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APPLICATION OF HERBICIDE 2,4-D BUTYL ETHER FOR THE IMPROVEMENT OF HILLY MEADOWS

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Herbicides are widely used in agriculture, but by now, there is no sufficient literature concerning the studies of complex effects of herbicides on hilly grasslands.

In the present work an attempt is made to find out the effect of herbicide 2,4-D butyl ether (2,4-DB) on microflora, fermentation activity of soil and chemical composition of plants.

Investigations were carried out in slightly skeletal subalpine hill-meadow soils in Caucasus at the altitude of 1900 m. Before the experiments the plots were intensive by grazed.

Grasses on the plots were in predominance; about a half of plants consisted of weeds, mostly *Veratrum Lobelianum* [1]. The plots with 2,4-DB were treated for a period of 3 years with the dose of 2 and 6 kg/ha.

RESULTS

After the treatment of 2,4-DB many species of weeds were either completely destroyed or considerably damaged, particularly *Veratrum Lobelianum* and *Cirsium* sp. L. One-year treatment reduced the yield of green matter, but the quality of fodder was improved due to the proportion of valuable grasses. In the third year at some decrease of productivity, the grassland was found free from weeds, particularly from poisonous *Veratrum*. The amount of grasses increased (Table 1). After 4 years of treatment with 2,4-DB in the dose of 2 and 6 kg/ha grass yield was reduced 2.6 or 12 times, respectively. But a part of grasses in the yield was improved by 1.3 and nearly 1.8 times.

The number of microorganisms in fresh and dry samples were de-

termined many times during 3 years. Generally, the soil characterized itself by a low number of microorganisms (Table 2). Bacteria were mostly represented by auxoautotrophic microorganisms, which consumed the mineral nitrogen of the Čapek medium with sucrose. The bacteria capable to use starch were less numerous and the auxoheterotrophic bacteria utilizing the organic nitrogen were found to be considerably fewer. Among the latter the spore-forming bacilli occurred abundantly, particularly many bacteria from the *Bacillus cereus group*. The least numerous were pigmented bacteria. Mycobacteria were moderately di-

Table 1

The effect of 2,4-D butyl ether on the productivity of grasslands

No. of treatments with 2,4-DB	Doses kg/ha	Dry matter q/ha	Decrease in yield %	Green matter				Weste		Bedding	
				Gramineus		Moltey grass		q/ha	%	q/ha	%
				q/ha	%	q/ha	%				
3	0	39.0	-	13.5	34.7	19.1	49.1	4.1	10.3	2.3	5.9
	2	30.0	23.1	18.0	60.0	8.1	26.9	2.0	6.7	1.9	6.4
	6	31.4	19.5	25.5	81.2	1.9	6.2	2.6	8.2	1.4	4.4
4	0	34.7	-	13.0	37.4	16.5	47.6	3.2	9.1	2.0	5.9
	2	27.9	19.6	17.0	61.1	6.4	22.8	2.8	10.0	1.7	6.1
	6	27.8	19.9	23.2	83.3	1.4	4.9	2.0	7.1	1.3	4.7

Table 2

Influence of 2,4-DB on the No. of microorganisms in subalpine meadow soils /in 1000 per gm of soil/

Treatment of the plots with herbicides	Doses kg/ha	Months	Meat-pepton agar		Starch-ammonium agar		Čapek medium	Malt extract agar
			Bacteria	Bac. mycoides	Bacteria	Actinomyces	Bacteria	Fungi
Without treatment	0	VII	192	28	367	44	1390	29
		IX	262	31	290	90	-	12
Treatment during 3 years	6	VII	219	28	710	241	2350	34
		IX	434	42	465	32	-	28
	2	VII	400	21	660	118	2800	16
		IX	439	42	1208	52	-	46
One time treatment	6	VII	255	26	670	176	3000	33
		IX	493	28	1510	-	-	69
	2	VII	283	35	508	99	1009	20
		IX	475	36	1244	52	-	-

stributed. Actinomyces were present in limited varieties with predominance of grey, white and sometimes brown and pink groups. Generic composition of the fungi association was very poor: *Penicillium simplicissimum, P. ochro-chloron, Mucor rammanianus* and *Zygorhynchus* sp., as predominant species. *Trichoderma* sp. was found to be the least numerous. The treatment with the herbicide did not cause any substantial change in the quantitative composition of bacteria, actinomyces and fungi associations, although a slight alternation was observed. This may be due to several factors: firstly, to the direct effect of herbicides on microorganisms and secondly to the effect of herbicides on the composition of phytocoenosis, which in turn may also affect the microflora. Just after the treatment with herbicide an increase of fresh organic matter might be observed as a result of damaging effect of herbicide on the plants.

In the post-treatment period some increase in auxoautotrophic and auxoheterotropic bacteria was noted, but, as a rule, it never exceeded 1.5- to 2-fold values. A considerable number of pigmented bacteria and some other bacteria forms, rarely found in the control plots, appeared in the treated soil. No direct relationship between the quantity and quality of microorganisms and the dose of the applied herbicide was observed, while conspicious qualitative changes were found at a dose of 6 kg/ha. The content of *Penicillium* sp. on these plots increased. After the treatment with herbicide the two species: *Mucor* sp. and *Aspergillus versicolor* disappeared.

