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THE INFLUENCE OF S-TRIAZINE AND UREA HERBICIDES ON THE DEVELOPMENT OF SOIL MICROORGANISMS IN VARIOUS TYPES OF SOIL¹

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INTRODUCTION

Atrazine and linuron are soil-applied herbicides widely used in agricultural practice. There are readily adsorbed and persistent in soil systems. Thus they remain present for long periods in the surface layers of the soils, where there is also the greatest density of microorganisms, and hence these chemicals are more likely to affect microbial populations and their activity than many other herbicides.

The influence of as-triazine and urea herbicides on soil microflora have been subject of extensive investigations [4, 12, 13, 14, 19, 23, 24, 25, 28, 31, 32, 33, 34]. Despite many years of studies it is difficult to identify effect of these herbicides on soil microorganisms. This effect is not fixed with property of the herbicide but it is influenced by factors such as physicochemical soil characteristic, the biological situation in the soil, climatic influences and agricultural practices [8, 10, 16]. Since these factors vary from site to site and from year to year, the results from field studies of herbicide effects are specific to one particular location and season [15]. Results from field trials may be difficult to interpret, because the microflora or its activity may respond mainly to changes in vegetation rather than to direct herbicide effects [11].

Detailed information on methods and effects of herbicides on soil microflora can be found in reports of Anderson [1] and others [2, 7, 8, 10, 17, 18, 26].

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The aim of the present study was to examine in laboratory experiments the influence of atrazine and linuron on the development of microorganisms in various types of soils.

MATERIALS AND METHODS

The soil used: the samples from surface (5—15 cm deep) of five different types of soil were used in our experiments. The characteristic of these soils are presented in Table 1.

Table 1

Some physical and chemical properties of the soils examined

Type of soil	% of the mechanical fraction /% in mm/			Total water capillary hold. capacity. ml/100 g	pH in H ₂ O	N	C	Organic matter
	1.0-0.1	0.1-0.02	< 0.02					
Pseudopodsolic soil developed from slightly loamy sand	65	10	5	32.45	6.2	0.051	0.97	1.67
Pseudopodsolic soil developed from light loam	52	27	21	35.44	6.5	0.081	0.76	1.34
Brown proper soil developed from loess	53	28	19	42.21	6.5	0.077	0.47	0.81
Alluvial soil developed from medium loam	7	46	47	44.57	6.7	0.113	0.95	1.64
Black earth developed from heavy loam	20	16	64	61.01	7.7	0.469	4.54	7.03

In order to study the effect of linuron and atrazine on development of soil microflora, the following series were set up with each soil: 1 — soil without herbicide — control, 2 — soil + 10 ppm of linuron, 3 — soil + 100 ppm of linuron, 4 — soil + 10 ppm of atrazine, 5 — soil + 100 ppm of atrazine. Soil samples were incubated in plastic pots covered with aluminium foil at room temperature $\pm 20^{\circ}\text{C}$. Soil humidity was kept constant at 50% of the total water capillary holding capacity. Experiments were carried out for 4 month.

Microbial and biochemical analyses: after 0, 7, 14, 28, 60, 90 and 120 days of soil incubation following determinations were performed: total numbers of bacteria and actinomycetes by plate method on medium containing soil extract and K_2HPO_4 , total number of fungi on Martin's medium, microorganisms decomposing urea on „urea agar base oxoid medium", Azobacter numbers on free nitrogen medium by Fenglerowa's method, cellulolytic microorganisms on mineral agar medium with discs of Wathman filter paper, by Winogradski's method, the most probable number of nitrifiers by Pochon's method, oxygen uptake measured by Warburg's technique (five gram of soil samples enriched with glucose —

0.2% were placed in Warburg's flask for a period 20 hours before proceeding) and the activity of dehydrogenase by Casida, Klein and Santoro's method.

Results of these analyses are presented in each combination, as numbers and percent of control value average of seven individual determinations (Fig. 1—9).

