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CONDITION OF SOIL MICROBIAL COMMUNITIES WHEN EXPOSED TO SOME CHLOROACETAMIDE HERBICIDES

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Abstract

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In the process of tobacco growing, during field tests, scientists studied the impact of chloroacetamide herbicides Butizan S (a.s. *metazachlore*) and Dual Gold 960 EK (a.s. *S-metolachlor*) on soil microbial communities. They determined dynamically the population density of trophic groups of microorganisms, which are indicators for the general sanitary condition of the soil. A suppression in the numerical development of ammonifying and immobilizing mineral nitrogen microorganisms was found. This was achieved without disturbing the mineral nutrition of tobacco plants. An increase was recorded in the spores and in the relative proportion of spore-like microorganisms, which indicates degraded life conditions in the soil. *Metolachlor* has a negative impact with a longer duration. The likely period of adaptation in microbial communities after treatment is about 15 days for Butizan S (*a.s. metazachlore*) and 35 days for Dual Gold 960 EK (*a.s. S-metolachlor*). The stimulation effect on the group of *Actinomycetes* and the changes in their dynamics defines them as the first biodegrading agents in both herbicides, followed by the ammonifying microorganisms.

Keywords: herbicides, *metazachlore*, *metolachlore*, soil microorganisms, *Actinomycetes*

Introduction

Application of herbicides to combat weed infestation continues to be a highly effective and irreplaceable measure even in modern agriculture (Dimeska et al., 2003). The accumulated knowledge on the state of the environment, the global pollution and the effects of intensive use of chemicals, require a rethinking of the way they are evaluated. In addition to reporting on their biological and economic efficiency, it is necessary to expand and refine the criteria for their effects on the environment (Zaharenko, 2000; Mannio et al., 2007; Kulikova and Lebedeva, 2010). This is particularly relevant for the use of soil herbicides, in order to preserve the soil as the main natural body and basic medium for production (Alexander, 1991; Smetnik et al., 2005).

The soil microorganisms are necessary for the existence and the functioning of the soil as a complex ecosystem. The soil fertility and its regenerative capacity depend on their performance. They are crucial to the biodegradation and detoxification of pesticides. Studies in this area date back to the mid-60s of the last century, but their relevance is increasing nowadays (Mishustin et al., 1964; Sokolov and Galiulin, 1987; Polyanskaya and Zvyagintsev, 2005). The behavior of pesticide in soil is influenced by many factors and has various aspects. The rate of degradation depends on the chemical composition of the preparations, the conditions of the soil (organic matter content, moisture, temperature, etc.) and primarily on the biological activity of the soil (Khan, 1978; Voynova et al., 1983; Naumova, 1999).

All trophic groups of soil microorganisms take part in the process of biodegradation. They use the pesticides as a source of energy and nutrients in the different stages of their transformation. Oxidative and reduction processes take place, carried out by the enzymes, secreted by microorganisms (Dec and Bollag, 2001; Colla et al., 2008). It has been found that products with a cyclic structure are more difficult to break down. With the increase of the number of atoms in the molecule of herbicides derived from the same substance, the scientists

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observe an increase of the toxicity as well (Holodov et al., 2005). A bacterial degradation of choloroacetamide products has been reported (Liu et al., 1989, 1991; Laue et al., 1996). Usually, before the degradation process, a period of adaptation of the microbial communities is required, after which the population number of the microorganisms that start the degradation increases sharply. In a later phase the quantities can decrease again or stabilize at a relatively constant level. According to some authors, the adaptation of microorganisms to a certain herbicide leads to increased decomposition rates at its next use (Prather et al., 2000; Hager and Refsell, 2008).

Different groups of microorganisms respond differently to a given herbicide. Non-spore-forming bacteria are considered the most resistant microorganisms to the toxic effects of herbicides, followed by spore bacteria and actinomycetes. The most susceptible are the microscopic fungi, the nitrifying bacteria, the nitrogen-fixing bacteria and others (Mishustin et al., 1964; Pothuluri, 1997). The prolonged inhibition in numbers is an indicator of a delayed degradation or the presence of toxic metabolites.

In modern ecological research scientists increasingly include microbiological indicators as permanent criteria in the evaluation and assessment of the environmental risk in the application of pesticides.

The aim of this study is to determine the impact of choroacetamide herbicides Butizan S (a.s. *metazachlore*) and Dual Gold 960 EK (a.s. *S-metolachlor*) on the population density of trophic groups of microorganisms, which are indicative for the general sanitary condition of the soil.

