Analele Universităţii din Craiova, seria Agricultură – Montanologie – Cadastru (Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series) Vol. L/2020

EFFECT OF SOME SOIL APPLIED HERBICIDES ON SOIL MICROFLORA

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Keywords: soil applied herbicides, microbial population, soil mineral nitrogen.

ABSTRACT

A study on the effect of some herbicides on the number of soil microbial populations and microbial activity was conducted. The herbicides Adengo (225 g/l Isoxaflutol + 90 g/l Thiencarbazone-methyl + 150 g/l Ciprosulfamide (antidote)), Dual Gold (960g/l S-metolachlor), Marlin Flexx (240 g/l isoxaflutol) were applied, in optimal and doubled dose. There was a decrease in the amount of ammonifying bacteria, actinomycetes, nitrifying bacteria, soil fungi in some of the treatments, and the strongest effect, especially in the initial stages of incubation is the suppression of nitrogen-fixing bacteria. After 120 days of incubation in most variants of the experiment, the suppressive effect of herbicides disappeared.

INTRODUCTION

The soil microflora is the basis of all processes taking place in the soil. Through the activity of microorganisms the biogeochemical cycles, the decomposition of organic residues, humus formation, soil structure formation, processes of detoxification, etc. are carried out.

A number of studies have been done on the effects of pesticides (Anderson et al., 1981, Singh et al, 2014, Subhani 2000), heavy metals (Donkova, R., 2008), the application of organic and inorganic fertilizers and soil amendments. (Nunes et al., 2012), various tillage systems (Soane et al., 2012) on soil microbiological activity.

Pesticides are commonly used in crop production to improve crop yields and quality and increasing economic returns. Herbicides are the most widely used class of pesticides in crop production (Newman et al., 2016). Weed control in cultivated plants is one of the main technological operations, as they have a direct negative impact on yields and quality of agricultural products. However, the intensive use of herbicides leads to environmental pollution, including accumulation in the soil and subsoil (Bakalivanov, D.1982., Monako et al. 2002., Subhani, et al., 2000).

In application of soil herbicides, it is possible for some of the active substances to remain undegraded, which may cause possible phytotoxicity on the next crops in the crop rotation and to suppress the activity of the soil microflora. The persistence of soil applied herbicides rarely exceeds 120 days. However, increasing the doses leads to a longer persistence of the herbicide (Sapundjieva et al., 2008, Taiwo et al. 1997). An important role in the detoxification of herbicides is played by the biological properties of the soil and, above all, the activity of soil microorganisms.

The results of a large number of experiments show that the action of some herbicides on individual groups of microorganisms is selective and has a variable nature (Kartalska et al., 2008). Devrinol 45, for example, has a stimulating effect on ammonifying microorganisms and on nitrifying bacteria. The herbicide also increases the amount of cellulose-decomposing bacteria in the soil layer from 0 to 10 cm. Dasomet and mancozeb suppress not only pathogenic microorganisms but also soil nitrifying activity for long periods of time (Feld et al., 2015). Glyphosate is one of the most widely used herbicides in agriculture with 1.35 million tons estimated amounts are used annually until 2017. A number of studies are now being done on the non-target effects of its application on soil microbial communities, which may have a negative impact on soil functions, plant health and crop productivity (Newman et al., 2016).

Ayansina, Oso (2005) found that oxadiargyl in increased doses had an inhibitory effect on the amount of azotobacteria, actinomycetes and soil fungi. Donkova et al. (1985) prove that metolachlor has a stimulating effect on ammonifying microorganisms in the soil layer from 0 to 10 cm, and in the soil layer from 10 to 20 cm the herbicide stimulates the development of nitrifying bacteria and increases the amount of cellulose-decomposing bacteria in the layer from 0 to 10 cm (Kartalska et al., 2008).

In testing the effect of 29 herbicides on soil respiration and nitrification from the activity of soil microorganisms, it was found that some of them do not have a significant effect in the soil (chlorinated hydrocarbons), some permanently suppress respiration and nitrification (carbamates, cyclodienes, phenylurea); others are toxic but are transformed by soil microorganisms (amides, anilides, organophosphates, phenylcarbamates, triazines). Some compounds of the latter type caused an initial increase and subsequent decrease in soil CO_2 production. The results of studies of model systems show that there is one or a combination of mechanisms and interactions in the soil between test compounds and soil respiration (Lanzilotta et al, 1967).