RESULTS AND DISCUSSION

The soil used in our studies differed in the respect to physical and chemical properties (Table 1) and to numbers of microorganisms (Fig. 1A—7A) and their activity (Fig. 8A—9A).

The development of microorganisms in studied soils to which herbicide at 10 or 100 ppm was added varied greatly, depending on the soil types, the kind and amount of herbicide, on the group of microorganisms and the level of their number in soils (Fig. 1B—7B). Atrazine and linuron inhibited or stimulated periodically the growth of microorganisms. It is difficult to generalize these results.

Many publications have reported both the stimulation and reduction in microbial populations at various range of herbicide concentrations [12, 25, 32]. Some authors demonstrated that microbial groups responded differently to these chemicals [18, 25].

The average total numbers of bacteria and actinomycetes ranged from 16 millions/g of pseudopodsolic soil developed from slightly loamy

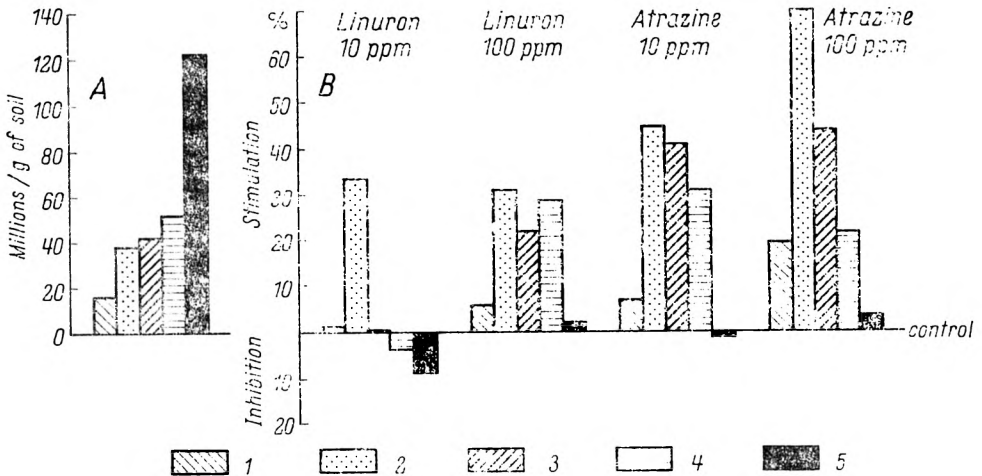


Fig. 1. Total numbers of bacteria and actinomycetes in various types of soil

A — average numbers in control soils, B — influence of herbicides — stimulation or inhibition per cent of control; soils: 1 — pseudopodsolic soil developed from slightly loamy sand, 2 — pseudopodsolic soil developed from light loam, 3 — alluvial soil from medium loam, 4 — brown proper soil developed from loess, 5 — black earth developed from heavy loam

sand to 121 millions per gram of black earth developed from heavy loam (Fig. 1A). Addition to soils 10 or 100 ppm of atrazine or linuron stimulated the development of these microorganisms. Atrazine had greater stimulatory effect than linuron (Fig. 1B).

Atrazine and linuron had highly variable effects on the soil fungi (Fig. 2B). Atrazine, especially used at higher concentration, increased their number, while linuron had inhibitory effect. Some negative action after the application of higher doses of substituted urea were observed also by Grossbard and Marsh [12], while chloro-s-triazine stimulated of the fungal population in many studies [26, 28, 37]. Some of herbicides used in normal doses, often did not modify the soil fungal population [6, 34].