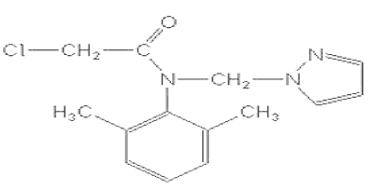
Materials and Methods

Description of the included in the study chloroacetamide herbicides

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

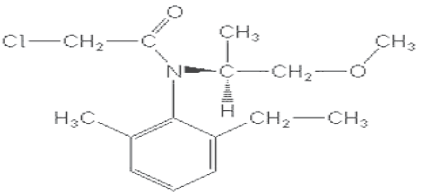
The mechanism of action of *metazachlore* is based on changes in the composition of fatty acids, the disruption of protein synthesis and the cell membrane permeability of embryos of susceptible weeds.

metazachlor



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

S-metolachlor



The *metoalachlor* inhibits the growth of susceptible weeds in the early stages of germination by affecting cell division through blocking mitosis.

Both herbicides are used widely for control of annual cereal and some dicotyledonous weeds. Their period of decay in the soil ranges from 25 to over 130 days, depending on the conditions. The major degradation byproducts, which have been identified in the soil, are compounds of metazachlor and metolachlor oxalic and sulfonic acids (Mathew et al., 1996; Hladik et al., 2006).

Formulation and analysis

Field tests have been made in a two-year period for the cultivation of Oriental tobacco on humus-carbonate soils (rendzinas) with the following agrochemical characteristics: total humus content (in Turin) - 2.68%, total nitrogen (Kjeldahl) – 0.154%, mobile phosphorus (in Egner-Riem) – 16.90 mg/100 g soil, assimilable potassium (on Miltcheva) – 36.5 mg/100 g. Soil reaction is mildly alkaline – pH (in H_2O) 7.8. The herbicides have been introduced before the planting of the tobacco with 20 liter of working solution per da. The following options have been set:

- Untreated control field
- Butizan S (a.s. 500g/l *metazachlore*) at a dose of 150 ml/da
- Dual Gold 960 EK (a.s. 960g/l *S-metolachlor*) at a dose of 150 ml/da

Soil samples for microbiological analyzes were taken from the soil layer 0 - 20 cm in the rhizosphere zone of tobacco plants, in the following dynamics – prior to treatment (0 days), 15, 35, 50 and 90 days after herbicide application.

Analyzes were made by the method of Koch, by introducing dilute soil suspensions on nutrient medias, specific for trophic groups of microorganisms. The amount is recorded after a certain period of incubation and is assessed as the most probable number of cells (MPN) in 1 g of absolutely dry soil (a.d.s.) (Koleshko, 1981). The following microbiological indicators are selected for the purpose of the study:

- Ammonifying microorganisms – on meat-peptone agar

- Immobilizing (assimilating) mineral nitrogen microorganisms – on starch-ammonia agar
- Mineralization-immobilization index – calculated as the ratio between assimilating mineral nitrogen microorganisms and ammonifying microorganisms
- Soil *Actinomycetes* – on starch-ammonia agar
- Spore microorganisms – measured on meat-peptone agar, after pasteurization of the soil suspension
- Ration of spores - (%) of ammonifying microorganisms.

The data is subjected to a single-factor dispersion analysis. For each trophic group of microorganisms are identified: force of impact (ηx^2) of the herbicide, as well as the confidence level (p), according to the Fisher (F) criterion. A correlation analysis is made between the quantities of actinomycetes and ammonifying microorganisms in microbial communities. A correlation coefficient (r) and its reliability are determined using the criteria of Student (t) (Plohinskiy, 1980). The presented data is average for the two-year period of the study.

Results and Considerations

The numerical growth of microorganisms in both trophic groups, which carry out the main transformations of nitrogen compounds in the soil – ammonifying microorganisms and immobilizing the mineral nitrogen microorganisms, is inhibited by the test herbicides. The reported values are generally lower than those in the untreated soil. In ammonifying microorganisms, without statistically proven difference with the control sample, they are in the range of tens and hundreds of millions. For the whole period the average reduction in Butizan S is 85.394x10^5/g a.d.s. ($F_{exp} = 2.527 < F_{tab} = 4.41$), and in Dual Gold – 146.203x10^5/g a.d.s. ($F_{exp} = 2.947 < F_{tab} = 4.41$ at p = 0.95). The adaptation period is about 15 days at Butizan S and 35 days at Dual Gold. After the 50th day the values are lower, but gradually approach those, recorded in the control sample (Figure 1).