The loss of microbial diversity, which may be the result of the application of herbicides, leads to a disturbance of soil functions. Studies are also being conducted on the simultaneous application of pesticides and organic materials on the amount and activity of soil microbial communities due to the large number of pesticides and organic residues used in agriculture (Karpouzas D. G, 2014, Xu J., 2014).

The aim of the present study is to trace the dynamics of the impact of some soil herbicides on the amount of ammonifying bacteria, actinomycetes, soil fungi, the amount of nitrogen-fixing and cellulose-decomposing microorganisms, for which purpose a pot experiment was conducted and their influence on the dynamics of mineral nitrogen in the soil.

MATERIAL AND METHOD

Experiment design

A pot experiment was conducted with calcic chernozem soil type from agricultural areas in the region of the town of Marten, Ruse district. The properties of the soil are presented in table. 1. The soil is sieved through a 2 mm sieve and 200 g of sample are placed in 1 liter flasks. The experiment is conducted in three replications. Throughout the period, the vessels were incubated, closed and shaded. Humidity of about 20% and temperature 20-22^{0}C is maintained.

Samples for microbiological analyzes were taken on the 1st, 15th, 30th and 120th day, taking a composed sample of all replicates.

Herbicides and soil treatment

At the beginning of the experiment, herbicides dissolved in sterile water were applied with a sprayer. The herbicides Adengo 465 SC 225 g/l Isoxaflutol + 90 g/l Thiencarbazone-methyl + 150 g/l Ciprosulfamide (antidote), Dual Gold 960 EC (960 g/l S-metolachlor), Marlin Flexx 480 SC(240 g/l isoxaflutol) were applied. in optimal and doubled dose.

Variants of treatment

1-Control, without application of herbicides; 2- Adengo at a dose of 44ml/da; 5 ml, 0.88 ml/l working solution

+ 5 ml distilled, sterile water were applied; 3- Adengo at a dose of 88 ml/da; 10 ml, 0.88 ml/l working solution was applied; 4 - Dual Gold in a dose of 150ml/da; 5 ml, 3.00 ml/l working solution + 5 ml distilled, sterile water were applied; 5 - Dual gold in a dose of 300ml/ da; 10 ml, 3.00 ml/l working solution were applied; 6 - Merlin Flexx in a dose of 42ml / da; 5 ml, 0.84 ml/l working solution + 5 ml distilled, sterile water were applied; 7 - Merlin Flex in a dose of 84ml/da; 10 ml, 0.84 ml/l working solution were applied

Microbiological and chemical analyzes

For agrochemical characteristics of soils the main parameters are determined - content of humus according to Turin, content of mineral nitrogen according to Kjeldal, pH of the soil (in H2O potentiometrically), content of available forms of phosphorus according to Murphy-Riley and available forms of potassium on a flame photometer .

The quantities of the following physiological groups of microorganisms were determined: ammonifying microorganisms - on meat-peptone agar; actinomycetes - on starch-ammonia agar; microscopic fungi - on Chapek agar; bacteria on starch-ammonia agar, nitrogen-fixing on Ashby's medium and cellulose-degrading on Hutchinson's medium.

RESULTS AND DISCUSSIONS

During the time of incubation the number of ammonifying bacteria is highest by the 15th day and begins to decrease by the 30th. The amount of ammonifying bacteria is the lowest at 120 days of incubation in all variants (tabl. 2, fig. 1). Twenty-four hours after application of the herbicides, there was a decrease in the total number of ammonifying bacteria in almost all variants, except for the variant with application of Merlin Flexx in a single dose, and in a double dose application, there was a decrease in the amount. After 15 days of incubation, a suppressive effect of the tested herbicides was observed again, as in Adengo and Dual, an increase in the effect was reported in double dose application, compared to a single dose. On the 30th day, lower amounts of saprophytic bacteria were reported, and show continuing action, but after the 120th day of the experiment, a decrease in the effect of inhibiting the development of ammonifying bacteria is already noticeable.