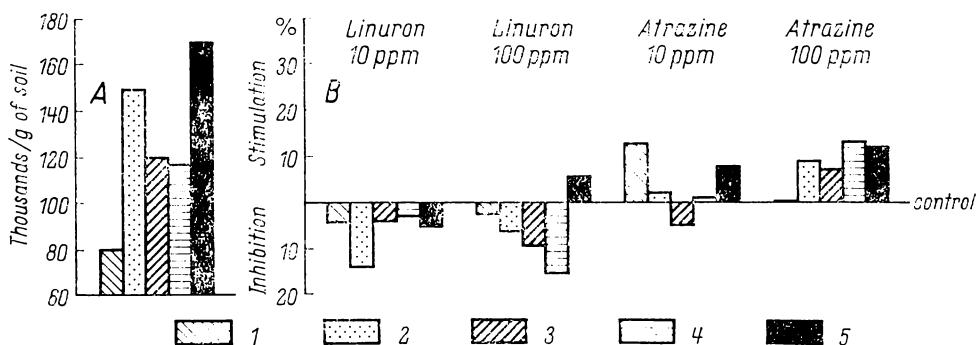


Fig. 2. Total numbers of fungi in various types of soil explanations — see Fig. 1

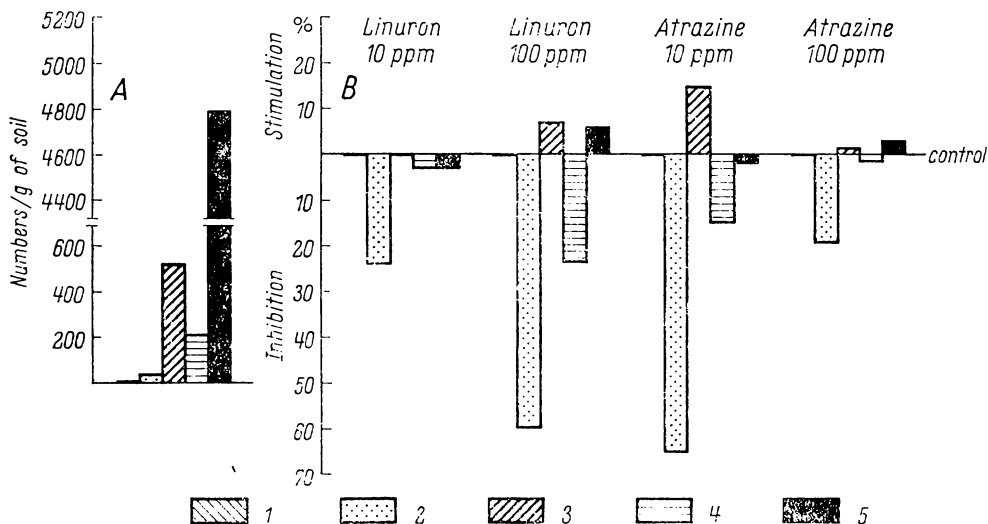


Fig. 3. Numbers of *Azotobacter* in various types of soil explanations — see Fig. 1

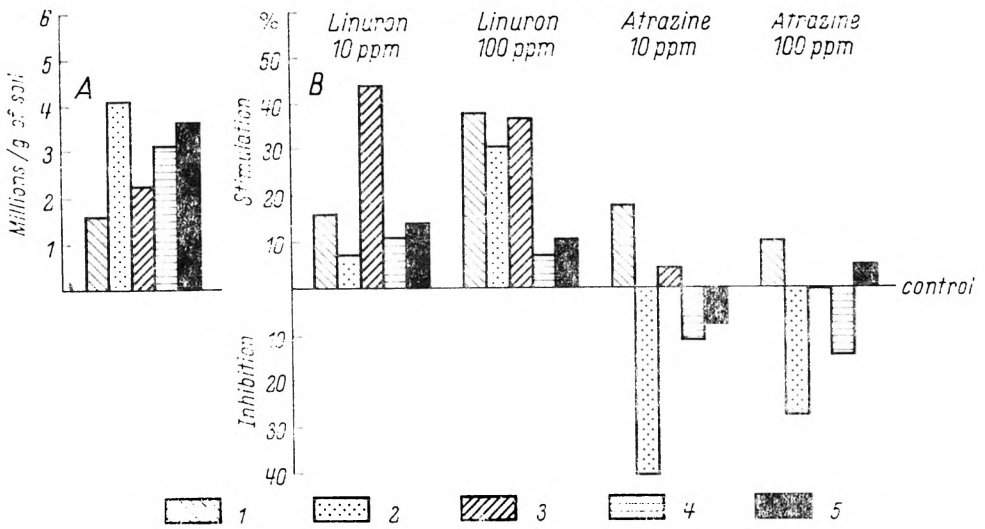


Fig. 4. Total numbers of urea bacteria in various types of soil explanations — see Fig. 1

