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RESEARCHES REGARDING THE MICROORGANISMS INFLUENCE ON GLYPHOSATE BIODEGRADATION

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Abstract

Glyphosate [N-(phosphonomethyl)-glycine] is a broad-spectrum, non-selective, post-emergence herbicide that is widely used in agricultural. The object of this work was to study the dynamic of glyphosate mineralization in different agricultural soils characteristic to the west part of Romania: Black Chernozem, Typical Gleysol, Phaeozom and Slight Vertisol with moderate carbonation. To follow the quantity of degraded product in the presence of micro-organisms, in microbiology its use radio-perspiration-metre of studied mineralization molecule, determining the soil perspiration potential in the presence of microorganisms.

The quantity of free CO2 as result of glyphosate biodegradation under the microorganisms action is bigger in all four analysed soils comparatively with blind sample (uncured soil), which demonstrate the microbiological biodegradation capacity of the herbicide.

Keywords: microorganisms, glyphosate, biodegradation

1. Introduction

Glyphosate, N-(phosphonomethyl)glycine (figure 1) is an organophosphorated nonselective agrochemical widely used for controlling perennial weeds through application after harvest. Have been patented for his herbicide action in 1969, and his proprieties have been described for the first time in 1971. Since 1971 until to these days the researchers have accorded a special attention to this herbicide regarding the technical and commercial proven success [3].

To glyphosate applied treatments, a part of active agent comes in contact with soil surface, adsorbing to soil components, while another part remain in soil solution. The adsorbing to soil compounds represents a feat importance conditioning the herbicide presence in soil solution and so, his availability to degradation and dispersion in the environment [1].. Glyphosate is moderately persistent in soil. It is strongly adsorbed to most soils, and therefore has a low potential for runoff except when adsorbed to suspended matter which can be washed off into water. Glyphosate remains tightly bound to the suspended matter even in water [2].

Microbes are responsible for most of the breakdown of the chemical. Glyphosate's primary route of decomposition in the environment is through microbial degradation in soil. The herbicide is inactivated and biodegraded by soil microbes at rates of degradation related to microbial activity in the soil and factors that affect this activity. The biological degradation process is carried out under both aerobic and anaerobic conditions by soil microflora. Rates of decomposition depend on soil and microfloral population types. In nonsterile conditions, as much as 55 percent of glyphosate is given off within 4 weeks using Lintonia Sandy Loam soil [4]. The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Degradation of AMPA is generally slower than that of glyphosate possibly because

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AMPA may adsorb onto soil particles more strongly than glyphosate and/or because it may be less likely to permeate the cell walls or membranes of soil microorganisms [5].

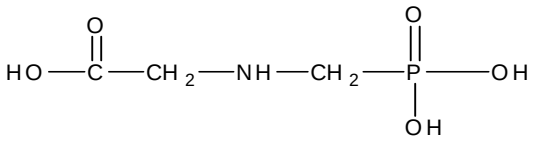

Figure 1. Glyphosate

2. Materials and Method

Four types of soils have been taken under study: Black Chernozem, Vertisol, Gleysol and Phaeozem with different characteristics.
The description of the analyzed soils is presented in table 1.

The analyzed soils have been taken from horizon A, from a depth of 10 cm. In order to obtain a representative sample, the samples have been taken from different points by splitting the surface in quarters, diagonally and on rows, through the carrots.

The assay and analysed samples achievement

To follow the quantity of degradation product in the presence of micro-organisms, in microbiology its use radio-perspiration-metre of studied mineralization molecule, determining the soil perspiration potential in the presence of micro-organisms. .
The perspiration metre is made of a bottle with a capacity of 150 ml in which introduce one after another, a cylindrical beaker fitted with two holes in which is a glass pipe (has a ventilation and drainage role) in which it introduce soil and upon another identical cylindrical beaker containing MnO_2 powder.
The soil introduced in cylindrical beaker respire the oxygen closed in the bottle and produce a small depression, as follow from the communicable its adsorbed a first oxygenation particle of water. This falls on MnO_2 and it decomposes dismissing oxygen which fills the space of the perspiration-metre. If the pressure has not re-establish in the beaker cylinder than it will be adsorbed a new particle of oxygenated water which through decomposition will dismiss the oxygen and the pressure from the perspiration-metre will be re-establish. The CO_2 resulted as follow of perspiration is adsorbed with NaOH from the glass resulting Na_2CO_3. Through addition of $BaCl_2$ it getting out CO_2 from combination with Na and it combines with Ba. Through addition of HCI is titrating the excess of NaOH the rest of un-combined with CO_2.