The amount of actinomycetes (tabl.3, fig.2) increases gradually and the highest quantity is observed in the final stage of incubation. In actinomycetes, inhibition was observed on the first and 15th day of incubation, after which it cannot be claimed that an effect of the herbicides tested was recorded. After 120 days of incubation there is a threefold increase in the amount of actinomycetes in some of variants treated with herbicides.

The development of soil fungi (fig.3, tabl.4) is not affected by the herbicides in the first 24hours after their application. In the later stages, a decrease in their amount was observed when Adengo was administered in a double dose, as well as in Dual in both doses. The suppressive effect is the highest at 30th day and disappears by the 120th day of incubation.

The strongest influence is observed on the amount of nitrogen-fixing bacteria. An inhibitory effect is observed for all types of herbicides at both doses, with the double dose having a stronger effect. The reduction in the number of nitrogen-fixing bacteria is from 4 to 25 times compare to the control variant.

There is a strong inhibition of the development of bacteria, using mineral nitrogen, which is observed at the beginning of the application of Adengo and Dual (fig.5, tabl.6). In Adengo application there is a decrease in the amount of the nitrifying bacteria, in the first hours of its application and up to 30 days, and in Dual the amount of these bacteria decreases more strongly after 15 days and the development is suppressed until the end of the experiment. When applying Merlin Flexx there is almost no suppression, only on the 15th day of its application there is a decrease of about 40-50%.

The number of cellulose-degrading microorganisms also decreased in all variants of the experiment, with the exception of Dual Gold at the recommended dose (tabl.7, fig.6). During incubation, the largest number of cellulose-degrading microorganisms is observed on the 30th day, with no suppressive effect of the herbicidal action, and on the 120th the amount of these microorganisms in all variants is very small or absent, probably due to depletion of the substrate.

After 120 days of incubation, the number of ammonifying bacteria decreases sharply in all variants of the experiment. The same is observed with microorganisms that break down cellulose. It can be assumed that the initially available organic residues in the soil samples have decreased. After the 120th day, their development is no longer suppressed by the applied herbicides. At this stage the inhibitory effect of the treatments on the development of actinomycetes, fungi, nitrogen-fixing and cellulose-degrading microorganisms has disappeared.

The analyzes of mineral nitrogen show that by the 30th day of incubation, a lower amount of ammonium ions was measured in all treated variants of the experiment, and only in the case of treatment with Dual the opposite effect was observed (tabl.8). In variant with Merlin Flexx, the decrease in ammonium ions is the strongest, but nitrates have increased and the total amount of mineral nitrogen is not affected. After the 120th day of incubation, the ratio between ammonium and nitrate ions approaches the control variant, only in Adengo and Merlin Flexx in double dose, it remains suppressed, but to a lesser extent.

CONCLUSION

The ammonifying bacteria are suppressed by the applied herbicides, as their amount decreases significantly compared to the control by the 30th day of incubation and till the 120th day their development is restored, as in the application of Dual in both doses remains suppressed by about 40% and after this period . In actinomycetes, inhibition was observed on the first and 15th day of incubation, and after 120 days even three times the amount was observed compared to the control variant. In soil fungi, there is almost no inhibition of development. There is a certain decrease in their quantity by the 30th day of incubation and they recover again after this period. The number of nitrifying bacteria decreases sharply, especially at double doses, but Merlin Flexx has no inhibitory effect. Variants, treated with this herbicide showed a slight decrease in their quantity in the 30th day. In cellulose decomposing microorganisms, a decrease is observed in all variants of treatment, but only at the beginning of incubation.

Nitrogen-fixing microorganisms are most strongly affected. There is initial decrease by 4 to 25 times, and the strongest reduction of quantity is in variant with Adengo applied in double dose. In general, for all groups of microorganisms, with some exceptions, there is elimination of the suppressive effect of the applied herbicides after 120 days of incubation.

The analyzes of the dynamics of mineral nitrogen show that there is a decrease in the release of ammonium ions in the beginning up to 30th day, and in some cases there is an increase in nitrates, so that a significant decrease in mineral nitrogen is observed only in Adengo and Dual in double dose up to the 30th day, and in Merlin Flexx application in double dose, the decrease continues after the 120th day.