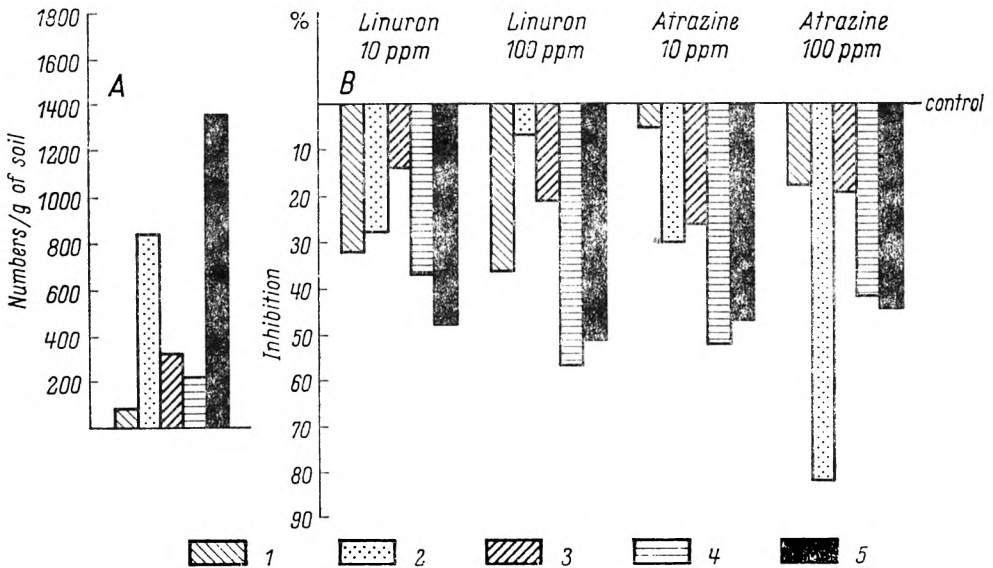


Fig. 5. Total numbers of nitrifying bacteria in various types of soil explanations — see Fig. 1

The number of Azotobacter in studied soils varies greatly depending on the soil types (Fig. 3A). In the soil developed from slightly loamy sand Azotobacter did not occur. In other soils from about 40 Azotobacter cells per gram (posolic soil) to 4800 cells per gram (black earth) was found (Fig. 3A). In soils containing a little number Azotobacter cells, the

herbicides influenced harmfully on this bacteria. In remaining soils, herbicides had little effect on number of *Azotobacter* (Fig. 3B). The results of studies on this subject are often controversial, some authors indicate favourable, other unfavourable action of herbicides [29, 30].

The study of the action of herbicides on the development of urea decomposing bacteria indicated, that soil treated with linuron contained much greater average number of these microorganisms than control soils (Fig. 4B). Atrazine added to studied soils inhibited growth of urea decomposing bacteria, especially in light loam and in medium loam. It is possible that urea herbicides may select the bacteria able to hydrolyze of urea bonds.

Among the investigated microorganisms, nitrifying and cellulolytic bacteria were the most susceptible to the presence of atrazine and linuron in the soils. The herbicides inhibited significantly the number of these microorganisms in all soils (Fig. 5A, 6A). The effect of herbicides on the nitrifying bacteria depended mainly on the soil properties (Fig. 5B).

