td></td>
</tr>
</tbody>
</table>

Factors | A | B | B* A | A *B |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DL P 5%</td>
<td>2,5979</td>
<td>3,2549</td>
<td>5,4548</td>
<td>5,6377</td>
</tr>
</tbody>
</table>

Figures in the same column, preceded by different letters are significantly different at P≤0.05
Figures in the same row, followed by different letters are significantly different at P≤0.05

The first observation about the partial influence of fertilization with organic matter is that it determined a IEAP %, superior to that from the variant unfertilized with stable manure or remains of sugar-beet. Regarding the chemical fertilization, one finds that in chemical unfertilized variant, in average of the 3 variants with organic fertilization, IEAP % diminished below the value of 33.22 a, in all the other variants (from N60 and then from N100 and N150) In the case of the interaction of anthropic influence by experimental factors we are finding a negative influence on pedo-enzymic potential determined by chemical fertilization together with stable manure (spontaneously composted), after 2 years from the last application. The influence of sugar-beet remains, applied in autumn (the soil samples were sampled in the spring which followed), after 19 years of experimentation, wasn’t clear, enough.

6. Interdependence between soil chemical reaction and Indicator of Enzymatic Activity Potential %

The oxidasic activity, developed by soil microflora on organic matter, was realized for obtaining the necessary energy for metabolic processes and for new organic syntheses. At the same time, in the soil, microflora and the various organic components of the vegetal cover enrich the soil in pedo-enzymes, of all kind, which contribute to the organic matter mineralization, especially residuals in soil from the cropping process. By mineralization, the content of cations increases in soil. These are assimilated by the vegetal cover, but they are also leached in the soil profile. By continuous partial disappearance of the cations (calcium, kalium, natrium, magnesium etc.), the soil becomes acid, first in microzones, then on large zones, influencing negatively bacterial microflora, but also some pedo-enzymes, especially those which have isoelectric point in the domain of neutral or alkaline pH (such as some phosphatases). The interdependence of pH x IEAP %, in the parcels with stable manure is presented in fig.1. For a good correlation we considered as repetition (all the 15 values obtained: 3 repetitions from the 5 doses of mineral fertilization).
Conclusions

1. Organic fertilization, with stable manure, spontaneously composted, stimulated amidasic activity, even after 2 years from the last application, and sugar-beet refuses stimulated total-phosphatasic activity.

2. The fertilization with ammonium nitrate inhibited both total-amidasic and total-phosphatasic activities.

3. The appreciation of the influence of fertilization with organic matter, made by IPAE %, points out the stimulation of enzymic potential and inhibition produced by ammonium nitrate, as the ammonium nitrate doses increased.

4. The inhibition activity of the acid reaction of fertilized soil, for a long-time, to ammonium nitrate, on the soil enzymatic potential, evaluated by the Indicator of Enzymic Activity Potential (IEAP %), is evident.

5. Though the values which measure pedo-enzymic activities without addition of specific substrates (like medical or plant enzymology) are very small. However, they are real. A proof (that we present under the form of a hypothetical comparison), is that these values vary very little and even exceed the quantities of N applied in agricultural practice, as fertilizers.

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