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Comparative Analysis of Some Herbicides from Amide and Dinitroaniline Families on the Soil Microorganisms

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Abstract

The changes under the influence of some herbicides from dinitroaniline family (Wing P – *a.s. dimethenamid-P + pendimethalin* and Benefin – a.s. *benfluralin*) and amide familiy (Butizan S – a.s. *metazachlore* and Dual Gold 960 EC – a.s. *S-metolachlor*) in the basic trophic groups of soil microorganisms, on population level, were traced. Herbicides were applied when cultivation of oriental tobacco on humus-calcareous (rendzina) soil. Microbiological analyzes were carried out in dynamics, during the period - before treatment, to the 15th-day, 35th-day, 50th-day and 90th-day after their submission in the soil. The numerical development of assimilating mineral nitrogen microorganisms were suppressed from the four herbicides was found. An action of amides herbicide was highly. The negative impact on ammonifying microorganisms was relatively weak. Treatment with Wing P reported even some stimulation. The effect of herbicides from the amide family of density of actinomycetes was excitatory and inhibitory of dinitroaniline family. The negative effect was durable and very strong at Benefin. Indicative of deteriorating living conditions in the soil were generally increased population density of the spore microorganisms and proportion of spores. The power of influence (η_x^2) of the factor herbicides was about 40% and was statistically significant. The comparative analysis of microbial communities in the presence of various herbicides shows disorders percentage distribution of the different groups of microorganisms. Stronger when they are dinitroaniline herbicides. The likely period of adaptation of microbial communities after treatment was about 15 days for Butizan S (a.s. *metazachlore*), 35 days for Dual Gold 960 EC (a.s. *S-metolachlor*) and 50 days for dinitroaniline herbicides. Dynamics over time suggests the possibility for participants in the early stages of biodegradation of amide herbicides were actinomycetes, and of dinitroaniline - assimilating mineral nitrogen microorganisms .

Key words: Amide, dinitroaniline, herbicides, soil microorganisms

Introduction

The application of pesticides, including herbicides, is part of the plant-protection systems in growing of crops. For weed control in tobacco growing farmers widely use soil herbicides from the group of amides and dinitroanilines. Their advantage is the possibility of introduction into the soil 24 – 48 hours before tobacco planting (Dimeska et al., 2003; Onkov et al., 2014). They have a broad spectrum of use and are highly effective against a large number of annual grasses and dicotyledonous weeds. The amide herbicides inhibit photosynthesis, respiration and cell division, as well as the synthesis of nucleic acids. Dinitrroaniline herbicides block seed germination and cell division in susceptible weeds. Their persistency in the soil is about 2-3 months. The main metabolites, which are formed at their degradation, are compounds of oxalic and sulfonic acids, as well as derivatives of benzimidazole (Mathew et al., 1996; Hladik et al., 2006). The dynamics of the herbicides in the soil are influenced by several factors, but crucial to their degradation and detoxification are the soil microorganisms. Studies in this direction and the use of soil microorganisms as evaluation criteria, and for prediction of environmental risk from the application of different herbicides, have expanded in recent years (Polyanskaya & Zvyagintsev, 2005; Smetnik et al., 2005; Mannio et al., 2007; Colla et al., 2008; Hager & Refsell, 2008; Kulikova & Lebedeva, 2010).

The aim is for a comparative analysis to be developed, which will study the impact of some dinitroaniline and amide herbicides on the

environmental purity of the soil by building quantitative characteristics of microbial communities.

Materials and Methods

The study includes the following herbicides:

1. Dinitroanilines

a. Wing (*a.s.* 212.5 g/l *dimethenamid-P +* 250g/l *pendimethalin)* - $C_{12}H_{18}ClNO_2S + C_{13}H_{19}N_3O_4$

b. Benefin (a.s. *benfluralin)* - $C_{13}H_{16}F_3N_3O_4$

2. Amides

a. Butizan S (a.s. 500g/l metazachlore) - $C_{14}H_{16}ClN_3O$

b. Dual Gold 960 EC (a.s. 960g/l S-metolachlor) - $C_{15}H_{22}ClN_3O$

The tests were conducted in the field, for a two years period during the process of growing Oriental tobacco on a humus carbonate soil (renndzina). The agrochemical parameters are: content of total humus (by Tyurin) – 2.68 %, content of total nitrogen (by Kjeldal) – 0.154 %, content of mobile phosphorous (by Egner-Ream) – 16.90 mg / 100 g of soil, content of absorbable potassium (by Miltcheva) – 36.5 mg/100g. Soil reaction is slightly alkaline - pH (in H2O) - 7.8. The herbicides have been applied before the planting of the tobacco, with 30 l. working solution per da. The following variants have been set:

- Untreated control field;
- Wing (a.s. 212.5 g / l dimethenamid-P + 250g / l pendimethalin) - at a dose of 350 ml / da;
- Benefin (a.s. benfluralin) - at a dose of 200 ml / da;
- Butizan S (a.s. 500g / l metazachlore) at a dose of 150 ml / da;
- Dual Gold 960 EC(a.s. 960g / l S-metolachlor) at a dose of 150 ml / da

Soil samples for microbiological analysis were taken from the 0-20 cm soil layer, from the rhizosphere zone of tobacco plants. The dynamics of sampling are – before treatment (day 0), days 15, 35, 50 and 90 after treatment with herbicides. For the purposes of the study the following microbiological parameters have been selected: Soil actinomycetes – on a starch-ammonia agar; Ammonifying microorganisms – on a meat-peptone agar; Assimilating mineral nitrogen microorganisms – on starch-ammonia agar; Spore microorganisms – recorded on a meat-peptone agar, after pasteurization of the soil suspension; Ration of spores – (%) of ammonifying microorganismsm.

The microbiological analysis are made by the method of Koch, by planting diluted soil suspensions on growing media, specific for the different trophic groups of microorganisms. The amount is recorded after a respective period of incubation and calculated as the most probable number of cells (M.N.C.) in 1 g of absolutely dry soil (a.d.s.) (Koleshko, 1981).

The data is subjected to a single-factor dispersion analysis. For each trophic group of microorganisms are defined the force of impact (η_x^2) of the factor – herbicide and a level of confidence (p), according to Fisher criterion (F) (Plohinskiy, 1980). The distribution of the different trophic groups of microorganisms as a percentage from the total population microbial communities is estimated. The presented data is average for a two-year study period.

Results and Discussion

The actinomycetes are one of the more precise and sensitive indicators for the general biological and ecological condition of the soil. Their biological characteristics – a powerful enzyme system, being producers of a variety of biologically active substances, their ubiquitous distribution, etc, makes them active participants in the mineralization of the organic matter in the soil and the biodegradation of many pesticides. The continuous inhibition of their numbers is an indicator of delayed degradation or the presence of toxic metabolites (Alexander, 1991; Pothuluri, 1997).

A different type of impact of the tested herbicides on the population density of actinomycetes has been observed (Table1 - A). The dinitroanilines lastingly suppress their numerical development. With a strong negative impact is Benefin (a.s. benfluralin). Average for the test period, the values compared to the control sample are lower with 15.280×10^5/ g a. d. s. for Wing (a.s. dimethenamid-P + pendimethalin) and 35.511×10^5/ g a. d. s. for Benefit (a.s. benfluralin). The differences were statistically proven at p = 0.95. The presence of Wing has a force impact of 33.34% (F_{exp}=4.751 > $F_{tab.}$=4.41), and that of Benefin - 19.40% (F_{exp} = 4.572 > F_{tab}). The effect of the amides is generally stimulating. The average density of the actinomycetes for the period in both herbicides is increased by tens of millions / g a.d.s. Differences are observed in the time of adaptation. In the case of Butizan S it is about 15 days after treatment, while in Dual Gold 960 EC (S-metolachlor) - after 35 days. The impact of Butizan S is 72.4% at confidence level p=0.95 (F_{exp} = 7.978 > $F_{tab.}$=4.41), and that of Dual Gold 960 EC - 92.7% at confidence level p=0.99 (F_{exp} = 9.138 > $F_{tab.}$=8.28).

The numerical development of microorganisms in both trophic groups, which carry out the main transformations of nitrogen compounds in the soil – the process of ammonification and immobilization of the mineral

nitrogen, is generally inhibited by the tested herbicides.