The inhibitory effect of the herbicides on the immobilizing the mineral nitrogen microorganisms is more pronounced. The differences in density for the entire period, for both herbicides, compared to the control sample are without being proven statistically, in the range of hundreds of millions (for Butizan S $F_{exp} = 1.861$, for Dual Gold $F_{exp} = 1.027 <$ Ftab $= 4.41$ at p = 0.95). Dual Gold has a stronger and more lasting negative impact. In the case of Butizan S, about 35-50 days after treatment, some stimulation in the numerical growth of microorganisms in this group has been observed (Figure 2).

In the structure of microbial communities, these two physiological groups are in a biological equilibrium. Their ratio is crucial for the availability of ammonium and nitrate ions, which are absorbed by plants. The values of the mineralization-immobilization index are illustrative. The values, accepted as favorable for plant nutrition, are around one (Koleshko, 1981). The results of this study show higher values (about 2.2) in the period 15-50 days and in the untreated soil. The introduction of herbicides does not have substantial impact on this indicator. Significant increase is reported on day 15 in the case of Butizan S – 4.70 and on day 35 in the case of Dual Gold – 8.01. This does not impair the mineral nutrition of tobacco plants, because in the period 50-90 days, which coincides with the phases of active growth, the values are favorable and in most cases lower than those in the control sample (Figure 3). The changes have been demonstrated in confidence level p = 0.99 for Butizan S - $\eta x^2 = 0.885$ (at $F_{exp} = 8.921 > F_{tab} = 8.28$) and Dual Gold with confidence p = 0.95, $\eta x^2 = 0.528$ (at $F_{exp} = 6.565 > F_{tab} = 4.41$).

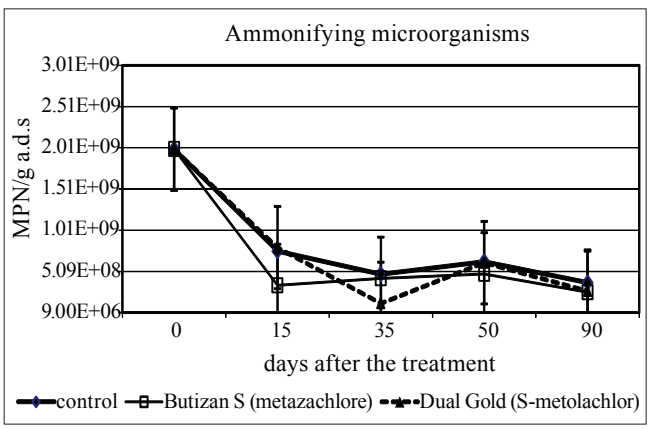


Fig. 1. Changes in population density of Ammonifying microorganisms (MPN/g a.d.s.) in dynamics

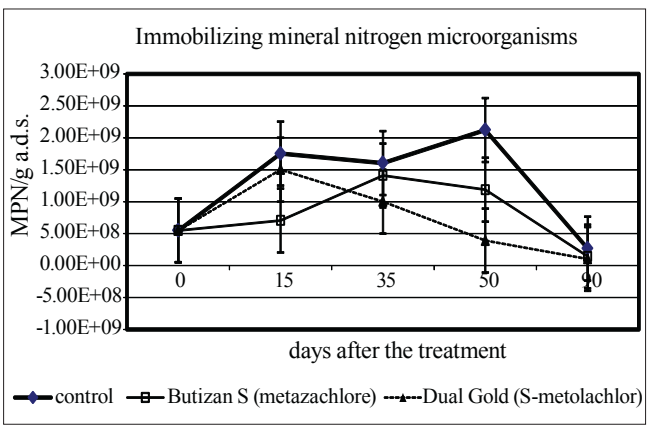


Fig. 2. Changes in population density of Immobilizing mineral-Nitrogen microorganisms (MPN/g a.d.s.) in dynamics

The group of soil *Actinomycetes* is considered one of the sensitive indicators for the environmental condition of the soil (Alexsander, 1991). The decrease in their density is an indicator for the presence of toxic substances in the soil. At the same time the actinomycetes possess a powerful enzyme preparation and produce many biologically active substances, which make them active participants in the mineralization of the organic matter in the soil and in the biodegradation of many pesticides.