Calculation
CO_2mg/l00 grams soil = (A- B) x 2,2 x 5 x F = {A- B) x 11 x F
which:
A – no. ml of HCI 0,1 n with which have been made the titration of the control perspiration metre content (instead of soil it introduce distillation water with free CO2).
B - no. ml. HCl 0,2 n with which have been made the titration with the soil sample.
2,2 - equivalent in mg of a ml HCI 0,1 n vis-e-vis of CO2.
5 – is the reference coefficient of 20 grams to 100 grams soil.
F – correction factor for normality soil of HCI 0,1 n.

Have been studied the micro-organisms activity in the absence and the presence of glyphosate in 2 different concentration (2ppm and 4ppm), and also in the presence of his methabolit AMPA.

The monitoring of the free CO_2 quantity dismissed in the presence of micro-organisms have been realised during 20 days.

Table 1. Chemical physical characteristics of analyzed soils

Horizont	**Ap**	**Am**
Depth	**0-25**	**25-45**
GLEYSOL		
dust <0,02 mm	26,0	27,0
clay < 0,01 mm	37,1	36,5
Sand (0,2-0,02 mm)	36,7	36,0
Sand (0,2-2 mm)	0,2	0,5
pH in H_2O	8,05	8,05
Humic matter (%)	3,35	3,78
$CaCO_3$	0,16	0,16
P (ppm)	30,5	24,8
K (ppm)	249	266
BLACK CHERNOZEM		
Clay < 0,01 mm	41,1	44,5
Sand (0,2-0,02 mm)	29,2	27,7
Sand (0,2-2 mm)	0,5	0,5
dust (0,02-0,001 mm)	29,2	27,3
pH in H_2O	6,45	6,74
Humic matter (%)	4,09	3,97
N total (%)	0,136	0,157
P (ppm)	28,8	12,1
VERTISOL, LOW GLEIZATED , MODERATE CARBONATATION		
Clay < 0,01 mm	41,8	42
Sand (0,2-0,02 mm)	30,5	32,7
Sand (0,2-2 mm)	0,5	0,3
Dust (0,02-0,001 mm)	27,2	25,0
pH in H_2O	6,51	7,15
P (ppm)	51,81	51,82
PHAEOZEM		
clay < 0,01 mm	35,5	29,5
Sand (0,2-0,02 mm)	29,2	27,8
Sand (0,2-2 mm)	0,4	0,3
dust (0,02-0,001 mm)	35,3	42,4
pH in H_2O	5,60	6,30
Humic matter (%)	2,60	2,36
P (ppm)	17,4	18,3
K (ppm)	149	137

3. Results and Discussion

The degradation capacity is influenced by the micro-biological soils particles and leads to the glyphosate primary methabolit formation, aminomethyl-phosphonic acide (AMPA), which is a low toxicity compound.

For determination of CO2 content, it shows the microorganisms action over the free glyphosate in soil.

Has been determinate the CO2 free quantity as sequel of glyphosate and AMPA bio-degradation, in soil on alternate days, during 20 days. The experimental results regarding the free CO2 quantity dismissed

after 4 days as follow of microorganisms actions on the glyphosate herbicide and his methabolit AMPA in all of the fourth analysed types of soils are given in table 2.

Table 2. The free CO_2 quantity dismissed after 4 days as follow of microorganisms actions on the glyphosate

