RESEARCHES CONCERNING THE STRUCTURE AND ACTIVITY OF EDAPHIC MICROFLORA COMMUNITY WHEN GLYPHOSATE IS ADDED

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Abstract. The study was realized on two soil types: Chernozem and typical Gleysol. The results highlighted the positive effect on the microflora in the addition of glyphosate case, which is an alternative source of C, N or P. Daily respiration rate registered highest value in the 4th day of the treatment. Quantitative and qualitative estimation of microflora revealed abundant development of the zymogene fraction exponent, respectively of bacterial species belonging to the *Pseudomonas* and *Arthrobacter* species.

Keywords: cultivable heterotrophic bacteria, actinomycetes, micromycetes, daily soil respiration rate

INTRODUCTION

Glyphosate is a total herbicide with frequent use in agricultural practice, is applied in the vegetation period, on the weeds [3, 15]. So, we considered convenient to study the impact of the active substance on the structure and edaphically microflora activity. To glyphosate treatments applied, a part of the active agent comes contact with soil surface, adsorbing to soil components, while another part remain in the 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 [2]. Research has highlighted the fact that soil moisture is affecting the availability of glyphosate molecules to microbial attack. Glyphosate can be used as an alternative source of C, N and P [2, 5, 10], although the link C-P is highly resistant to microbial degradation, micro-organisms involved must have specific adaptive enzymes [6]. The frequent use of glyphosate is claimed, regarding nutritional, microbial community segment that is capable of mineralization, but other functional groups that can be seriously affected [1, 8, 12].

MATERIALS AND METHODS

It was study two soil types: Chernozem and typical Gleysol, whose physicochemical properties are presented in Table 1. The soil taken from the first 20 cm was kept in the refrigerator up to start the experiment. Addition of glyphosate, with commercial product Roundoup, was achieved by spraying the solution containing 4 ppm glyphosate, and 2ppm glyphosate. Soil variants were mounted in airtight container, and ensuring the permanent need of oxygen, Stefanic Gh. method [13], each variant was set up into three repetitions.

The determination of carbon dioxide quantity resulted from the herbicide biodegradation was realized in two, four and six days since soil in airtight container were mounted.

Table 1. Physical-chemical characteristics of analyzed soils.

Soil Depth	**Black chernozem** 0-25 cm, horizon Ap	**Gleyosol** 0-25 cm, horizon Ap
Dust<0,02 mm	29.2	26.0
Clay <0,01 mm	41.1	37.1
Sand 0,2-0,02 mm	29.2	36.7
Sand 0,2-2mm	0.5	0.2
pH in H_2O	6.45	8.05
Humic mater (%)	4.09	3.35
$CaCO_3$	N/A	0.16
N total (%)	0.136	0.112
P ppm	28.8	30.5
K ppm	138	249

The change of microbial community structure was done by culture method [11] of soil dilutions on specific culture media. As a dispersant medium for dilution, was used sodium pyrophosphate solution of 0.1%. Cultivation media were Topping for eubacteria [11], Gause [14] for actinomycetes and Martin for micromycetes isolation [11]. The quantitative determination of micro-cultures was realized after 4 and 10 days after herbicide application. Isolated bacterial species assessment was done by establishing the frequency of occurrence on the glass boards. High-frequency species were isolated in pure culture for examine the colonial morphological characteristics, microscopic examination to assess the cell morphology, the mode of assembly and staining properties.

RESULTS

The Community size of edaphic microorganisms. As can be seen in Figures 1 and 2, edaphically microflora community structure and size is varying

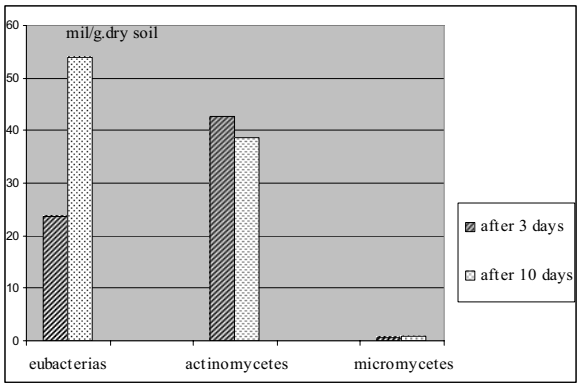

Figure 1. The number of culturable edaphic microflora in the black Chernozen soil.