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Table 1

Soil chemical properties

Parameters	Content
Mineral nitrogen, mg/kg	94,25
Available P_2O_5 , mg/100g	4,13
Available K_2O, mg/100g	23,84
Humus, %	2,64
pH, in H_2O	7,30

Table ***2***

Number of ammonifying bacteria, after application of herbicides, CFU (colony forming units) x10^6 / g absolutely dry soil.

Variants of treatment	Dose	In CFUx 10^6				In % compare to the control			
		1st day	15th day	30th day	120th day	1st day	15th day	30th day	120th day
Control		3123,20 ±36,9	10304,00 ±34,6	7357,33 ±25,4	91,33 ±0,33	100,00	100,00	100,00	100,00
Adengo	single dose	1749,49 ±46,8	5433,07 ±248,1	1640,00 ±5,7	127,00 ±0,57	56,02	52,73	22.29	139,06
Adengo	double dose	666,64 ±3,2	3806,40 ±57,7	8901,00 ±31	63,50 ±0,29	21,34	36,94	120,98	69,53
Dual	single dose	913,68 ±20,8	5687,47 ±227,0	3250,00 ±121	61,50 ±0,28	29,25	55,20	44,17	67,34
Dual	double dose	912,12 ±5,7	3058,67 ±93,8	1906,67 ±3,2	43,00 ±0,57	29,20	29,68	25,92	47,08
Merlin Flexx	single dose	3780,00 ±11,5	1529,07 ±24,1	1270,00 ±5,8	131,00 ±1,45	121,03	14,84	17,26	143,44
Merlin Flexx	double dose	884,77 ±9,0	5164,67 ±257,0	1763,00 ±3,8	43,33 ±0,86	28,33	50,12	23,96	47,44

Table ***3***

Number of actinomicetes, after application of herbicides, CFU (colony forming units) x10^6/g absolutely dry soil.

Variants of treatment	Dose	In CFUx 10^6				In % compare to the control			
		1st day	15th day	30th day	120th day	1st day	15th day	30th day	120th day
Control		0,20 ±0,03	0,58 ±0,01	3,31 ±0,88	1,24 ±0,03	100,00	100,00	100,00	100,00
Adengo	single dose	0,09 ±0,03	0,69 ±0,06	0,00	0,00	41,87	118,97	0,00	0,00
Adengo	double dose	0,00	0,31 ±0,01	1,29 ±0,58	4,51 ±0,01	0,00	53,45	38,97	363,71
Dual	single dose	0,13 ±0,06	0,00	3,25 ±0,14	1,23 ±0,06	62,56	0,00	98,19	99,19
Dual	double dose	0,04 ±0,03	0,04 ±0,03	0,43 ±0,03	3,87 ±0,14	20,69	6,90	12,99	312,10
Merlin Flexx	single dose	0,04 ±0,03	0,00	3,81 ±0,58	3,93 ±0,58	19,70	0,00	115,11	316,94
Merlin Flexx	double dose	0,34 ±0,04	0,25 ±0,03	0,00	0,00	167,49	43,10	0,00	0,00

Table ***4***

Number of soil fungi, after application of herbicides, CFU (colony forming units) x10^3 / g absolutely dry soil.

Variants of treatment	Dose	In CFUx 10^6				In % compare to the control			
		1st day	15th day	30th day	120th day	1st day	15th day	30th day	120th day
Control		6,51	4,10 ±0,3	3,1 ±0,74	1,5 ±0,23	100,00	100,00	100,00	100,00
Adengo	single dose	4,73 ±1,2	6,30 ±2,3	3,1 ±0,23	2,2 ±0,03	72,31	153,66	100,00	146,67
Adengo	double dose	7,90 ±2,8	2,80 ±0,5	1,3 ±0,03	1,8 ±0,12	121,54	68,29	41,94	120,00
Dual	single dose	6,60 ±0,23	3,1 ±0,5	0,8 ±0,03	1,2 ±0,03	101,54	75,61	25,81	82,00
Dual	double dose	8,01 ±1,45	2,9 ±0,3	0,0	2,1 ±0,58	123,08	70,73	0,00	140,00
Merlin Flexx	single dose	7,93 ±2,67	4,2 ±0,3	1,1 ±0,36	1,0 ±0,15	121,54	102,44	35,48	66,67
Merlin Flexx	double dose	8,60 ±0,39	5,1 ±0,2	1,8 ±0,20	1,8 ±0,03	132,31	124,39	58,06	120,00