Under the impact of the tested herbicides the changes in the population density of actynomycetes have similar trend, which changes in time. Butizan S stimulates a rise in the quantities around day 15, than the numbers decrease rapidly and around day 50 they rise again. At the end of the period the values are close to those in the untreated soil. In the case of Dual Gold the strong stimulation comes later – around day 35, followed by a sharp decrease on day 50 and afterwards the quantities rise again to values, similar to those of the control sample (Figure 4). The average number of actinmycetes for the entire period, in the presence of herbicides, is higher than those established in the control sample. In the case of Butizan S the increase is in the range of 7.67×10^5, and in Dual Gold – 10.38×10^5. The differences are statistically proven. The power of the impact in treatment with Butizan S is $\eta x^2 = 0.724$ at confidence level p = 0.95 ($F_{exp} = 7.978 > F_{tab} = 4.41$). Dual Gold influences by force $\eta x^2 = 0.927$ at confidence level p = 0.99 ($F_{exp} = 9.138 > F_{tab} = 8.28$). The stimulation period of actinomycetes in both herbicides coincides with the period of adaptation, reported in the group of ammonifying microorganisms. It is possible, that the actinomycetes are the first to begin the biodegradation of herbicides, followed by the ammonifying microorganisms.

The correlation analysis between the quantities of actinomycetes and ammonifying microorganisms indicates a weak negative, statistically unproven correlation in the control sample ($r = -0.120$ $t_{exp} = -0.273 < t_{tab} = 2.571$) and in Butizan S ($r = -0.182$ $t_{exp} = -0.420 < t_{tab} = 2.571$). In the sample, treated with Dual Gold the correlation between the quantities in both groups of microorganisms is average in power, negative, statistically proven at confidence level p = 0.95 ($r = -0.665$ $t_{exp} = -2.669 < t_{tab} = 2.571$).

The tested herbicides stimulate as a whole the numeric development of spore microorganisms. The average increase compared to an untreated soil is in the range of tens of millions. The differences are demonstrated at a level p = 0.95. The power of impact of Butizan S is $\eta x^2 = 0.413$ ($F_{exp} = 5.549 > F_{tab} = 4.41$), and that of Dual Gold is $\eta x^2 = 0.305$ ($F_{exp} = 4.445 > F_{tab} = 4.41$). The stimulating effect of Butizan S occurs after day 15 from the treatment and is especially strong in the period between day 35 and day 50. In the case of Dual Gold an increase is observed as early as day 15, around day 35 the numbers sharply decrease and afterwards they rise again and remain high until the end of the period (Figure 5). The observed quantitative dynamic of spore microorganisms is proportional to the dynamic of the ammonifying microorganisms and inversely proportional to the dynamic of actinomycetes. In the untreated control sample the dynamic of all surveyed microorganisms is proportional. The data support the likelihood that actinomycetes carry out the initial stages of the degradation of both herbicides.

The increase in density of spore microorganisms during herbicide treatment also affects the percentage increase in the proportion of spores from ammonifying microorganisms. The values are high until the end of the period (Figure 6).

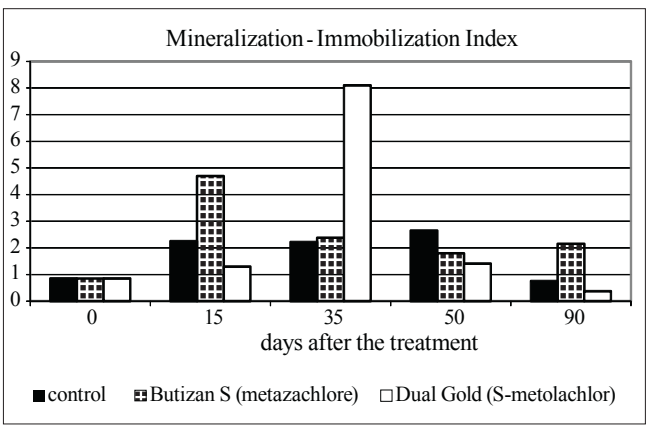


Fig. 3. Changes in the values of Immobilization-Mineralization Index in dynamics

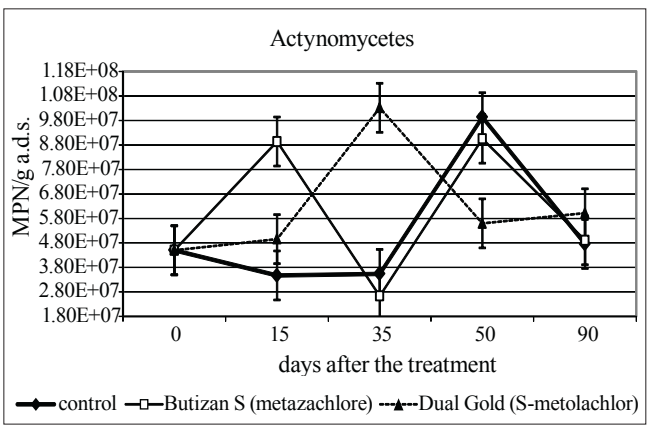


Fig. 4. Changes in population density of *Actinomycetes* (MPN/g a.d.s.) in dynamics