In the case of the ammonifying microorganisms up to the 35th day the readings are lower than those in the untreated soil and in the four tested herbicides (Table 1 - B). In the dinitroaniline herbicides there is an increase in the values, clearly defined around day 50 of treatment with Wing. In the amide herbicides - Butizan S and Dual Gold 960 EC the depressing effect lasts until the end the study period. The impact of the herbicides on this trophic group of microorganisms is not proven statistically (for Wing F_{exp}=4.017; Benefin for F_{exp}=3.351; for Butizan S F_{exp}=2.527; for Dual Gold 960 EC F_{exp}=2.947 < $F_{tab.}$=4.41 at p = 0.95). The period of adaptation of microorganisms is about 15 days with Butizan S, and 35 days with Dual Gold 960 EC and Wing. In the case of Benefin the values throughout the study period are very close to those in the control sample.

The suppressive effect of the herbicides on the density of the immobilizing mineral nitrogen microorganisms is more pronounced (Table 1 - C). Average for the entire period, the differences to the control sample in all four herbicides are of the order of hundreds of millions in g a.d.s., but not being able statistically to bring any prove (for Wing F_{exp}=1.834; for Benefin F_{exp}=2.008; for Butizan S F_{exp}=1.861; for Dual Gold 960 EC F_{exp}=1.027 < $F_{tab.}$=4.41 at p = 0.95). Despite the reduction in the population density the effects of the herbicides from both groups are different. Dinitroaniline herbicides exhibit stimulatory effect with values higher than those in the control sample – at day 15 in the case of Benefin, and at day 50 in the case of Wing, which is the likely period of adaptation. In the amide herbicides, regardless of fluctuations in time, the numbers do not exceed those in the untreated variant.

Table 1. Changes in population density of the trophic groups microorganisms (MPN/g a.d.s.) in dynamics

Variants	Density of the trophic groups microorganisms (MPN/g a.d.s.) in dynamics				
	0 day	15 days	35 days	50 days	90 days
	(A) Soil *Actinomycetes*				
Untreated control	45008509	347124967	35381322	99367563	47557578
Wing	45008509	25624334	41317273	35110772	38567972
Benefin	45008509	11052786	7994537	12672439	7745594
Butizan S	45008509	89396055	26339270	90535033	49091274
Dual Gold 960 EC	45008509	49582496	103204946	55968005	60147571
	(B) Ammonifying microorganisms				
Untreated control	1993231235	753982584	476138055	627602362	372198441
Wing	1993231235	545297559	485278314	1648980830	192542176
Benefin	1993231235	774384016	505229123	600533911	162514689
Butizan S	1993231235	339797521	425071953	477864074	256170451
Dual Gold 960 EC	1993231235	798106033	120914440	614372926	269544651
	(C) Assimilating mineral nitrogen microorganisms				
Untreated control	550434865	1753372353	1605342691	2121986465	267134882
Wing	550434865	607719207	570418318	2318146450	132147995
Benefin	550434865	2145687902	336683161	1062914863	151410106
Butizan S	550434865	706563100	1411433940	1188106427	142364695
Dual Gold 960 EC	550434865	1502769920	1002306835	392031062	104571183
	(D) Spore microorganisms				
Untreated control	311130649	253364186	120671328	152297728	22023471
Wing	311130649	465592144	333860727	603360510	28015471
Benefin	311130649	343623227	303853276	205858225	22209401
Butizan S	311130649	229931053	314145363	242408810	76647461
Dual Gold 960 EC	311130649	557118663	85477437	224723586	50069332

Under the impact of the herbicides there is an increase in the population density of spore microorganisms throughout the entire study (Table 1 - D). The average differences to the untreated soil are over 60 – 70 millions, and in the case of Wing – over 170 millions / g a.d.s. The differences were shown at the p=0.95. The force of influence in Wing is 39.56% (F_{exp}=5.386); in Benefin 37.80% (F_{exp}=5.212); in Butizan S - 41.26% (F_{exp}=5.549) and Dual Gold 960 EC - 30.54% (F_{exp}=4.445 > $F_{tab.}$=4.41). The dynamics of the spore microorganisms in Wing has a pronounced peak around day 50, in Dual Gold 960 EC – day 15, and Butizan S – day 35. In the case of Benefin the increase of spore microorganisms is

relatively evenly until day 50, without pronounced peaks in the dynamics. The observed differences in the dynamics of the individual herbicides are probably due to the different periods of adaptation. The increase in the density of spore microorganisms during treatment with herbicides also affects the proportion of spores from ammonifying microorganisms. The values are higher than those in the untreated control sample at the end of the period (Fig. 1). The differences are statistically proven at confidence level p=0.95. The force of impact of Wing is 39.40 % (F_{exp}=5.370); of Benefin - 52.19% (F_{exp}=6.515); of Butizan S - 51.91% (F_{exp}=6.492) and Dual Gold 960 EC - 49.91% (F_{exp}=6.326 > $F_{tab.}$=4.41). The increase of the proportion of spores indicates deterioration of life conditions in the soil environment in the presence of herbicides. The negative impact of Dual Gold 960 EC lasts longer.