Table ***5***

Number of nitrogen fixing bacteria, after application of herbicides, CFU (colony forming units) x10^6 / g absolutely dry soil.

Variants of treatment	Dose	In CFUx 10^6				In % compare to the control			
		1st day	15th day	30th day	120th day	1st day	15th day	30th day	120th day
Control		27,57 ±2,4	25,67 ±1,3	62,83 ±15,6	23,56 ±1,7	100,00	100,00	100,00	100,00
Adengo	single dose	3,48 ±1,4	24,48 ±1,7	22,18 ±1,02	13,97 ±0,88	12,62	95,36	35,30	59,30
Adengo	double dose	1,20 ±0,08	2,85 ±0,5	6,88 ±0,88	15,24 ±1,5	4,35	11,10	10,95	64,69
Dual	single dose	7,87 ±1,1	15,95 ±0,7	39,87 ±6,9	14,76 ±3,7	28,55	62,13	63,46	62,65
Dual	double dose	2,50 ±6,7	3,22 ±1,1	23,83 ±3,76	36,98 ±0,03	9,07	12,54	37,93	156,96
Merlin Flexx	single dose	3,32 ±1,21	2,77 ±0,2	4,23 ±0,88	36,68 ±5,6	12,04	10,79	6,73	155,69
Merlin Flexx	double dose	5,93 ±1,9	3,43 ±0,2	9,03 ±0,03	48,97 ±5,8	21,51	13,36	14,37	207,85

Table ***6***

Number of bacteria on starch ammonium agar, after application of herbicides, CFU (colony forming units) x10^6 / g absolutely dry soil.

Variants of treatment	Dose	In CFUx 10^6				In % compare to the control			
		1st day	15th day	30th day	120th day	1st day	15th day	30th day	120th day
Control		0,853 ±0,02	412,74 ±17,2	44,64 ±7,8	88,87 ±7,4	100,00	100,00	100,00	100,00
Adengo	single dose	0,515 ±0,03	249,57 ±70,4	0	81,28 ±5,6	60,38	60,47	0,00	91,46
Adengo	double dose	0	218,93 ±11,4	14,19 ±3,4	80,01 ±5,8	0,00	53,04	31,79	90,03
Dual	single dose	0,887 ±0,06	126,48 ±17,8	30,55 ±6,2	18,45 ±1,2	103,99	30,64	68,44	20,76
Dual	double dose	0,592 ±0,09	210,84 ±19,9	7,8 ±0,3	45,15 ±5,8	69,40	51,08	17,47	50,80
Merlin Flexx	single dose	0,880 ±0,03	250,48 ±11,6	36,38 ±8,3	113,97 ±8,08	103,17	60,69	81,50	128,24
Merlin Flexx	double dose	0,930 ±0,09	202,98 ±19,05	32,25 ±2,8	127,4 ±0,0	109,03	49,18	72,24	143,36

Table 7

Number of cellulose decomposing microorganisms, after application of herbicides, CFU (colony forming units) x10^6 / g absolutely dry soil.