Many of the authors [3, 12, 23, 33, 34, 35] reported about some influence of s-triazine and urea herbicides on nitrification activity or on the number of nitrifying bacteria. The results of these studies are controversial, some indicate no effect, other favourable or unfavourable effect. Bartha et al. [3] have reported that atrazine is an effective inhibitor of nitrification. Greaves et al [7] stated that nitrifying microorganisms are extremely sensitive to environmental changes. According to our studies and to Naidu [22] these nitrogen transformation is probably the best indicator of the detrimental effect of a herbicide on the biological equilibrium in soil. Furthermore, nitrification appear to have more direct relevance to practical agriculture, than many of the processes investigated in studies of the effect of herbicides on soil microorganisms.

Cellulolytic organisms are important microorganisms contributing to the humification processes in soils. The effect of herbicides on this process have been studied mainly by direct observations of changes in populations of cellulolytic organisms, and also by following decomposition of cellulosic substrated buried in treated soils [2, 12, 21, 28].

Composition of microbial population, taking part in cellulose decomposition in studied soils, indicate that there were mainly bacteria from *Cytophaga* genera, and in some soils little number of bacteria from *Celvibrio* sp.. The fungi were represented by not numerous *Humicola* and *Stachybotris* sp.. There were also some *Actinomyces*. The herbicides caused the change in composition of cellulolytic microflora. In all soils treated with herbicides the number of cellulolytic bacteria strong decreased (Fig. 6B), while the number of *Actinomyces* and fungi increased (Fig. 7B). The greatest increasing the number of *Actinomyces* was ob-

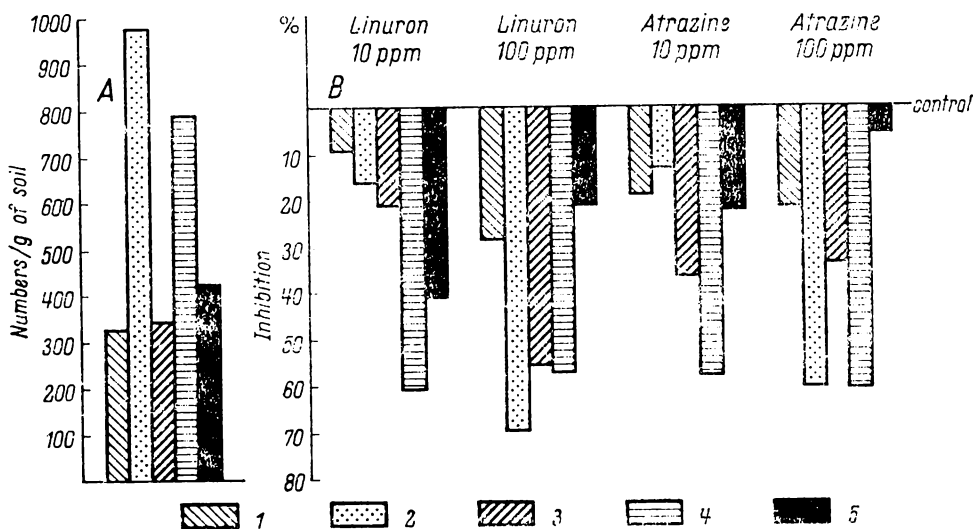


Fig. 6. Total number of cellulolytic bacteria in various types of soil explanations — see Fig. 1

served in black earth and in loess, the least in light soil (Fig. 7B). *Cytophaga hutchinsoni* was more resistant to the action of herbicides, than *Cotophaga tenuissima*.

Microbiological studies on the microorganisms active in carbon cycle, especially on the cellulolytic organisms are relatively limited. The study on the action of herbicides on cellulolytic bacteria, carried out by Grossbard and Marsh [12] and others showed that atrazine and linuron, at doses higher than used in agriculture practice, had inhibitory effect.

The effect of herbicides on microbial activity of soils was determined by oxygen uptake measurement as well [5, 9, 11, 12, 20].