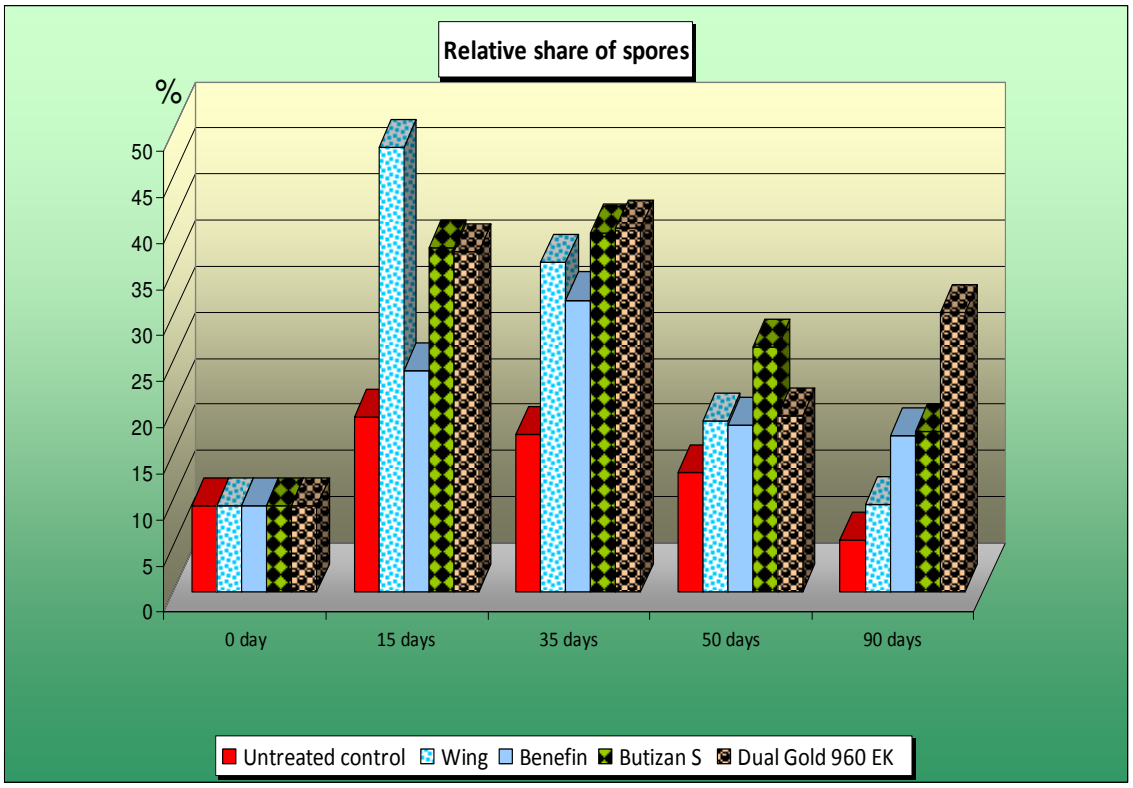
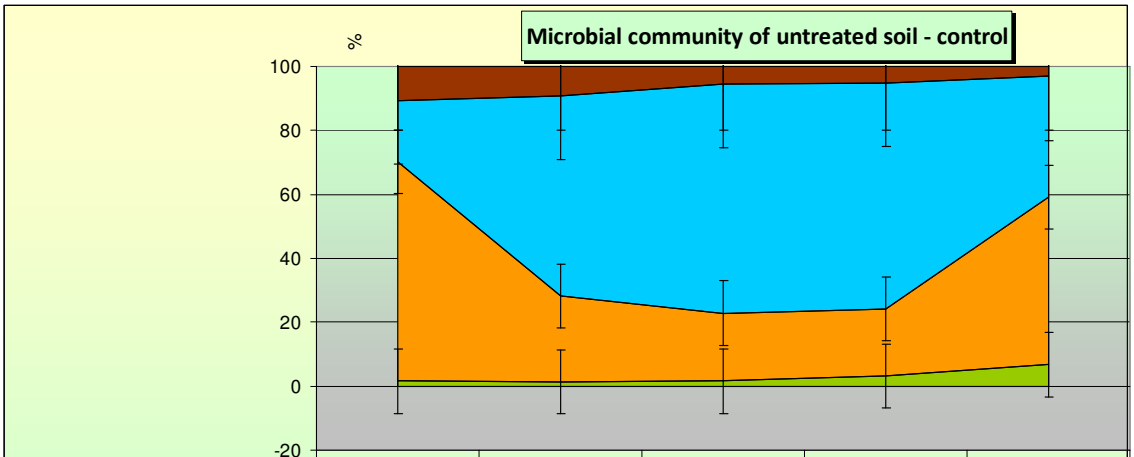