Variants of treatment	Dose	In CFUx 10^6				In % compare to the control			
		1st day	15th day	30th day	120th day	1st day	15th day	30th day	120th day
Control		1,30 ±0,35	1,15 ±0,04	4,09 ±0,43	0,21 ±0,03	100,00	100,00	100,00	100,00
Adengo	single dose	0,63 ±0,06	0,61 ±0,02	6,66 ±0,25	0,00	48,46	53,04	162,84	0,00
Adengo	double dose	0,64 ±0,06	2,07 ±0,09	3,35 ±0,28	0,25 ±0,02	49,23	180,00	81,91	119,05
Dual	single dose	2,28 ±0,81	0,49 ±0,01	3,77 ±1,01	0,00	175,38	42,61	92,18	0,00
Dual	double dose	0,25 ±0,11	0,74 ±0,23	5,20 ±1,38	0,00	19,23	64,35	127,14	0,00
Merlin Flexx	single dose	0,50 ±0,05	0,50 ±0,15	11,51 ±1,50	0,26 ±0,03	38,46	43,48	281,42	123,81
Merlin Flexx	double dose	0,51 ±0,06	0,51 ±0,05	7,22 ±1,76	0,00	39,23	44,35	176,53	0,00

Table ***8***

Soil mineral nitrogen (mg/kg), N-NH_4, N-NO_3 after treatment with herbicides

Days	Treat-ment	Dose	NH_4, mg/kg	NO_3, mg/kg	NH_4/ NO_3
1	Control	-	13,99	69,93	0,20
30	Control	-	16,54	62,04	0,27
	Adengo	single dose	3,56	24,91	0,14
	Adengo	double dose	10,71	60,66	0,18
	Dual	single dose	39,41	50,16	0,79
	Dual	double dose	10,77	21,54	0,50
	Merlin Flexx	single dose	3,57	93,30	0,04
	Merlin Flexx	double dose	3,56	71,16	0,05
120	Control		28,63	96,64	0,30
	Adengo	single dose	14,36	96,93	0,15
	Adengo	double dose	14,11	84,63	0,17
	Dual	single dose	14,36	75,39	0,19
	Dual	double dose	25,03	107,27	0,23
	Merlin Flexx	single dose	28,60	82,24	0,35
	Merlin Flexx	double dose	7,16	60,85	0,12

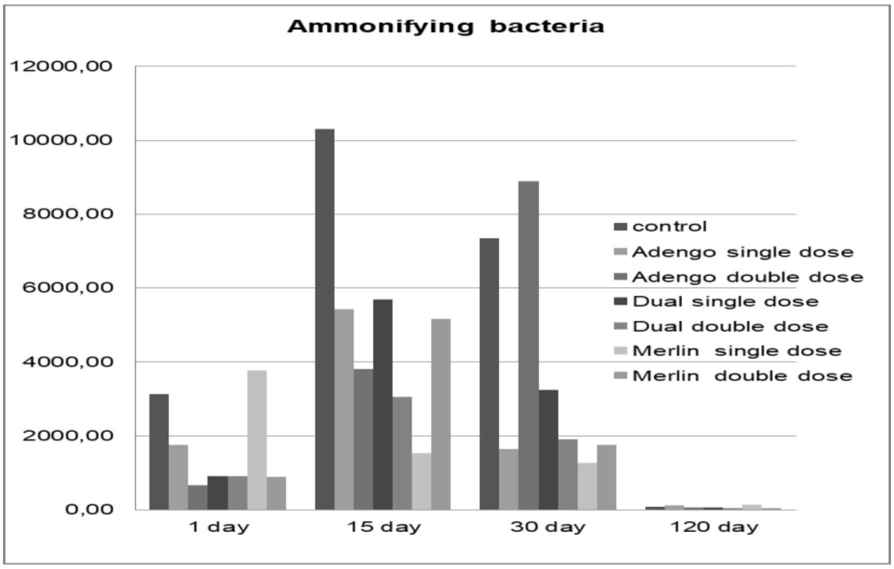

Figure 1 Number of ammonifying bacteria, after application of herbicides, CFU (colony forming units) x10^{6}