In our study the oxygen uptake depended on the type of soil (Fig. 8A), kind and doses of herbicide and time of incubation (Fig. 8B). Results of analyses presented as average per cent of stimulation or inhibition of oxygen uptake during seven individual determinations showed that herbicides had stimulatory effect on this process (Fig. 8B). However atrazine had greater stimulatory effect than linuron. Respiration studies can give a reasonable indication of the response of the soil microbial populations to pesticide treatment, although problems in interpretation can arise [9, 11]. The results of Grossbard and Marsh [12] showed that soil treated with 500 ppm linuron caused a consistent and significant depression in CO_2 output and O_2 uptake. Addition of 5 and 50 ppm had no significant effect. By contrast, stimulating effect of linuron in concentration up to $20 \mu\text{g/ml}$, on O_2 uptake was observed by Rostlycky [24].

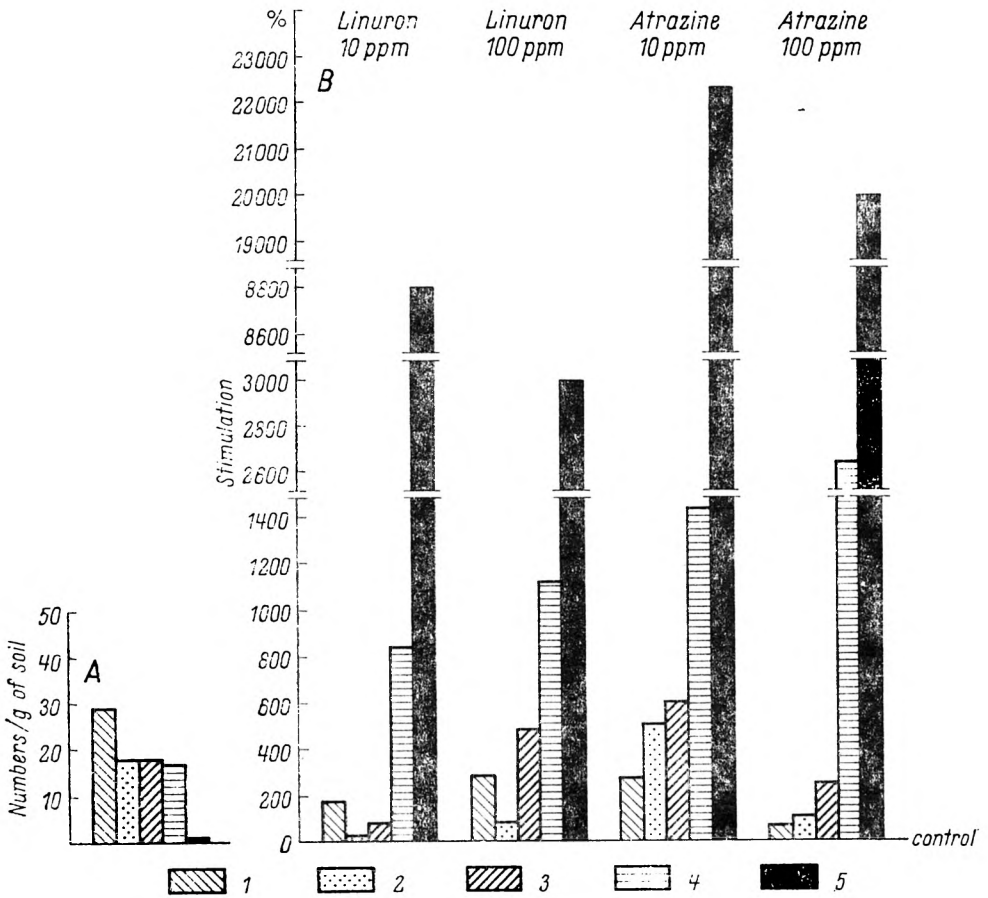


Fig. 7. Total number of cellulolytic fungi and actinomycetes in various types of soil explanations — see Fig. 1

The enzyme assay had shown that herbicides had no significant effect on dehydrogenase activity in studies soils. Linuron, especially in higher concentrations inhibited this enzyme activity, while 10 ppm of atrazine stimulated it (Fig. 9B). Moreover it is necessary to say, that measurement of dehydrogenase activity as an index of effect of herbicides on microbial activity in black earth is difficulty for interpretation, because this soil adsorbed strongly both herbicides and tetrazolium (Fig. 9). The study of soil enzymes has been one main areas of interest in soil biochemistry during last years, and a number of correlations between microbial activity have been found [27]. Yeast et al. [35] have shown no significant effect of herbicides on dehydrogenase activity. According to Tag El-Din et al (30) atrazine caused 50% inhibition of soil dehydrogenase.