Figure 1. Changes in the relative share of spores (%) in dynamics

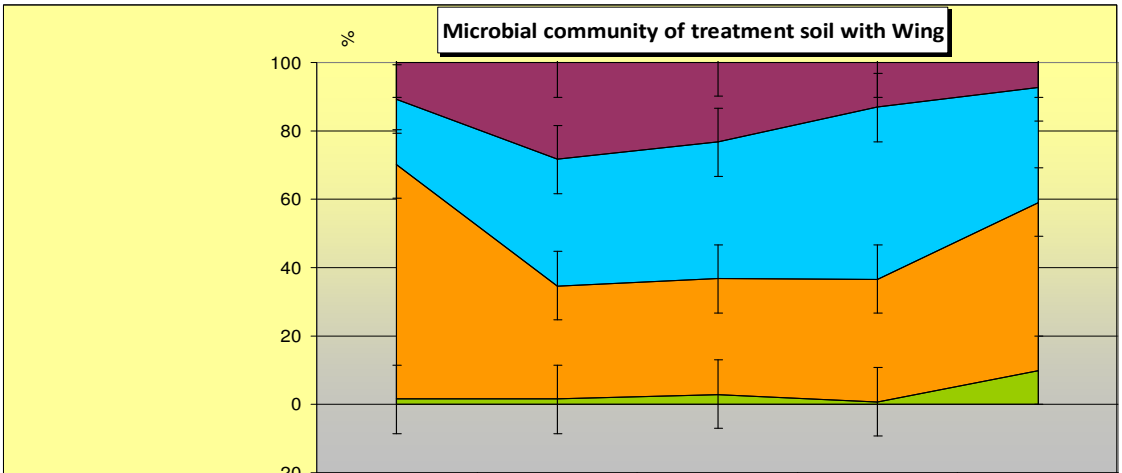
The changes in the different trophic groups of microorganisms lead to changes in the quantitative characteristic of microbial communities as a whole. The density of the different groups of microorganisms in an untreated soil, during the test period, is at a relative constant level. The microorganisms, absorbing mineral nitrogen are prevalent, followed by the ammonifying microorganisms. Average for the period, the share of actinomycetes is 2.87%, and that of the spores – 6.67% (Fig. 2).



	0day	15 days	35days	50days	90days
Spore microorganisms	10,729	9,064	5,393	5,074	3,107
Assimilating mineral nitrogen microorganisms	18,982	62,723	71,746	70,703	37,682
Ammonifying microorganisms	68,737	26,972	21,28	20,911	52,503
Actinomycetes	1,552	1,242	1,581	3,311	6,709