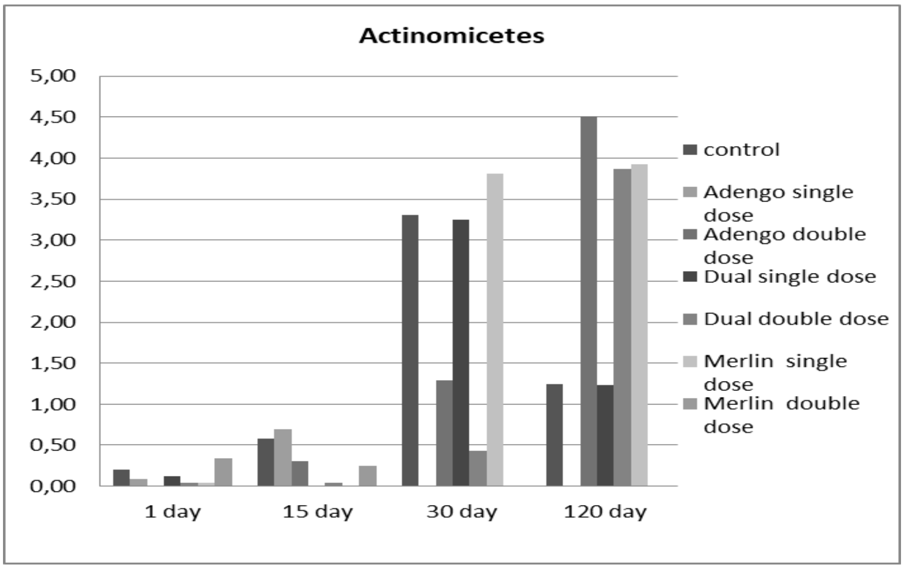

Figure 2 Number of actinomicetes, after application of herbicides, CFU (colony forming units) x10^{6}

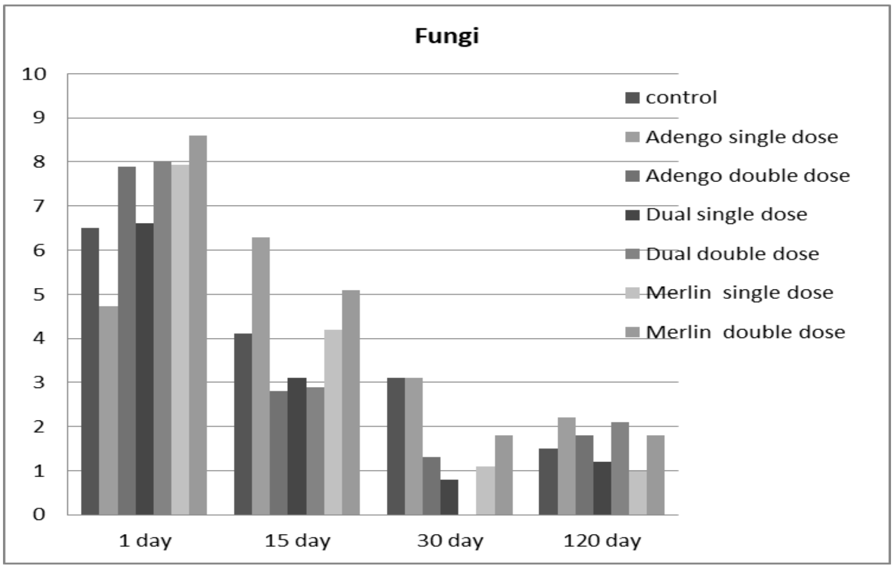

Figure 3 Number of soil fungi, after application of herbicides, CFU (colony forming units) x10^{3}