Sample	HCl 0,1 N quantity used to titration of control sample (ml)	HCl 0,1 N quantity used to titration of sample for analysis (ml)	CO2 quantity dissmised (mg/100 g sol)
Chernozem blind sample	38,6	37,55	11,55
Chernozem + Gly 2 ppm	38,6	38,20	4,40
Chernozen + AMPA 2 ppm	38,6	38,15	4,95
Chernozem + Gly 4 ppm	38,6	38,30	3,30
Gleysol blind sample	38,6	35,70	31,90
Gleysol + Gly 2 ppm	38,6	37,10	16.50
Gleysol + AMPA 2 ppm	38,6	37,30	14.30
Gleysol + Gly 4 ppm	38,6	37,10	16.50
Vertisol blind sample	38,6	36,40	24.20
Vertisol + Gly 2 ppm	38,6	36,70	20.90
Vertisol + AMPA 2 ppm	38,6	35,90	29.70
Vertisol + Gly 4 ppm	38,6	36,90	18.70
Phaeozem blind sample	38,6	36,40	24.20
Phaeozem + Gly 2 ppm	38,6	36,00	28.60
Phaeozem + AMPA 2 ppm	38,6	36,35	24.75
Phaeozem + Gly 4 ppm	38,6	35,60	33.00

The CO2 quantity release as sequel of glyphosate bio-degradation under the micro-organisms action is higher in all the four analysed soil comparatively with blind sample (pure soil), which demonstrates the microbial bio-degradation capacity of the herbicide.

The experimental results show microbial bio-degradation of glyphosate and of his methabolit AMPA after 96 hours (4 days) since the treatment application, when the CO2 quantity release is maximum.

In the first 2 days it was detected a low growth of CO2 quantity released comparing with the blind variant, because of the metabolic adaptability, of the soil micro-organisms, the purpose being glyphosate and AMPA substratum degradation, added.

The released CO2 quantity grows until day six, than it reaches a constant level regarding the glyphosate degradation, and the mineralization speed decrease.

Regarding the herbicide quantity added, it discovers that the free glyphosate from soil is directly and rapidly degraded by micro-organisms and not affect the microbiological activity, even at the high concentrations applied, double comparing with the quantity used in field.

The soil proprieties influence the degradation capacity of the glyphosate and of his methabolit AMPA, in the presence of micro-organisms.

The obtained experimental results and bio-degradation curves, indicates a bio-degradation capacity in the presence of micro-organisms, high in soils with lower clay and humus content.

The increasing of the humus and clay content in the soil structure leads to glyphosate and AMPA adsorbtion on the soil elements, decreasing the glyphosate fraction free and available for micro-organisms.

The bio-degrated glyphosate quantity, express through CO2 fraction released under the micro-organisms action is higher on soils which have lower humus content.

So, to Phaeozem soil (humus 2,6%, clay 35,1%), the CO2 quantity developed in the first 48 hours is 28,6 mg/100 g soil, when we applied 2 ppm quantity of glyphosate respectively 33 mg/100g soil for 4 ppm.

The bio-degradation capacity of the glyphosate in the presence of micro-organisms decrese in this order: Phaeozem, Gleysol, Vertisol and Black Chernozem. The Black Chernozem characterized through high humus and clay content (4,09% respectively clay 39,1%) presents the lower bio-degradation rate.

Regarding the AMPA behaviour in time under the action of micro-organisms, this keeps the same profile of bio-degradation curve like glyphosate, the CO2 quantity released reach the maximum level after 4 days since the treatment application, but the decrease is slower in time comparing with glyphosate bio-degradation.

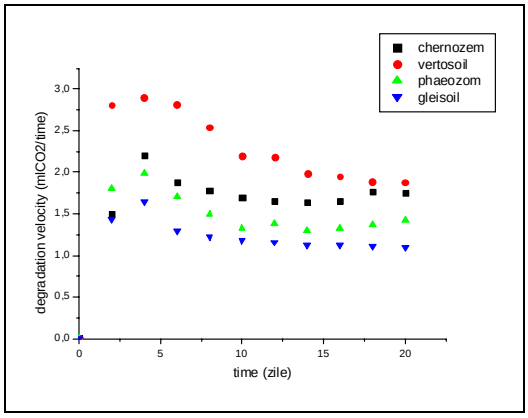

Figure 2. Degradation velocity of glyphosate

3. Conclusion

Even if the perspiration capacity of the soil grows once with application of glyphosate treatments, showing his bio-degradation capacity under the action of microorganisms, CO2 fraction released is still reduced, because of the high adsorbtion capacity of glyphosate on the soil elements, which diminish the glyphosate fraction free and available for micro-organisms.

The soil characteristics influence the glyphosate and his methabolit AMPA degradation capacity in the presence of microorganisms, which grows in soils with low humus and clay content.

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