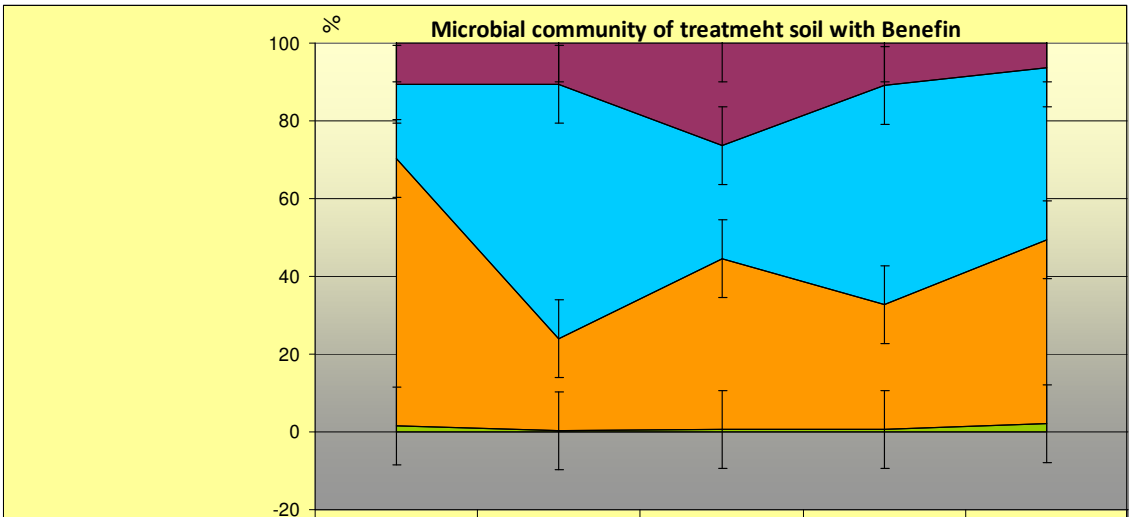
Figure 2. Percentage distribution of the trophic groups microorganisms in microbial community of untreated soil - control

After treatment with dinitroaniline herbicides, the percentage share of the absorbing mineral nitrogen microorganisms in the microbial communities decreases, while the share of the ammonifying microorganisms increases slightly. The actinomycetes decrease particularly under the impact of Benefin – 1.10% from the total microbial density of the communities. High remains the microbial share after treatment with Wing – 16.53% and Benefin – 12.99% (Fig. 3 and 4).



	0day	15 days	35days	50days	90days
Spore microorganisms	10,729	28,317	23,333	13,101	7,16
Assimilating mineral nitrogen microorganisms	18,982	36,961	39,865	50,333	33,774
Ammonifying microorganisms	68,737	33,164	33,915	35,804	49,209
Actinomycetes	1,552	1,558	2,888	0,762	9,857

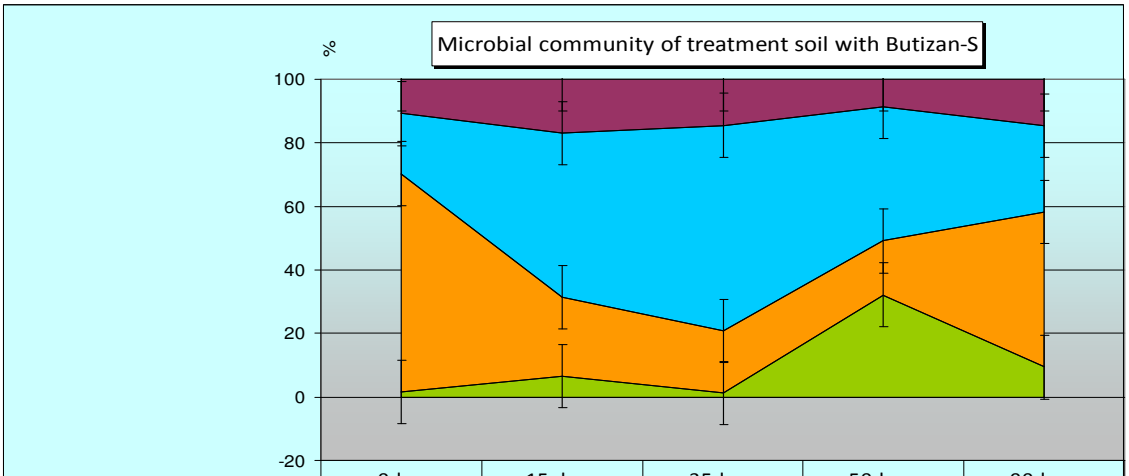
Figure 3. Percentage distribution of the trophic groups microorganisms in microbial community of treatment soil with herbicide Wing - dinitroaniline



	0day	15 days	35days	50days	90days
Spore microorganisms	10,729	10,493	26,334	10,938	6,458
Assimilating mineral nitrogen microorganisms	18,982	65,522	29,181	56,479	44,03
Ammonifying microorganisms	68,737	23,647	43,79	31,91	47,259
Actinomycetes	1,552	0,338	0,693	0,673	2,252