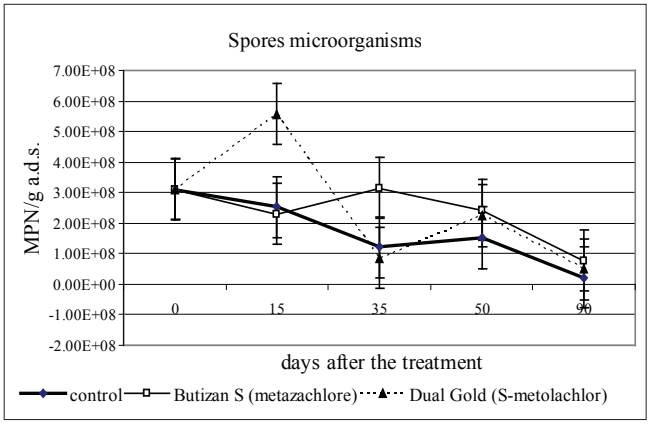


Fig. 5. Changes in population density of Spores microorganisms (MPN/g a.d.s.) in dynamics

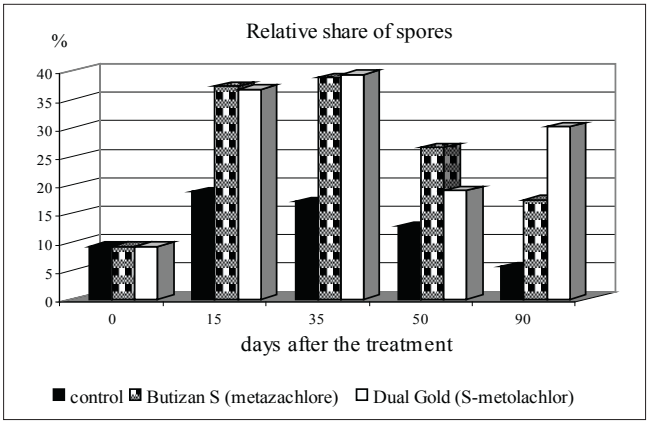


Fig. 6. Changes in the relative share of spores (%) in dynamics

The differences with the control sample are statistically proven at confidence level p = 0.95. The power of the impact in Butizan S is $\eta x^2 = 0.519$ ($F_{exp} = 6.492 > F_{tab} = 4.41$), while in Dual Gold - $\eta x^2 = 0.499$ ($F_{exp} = 6.326 > F_{tab} = 4.41$).

The data shows a deterioration of living conditions in the soil in the presence of herbicides with the negative impact being longer in the case of treatment with the herbicide Dual Gold 960 EC (a.s. *S-metolachlor*). Such changes in the dynamics of the soil microflora have been observed under the impact of some acetochlor herbicides, which also belong to the group of chloracetamides (Hristeva & Kalinova, 2002).

Conclusions

The chloracetamide herbicides Butizan S (a.s. *metazachlore*) and Dual Gold 960 EC (a.s. *S-metolachlor*) suppress the numerical development of ammonifying and immobilizing mineral nitrogen microorganisms, without disturbing the mineral nutrition of plants.

The herbicides increase the population density of spore microorganisms and the relative proportion of spores in microbial communities, which is an indicator of deteriorating living conditions in the soil. The herbicide Dual Gold 960 EC (a.s. *S-metolachlor*) has a longer negative impact.

The effect of herbicides on the group of *Actinomycetes* as a whole, is stimulating, however the changes in its dynamics in time are mixed. The outlined trends set the actinomycetes as the initial biodegradators of both herbicides, followed by the ammonifying microorganisms.

The data indicates, that the most likely period of adaptation of the studied trophic groups of microorganisms is around 15 days after treatment with Butizan S (a.s. *metazachlore*) and 35 days in Dual Gold 960 EC (a.s. *S-metolachlor*).

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