The results of studies allow to draw the following conclusions:

1. Atrazine and linuron added to soil at 10 or 100 ppm affect the

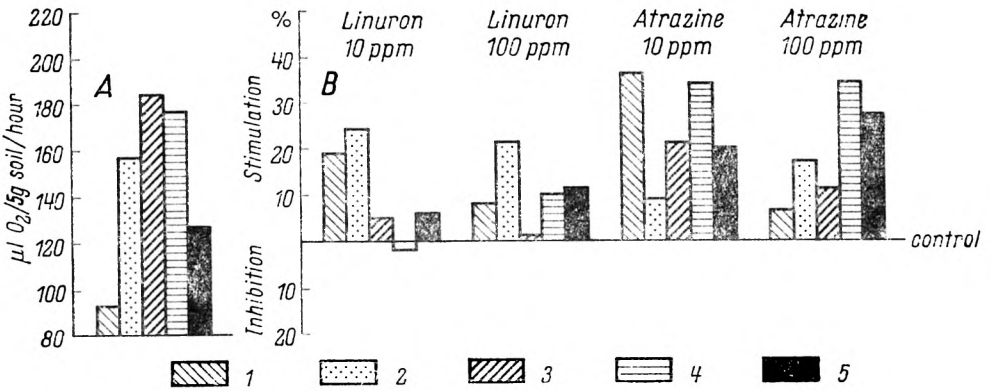


Fig. 8. Oxygen adsorption by various types of soil explanations — see Fig. 1

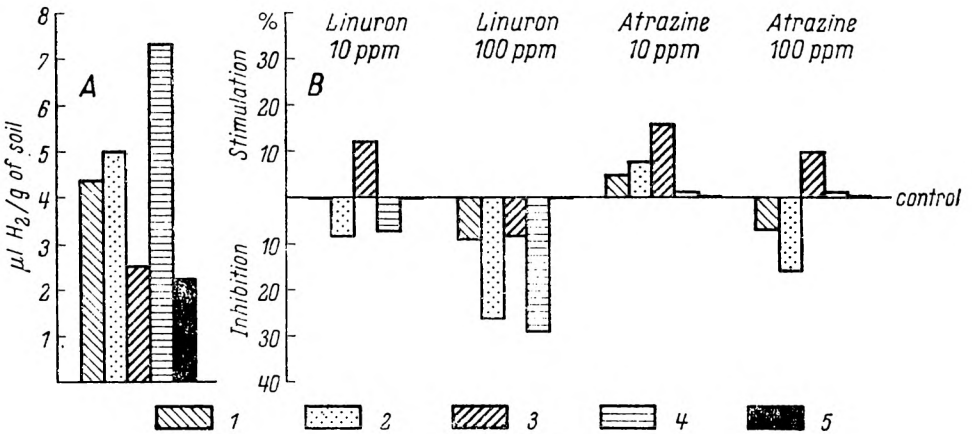


Fig. 9. Dehydrogenase activity in various types of soil explanations — see Fig. 1

development and selection of soil microorganisms, amount O_2 uptake by soil samples and dehydrogenase activity.

2. The influence of herbicides depends on their kind and doses, on the groups of microorganisms, and on the soil type.

3. Among the investigated microorganisms nitrifying and cellulosytic bacteria proved to be the most susceptible to the presence of atrazine and linuron in the soils. The most resistant to this herbicides were the soil fungi.