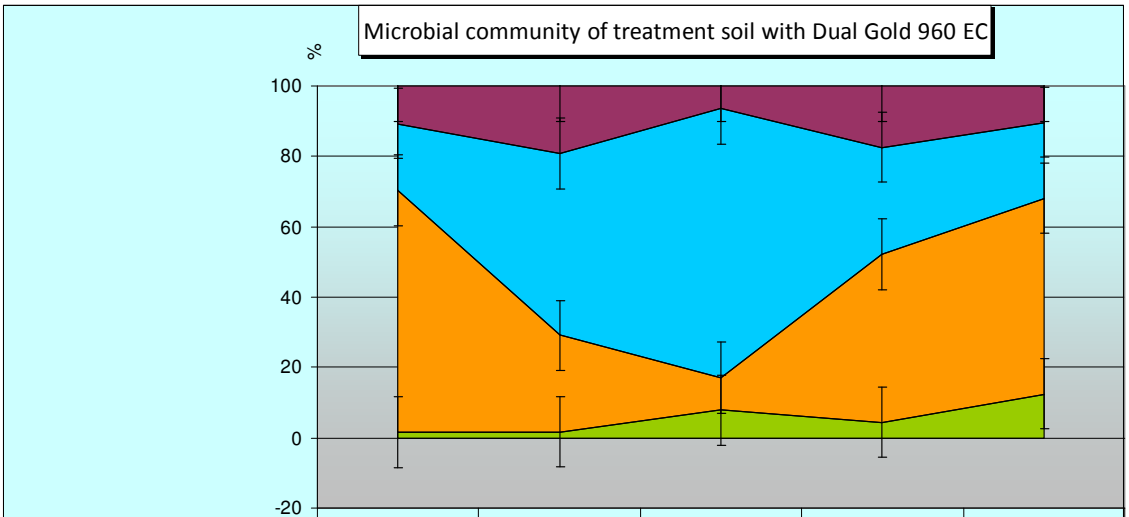
Figure 4. Percentage distribution of the trophic groups microorganisms in microbial community of treatment soil with herbicide Benefin – dinitroaniline

The changes in the microbial communities under the impact of the amide herbicides are similar compared to the percentage share of the spores – around 13.00%. The changes of the ammonifying and the absorbing mineral nitrogen microorganisms are on average for the period around 40%. Significantly increases the share of actinomycetes after treatment with Butizan S – 10.17% and Dual Gold 960 EC – 5.58% (Fig. 5 and 6).



	0day	15 days	35days	50days	90days
Spore microorganisms	10,729	16,836	14,43	8,615	14,62
Assimilating mineral nitrogen microorganisms	18,982	51,737	64,834	42,226	27,155
Ammonifying microorganisms	68,737	24,881	19,526	16,983	48,862
Actinomycetes	1,552	6,546	1,21	32,176	9,364

Figure 5. Percentage distribution of the trophic groups microorganisms in microbial community of treatment soil with herbicide Butizan-S – amide



	0day	15 days	35days	50days	90days
Spore microorganisms	10,729	19,161	6,516	17,46	10,338
Assimilating mineral nitrogen microorganisms	18,982	51,685	76,401	30,459	21,591
Ammonifying microorganisms	68,737	27,449	9,217	47,733	55,653
Actinomycetes	1,552	1,705	7,867	4,348	12,419

Figure 6. Percentage distribution of the trophic groups microorganisms in microbial community of treatment soil with herbicide Dual Gold 960 EC – amide

The changes in the density of the different trophic groups over time, as well as the changes in their distribution in the microbial communities, suggests, that the initial stages of biodegradation of dinitroaniline herbicides is carried out by the absorbing mineral nitrogen microorganisms. The initial stages of biodegradation of amide herbicides are carried out by the actinomycetes.

Conclusion

The studied dinitroanilinie and amide herbicides change the percentage distribution of the different trophic groups of microorganisms in microbial communities and lead to the deterioration of life conditions in the soil. With more prolongled negative effect are the herbicides Dual Gold 960 EC and Benefin;

Dinitroaniline herbicides have an inhibiting effect on the quantitative development of actinomycetes, while the amide herbicides – a stimulating one;

The likely period for adaptation of microbial communities after treatment is about 50 days for dinitroaniline herbicides, 15 days for Butizan S, and 35 days for Dual Gold 960 EC;

The trends of changes in the population density in different trophic groups of microorganisms and in the microbial communities in general, indicates that probably the initial stages of biodegradation of dinitroaniline herbicides are carried out by the absorbing mineral nitrogen microorganisms. The initial stages of biodegradation of amide herbicides are carried out by the actinomycetes.

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