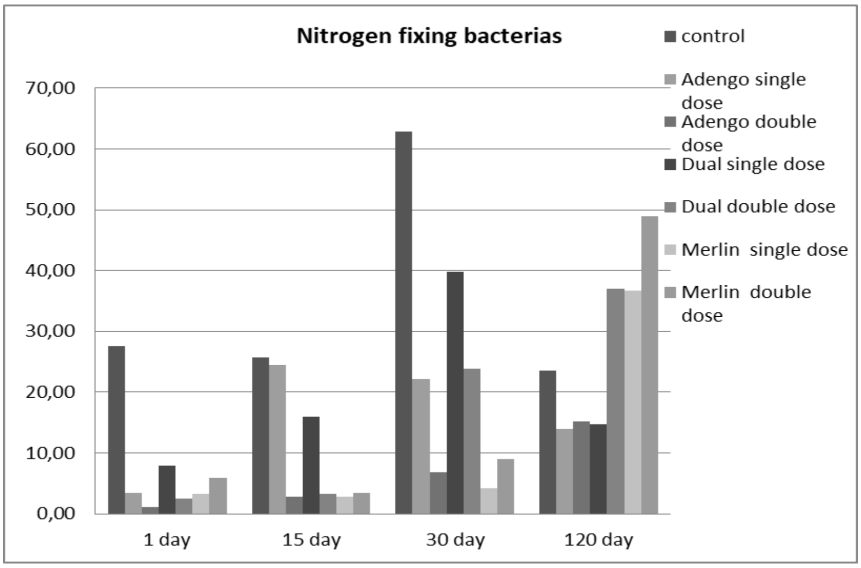

Figure 4 Number of nitrogen fixing bacteria, after application of herbicides, CFU (colony forming units) x10^{6}

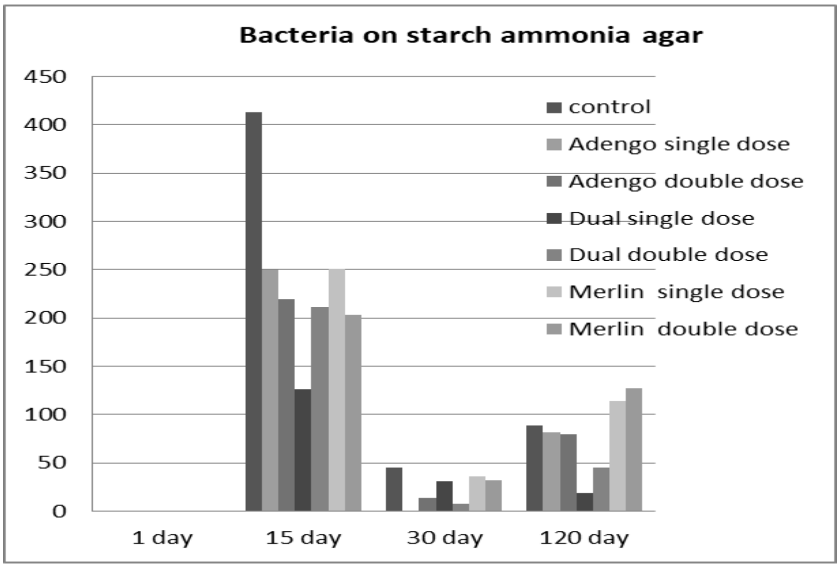

Figure 5 Number of ammonifying bacteria, after application of herbicides, CFU (colony forming units) x10^{6}

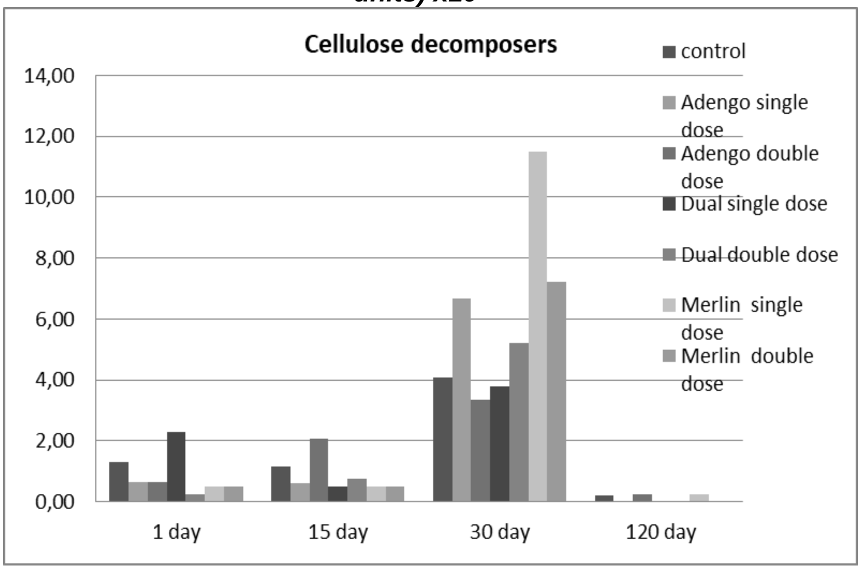

Figure 6 Number of cellulose decomposing microorganisms, after application of herbicides, CFU (colony forming units) x10^{6}