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A. СТЩЕЛЕЦ, Я. КОБУС, Я. ЧАБАН

ВЛИЯНИЕ С-ТРИАЗИНОВЫХ И МОЧЕВИННЫХ ГЕРБИЦИДОВ НА РАЗВИТИЕ
МИКРОФЛОРЫ В РАЗНЫХ ТИПАХ ПОЧВ

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Резюме

В лабораторном опыте исследовали влияние атразина и линурона (10 и 100 ppm) на развитие микрофлоры в пяти почвах с разными физико-химическими свойствами и с разной биологической активностью. Контроль составляли почвы не обработанные гербицидами. Результаты представлены как средние для анализов проводимых через 0, 7, 14, 28, 60, 90 и 120 дней инкубации почв.

Влияние гербицидов на развитие и активность микрофлоры зависело от свойства исследуемых почв, вида и концентрации внесенных гербицидов, вида микроорганизмов и их численности в данной почве.

В почвах обработанных гербицидами среднее число бактерий и актиномицетов было выше, чем в контрольных почвах. Атразин стимулировал развитие этой группы микроорганизмов, а линурон задерживал его.

Численность азотобактера не показывала существенных изменений под влиянием гербицидов. Исключение составляла только почва с наименьшей численностью этих бактерий. В указанной почве гербицид четко снижал их численность.

Внесение в исследуемые почвы линурона приводило к более сильному повышению содержания в них целлюлолитических бактерий.

Среди исследуемых микроорганизмов наиболее восприимчивыми к гербицидам оказались нитрификаторы и целлюлолитические бактерии. Во всех почвах гербициды четко задерживали их развитие. В почвах обработанных гербицидами изменялся состав целлюлитической микрофлоры.

Гербициды, в частности атразин, повышали количество усваиваемого почвами азота. Они, однако, не оказывали существенного влияния на активность дегидрогеназы.

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WPŁYW HERBICYDÓW S-TRIAZYNOWYCH I MOCZNIKOWYCH NA ROZWÓJ
MIKROFLORY W RÓŻNYCH TYPAH GLEB

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Streszczenie

W doświadczeniu laboratoryjnym badano wpływ atrazyny i linuronu (10 i 100 ppm) na rozwój mikroflory w pięciu glebach o różnych właściwościach fizykochemicznych i różnej aktywności biologicznej. Kontrolami były gleby nie traktowane herbicydem. Wyniki przedstawiono jako średnie z analiz przeprowadzonych po 0, 7, 14, 28, 60, 90 i 120 dniach inkubacji gleb.

Wpływ herbicydów na rozwój i aktywność mikroflory zależał od właściwości badanych gleb, rodzaju i koncentracji wprowadzonego do nich herbicydu, rodzaju drobnoustrojów i ich liczebności w danej glebie.

W glebach traktowanych herbicydami średnie liczebności bakterii i promienioców były wyższe niż w glebach kontrolnych. Atrazyna stymulowała rozwój

tej grupy drobnoustrojów w większym stopniu niż linuron. Wpływ herbicydów na ogólną liczebność grzybów glebowych był bardzo różny. Atrazyna stymulowała rozwój tej grupy drobnoustrojów, gdy tymczasem linuron hamował go.

Liczebność azotobaktera nie uległa pod wpływem herbicydów większym zmianom. Wyjątek stanowiła jedynie gleba o najmniejszej liczebności tych bakterii. W glebie tej herbicyd wyraźnie zmniejszał ich liczbę.

Wprowadzenie do badanych gleb linuronu powodowało silniejszy wzrost w nich bakterii urolitycznych.

Wśród badanych drobnoustrojów najwrażliwsze na herbicydy okazały się nityfikatory i bakterie celulolityczne. We wszystkich glebach herbicydy wyraźnie hamowały ich rozwój. W glebach traktowanych herbicydami ulegał zmianie skład mikroflory celulolitycznej.

Herbicydy, a w szczególności atrazyna, zwiększały ilość pobranego przez gleby tlenu. Nie miały one natomiast większego wpływu na aktywność dehydrogenaz.

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