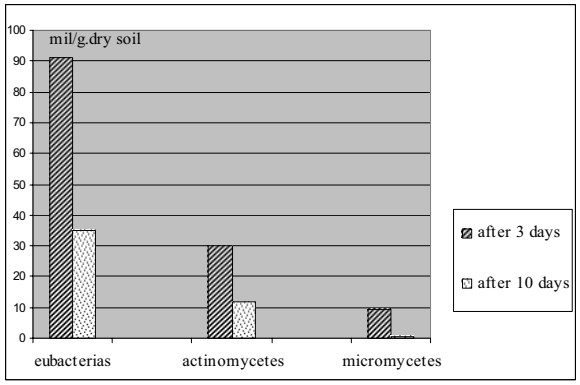

Figure 2. The number of culturable edaphic microflora in the Gleysol.

according to soil type. After three days of glyphosate application, highest number of microorganisms on the Chernozem was estimated to actinomycetes, about 42.6 million CFU / g dry soil, while the large number of microorganisms to Gleysol was determined for eubacteria, of 90.89 million / g dry soil.

Regarding the micromycetes after 3 days from the glyfosate application their number is reduced to Chernozem, 569 thousand CFU / g dry soil, while on Gleysol micromycetes are present in large numbers, of 9.5 million / g soil. After 10 days of treatment, while the soil was incubated on 23-25 ^{0}C, the determinations have highlighted changes concerning the number and the structure of edaphically microflora. Thus, Chernozem case was determined the highest number for eubacteria 53.9 million / g soil, followed by actinomycetes, 38.6 million / g soil. On Gleysol all the microorganisms isolated were represented in a smaller number compared with the previous determination.

The effect on bacterial community structure. Through comparative analysis of bacterial microflora on soils with and without herbicide, applied in doses of 2 ppm, were identified bacterial species, given in Table 2, and their occurrence frequency on the plate's media

It can be observed high frequency of occurrence of the *Arthrobacter globiformis* on plate's media, on Chernozem 71% and 72% on Gleysol, to variants with glyphosate addition compared with their frequency in untreated soils. On variants without herbicide the

Table 2. Bacterial species frequency on plate's media.

Nr Crt	BACTERIAL species	GRAM character	CHERNOZEM		GLEYOSOL	
			with glyphosate %	without glyphosate %	with glyphosate %	without glyphosate %
T0	*Unindentified*	N/A	N/A	5	N/A	7
T1	*Arthrobacter globiformis*	G+	71	51	72	45
T2	*Sarcina flava*	G+	N/A	N/A	5	3
T3	*Pseudomonas lemonnieri*	G-	14	3	1,5	5
T4	*Pseudomonas aurantiaca*	G-	15	2	3	2
T5	*Arthrobacter simplex*	G+	N/A	N/A	18,5	11
T6	*Bacillus mycoides*	G+	N/A	15	N/A	7
T7	*Bacillus megaterium*	G+	N/A	16	N/A	10
T8	*Arthrobacter oxidans*	G+	N/A	8	N/A	10

bacterial biodiversity microflora is higher, were identified 8 species on Chernozem and 6 on Gleysol.

Study of Glyphosate bio-degradation in soils. After titration and estimation, were obtained data presented in Table 3, for Chernozem and in Table 4 for Gleysol. In all variants with herbicide the CO_2 quantity exceeds the blind sample value, for Chernozem and for Gleysol. On both soils the highest value was deter-

Table 3. Biodegradation of glyphosate in the Blak Chernozem (mg CO_2 / 100 g dry soil).

Time / statistics	After 2 days	After 4 days	After 6 days
V_1 - without herbicide			
$\bar{x} \pm S_x$	22.55±0.26	33.0±0.20	25.3±0.60
s	1.15	2.08	1.86
S%	5.10%	6.30%	7.35
V_1 – 2 ppm			
$\bar{x} \pm S_x$	25.3±1.20	34.65±0.60	24.75±1.0
s	1.70	2.04	1.30
S%	6.71%	5.88%	5.25%
V_1 - 4 ppm			
$\bar{x} \pm S_x$	26.4±2.00	41.8±1.20	30.8±0.80
s	1.80	2.10	1.80
S%	6.81%	5.02%	3.50

Table 4. Biodegradation of glyphosate in Gleysol (mg CO_2 / 100 g dry soil).

Time / statistics	After 2 days	After 4 days	After 6 days
V_2 - without herbicide			
$\bar{x} \pm S_x$	25.3±2.40	36.3±1.40	24.2±0.40
s	1.8	2.30	3.20
S%	7.11	6.33	13.22
V_2 – 2 ppm			
$\bar{x} \pm S_x$	28.05±1.20	43.40±2.20	20.35±0.22
s	2.30	1.40	1.80
S%	8.00	3.22	8.84
V_2 - 4 ppm			
$\bar{x} \pm S_x$	26.95±0.60	43.45±1.80	21.45±1.20
s	2.06	1.60	2.30
S%	7.64	3.68	10.72

mined after 4 days from herbicide application in the variant with 4 ppm glyphosate addition, respectively 41.8 mg CO_2 on Chernozem and 43.45 mg CO_2 Gleysol.

Evolution of glyphosate mineralization pass off unlike on two soils, which can be observed in Figure 3 where shown daily mineralization rate (DMR) of glyphosate is calculated according of the CO_2 amount released in 24 hours. The highest values of the DMR were determinate within 3-4 days from making glyphosate treatment, respectively 21.7 mg CO_2/day on Gleysol and 20.9 CO_2/day on Chernozem. DMR low values were determined during 5-6 days, comparatively to those obtained in the first 2 days, observation valuable to both types of soil indicates that herbicide mineralization was closed.

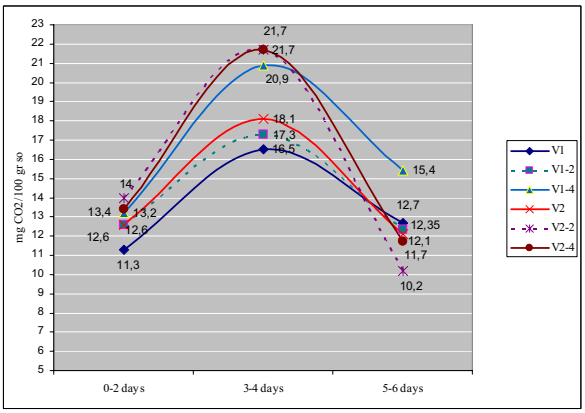

Figure 3. Glyphosate daily mineralization rate (DMR).

DISCUSSIONS

Chemical characters of soil, influence soil biological activity when herbicide is added [4]. So, in Chernozem case, rich in humus, whose predominant microflora is represented by actinomycetes through glyphosate treatment these organisms growths of. As major producers of antibiotics actinomycetes determine an inhibitory effect on eubacteria and micromycetes growth, which is highlighted by estimating a relatively small number of them. After 10 days, once with decreasing of glyphosate content in soil, decreases and the number of active actinomycetes, therefore we are witnessing to a numerical growth of bacterial population [8]. In Gleysol case the indigenous microflora is represented by eubacteria, so when we add glyphosate is registered a high growth of these organisms fraction It is about a quantitative growth with promotion of a selective growth of certain bacterial species with greater capacity to adapt to the herbicide biodegradation [12].

Through comparative analysis of bacterial species isolated from soil (Table 2) it can be observed that the addition of herbicide favours multiplication of bacteria of the *Arthrobacter* and *Pseudomonas* genus in a differently way for each type of soil, respectively *A. globiformis* and *A. simplex* for Gleiosol and only *A. globiformis* for Chernozem. Glyphosate biodegradation on Chernozem case is assured by *Pseudomonas lemonierii,* whose frequency of isolation on glass plates increased from 3% to 14% and *Pseudomonas aurantiaca* with a frequency increase from 2% to 15%.

It can be note that the herbicide addition restricts bacterial microflora biodiversity, fact that is confirmed by the absence of bacterial species such as *Bacillus mycoides, Bacillus megaterium* and *Arthrobacter oxidans* existed on both types of soils until the glyphosate addition, a fact recorded by other researchers in the domain [8, 9, 12].

Microbial biodegradation of the herbicide takes place with maximum intensity in a 4th day from the glyphosate treatment application. In the first 2 days there was a small increase of CO_2 compared with control sample, which leads us to the idea that the soil microflora is adapted for metabolic point of view for glyphosate herbicide degradation. Glyphosate appeared to be directly and rapidly degraded by microbes, even at high application rates, without adversely affecting microbial activity [7]. By reducing the amount of CO_2 caused in the 6th day after the treatment application indicates that biodegradation of glyphosate enters on the downward curve of mineralization.

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