The number of actinomyces varied, but it cannot be concluded that the herbicide application inhibited their development.

The number of fungi did not change significantly. In view of the demonstrated selective effect of the 2,4-DB on the microorganisms in soil, experiments were carried out to determine their reaction in pure culture.

The herbicide in the concentration of 0.1 mg/ml had an inhibitory effect almost on all the species of mycobacteria, actinomyces and fungi. Among yeasts and bacteria resistant to this high concentration were: *Cryptococcus albidus, Debaryomyces* sp., *Bacillus subtilis, B. cereus, B. megaterium, Serratia marcescens, B. mesentericus.*

At the concentration of 0.01 mg/ml the herbicide suppressed the growth of some species of bacteria *(E. coli, Bacillus subtilis),* actinomyces *(Actinomyces lavendulae, A. globisporus, A. aureoverticillatus, A. fradiae),* mycobacteria *(Mycobacterium lacticolum, M. mucosum),* fungi *(Alternaria humicola)* and yeasts *(Torulopsis aeria, Rhodotorula mucilaginosa, Lipomyces starkeyi, Schizoblastosporin starkeyi-henricci).*

No adverse effect on the growth of microorganisms was observed

at the concentration of the herbicide from 0.00001 up to 0.001 mg/ml. Consequently as a rule the doses of 2,4-DB applied in the large scale farming have been found to be non-toxic in the laboratory conditions in the experiments with the pure culture of soil microorganisms.

The herbicide application may change the biochemical processes, taking in the soil, and this may influence the fermentation activity of the soil.

Natural subalpine hill-meadow soils are characterised by a high invertase activity. On the other hand, the phosphatase activity was low. The activity of oxido-reductases is rather slight (Table 3).

Table 3

Influence of 2,4-DB on the enzyme activity in subalpine meadow soil

Treatment of the plots with herbicides	Doses kg/ha	Dehydrogenases, mg on 10g of soil per 24 hours	Phosphatase, mg P_2O_5 on 1 g of soil per hours	Invertase, glucose mg/g	Catalase ml O_2 per min
Without treatment		1.03	0.09	49.1	4.4
Triple treatment	6	1.34	0.12	79.6	5.1
	2	1.61	0.13	74.5	5.7
Once treatment	6	1.07	0.10	59.3	3.5
	2	1.04	0.10	55.3	3.4

Under the influence of 2,4-DB the activity of all studied enzymes slightly increased; this correlates with some increase of microorganism number.

Maximal increase of the fermentative activity was found, when the triple treatment of the herbicide was applied. Single application the herbicide had no effect on the fermentative activity of soil. Probably, the effect of the herbicide 2,4-DB on the fermentative activity is not direct, being connected with the more processes, like the change in the composition of plant association and that of microflora.

In connection of a wide application of the herbicide it is of interest to know the character of the herbicide accumulation and possibility of it decomposition in soil.

It has been stated that the introduction of herbicide into soil is related with the dose and period of its application. The herbicide applied continuously for 3 years in the doses of 2 and 6 kg/ha found to be accumulated in soil in the amount of 13-40% of its total quantity. During 3 years after single treatment with the herbicide at the dose of 6 kg/ha

it has been found in a trace quantity while at the dosis of 2 kg/ha the herbicide was not found.

Unfortunately, it is still unknown, what part of the herbicide is decomposed or indicativated and what is washed out into underlying layers and subsoil waters.

According to literature data the herbicides of the phenoxy-acetic acid group are very rapidly decomposed by microorganisms [4]. The prolonged preservation of 2,4-DB in hilly alpine soils is probably connected with a low microorganism quantity, and particularly with climatic conditions.

The herbicide brings about some changes in metabolism of gramineous plants. It has been found that the herbicide 2,4-DB affected the carbohydrate content, the nitrogenous compounds to a less extent, and changed the chlorophyll quantity in the sown grass *(Calamagrostis silvatica)*.

The herbicide caused an increase of the amount of nitrogenous compounds in plants: the total nitrogen from 1.57% to 1.95% and nonprotein nitrogen from 0.05 to 0.82 (Table 4).

Table 4

Influence of buthyl 2,4-D ether on the content of the nitrogenous compounds and the carbohydrate content in the sown grass /% of their dry matter/

Treatment	Nitrogenous compounds			Carbohydrates			
	Total	Proteine	Not proteine	Total	Monosaccharides	Disaccharides	Cellulose
0	1.57	1.07	0.50	34.90	5.56	9.52	19.82
6 kg/ha 3 years	1.95	1.13	0.81	29.90	4.54	5.28	20.08

The increase of the nitrogenous compounds is mainly due to the fraction of free amino-acids. The determination of total protein showed that its level remained unchanged, whereas some amino-acids underwent certain changes. The herbicide caused some increase of glutamic acid, threonine, serine and phenylalanine content, while the content of alanine, methionine and isoleucine decreased. The herbicide did not affect the content of other amino-acids of the plant proteins.

The occurrence of the free amino-acids under the herbicide influence is due to the inhibition of their inclusion into the protein molecules. This is closely connected with energetic processes, taking place in cells. There are sufficient data indicating that the herbicide 2,4-DB increases

the activity of adenosinetriphosphatase considerably, what brings about a decrease of the ATP content [2].

The effect of many herbicides consists in an intensification of plant respiration, which in consequence decreases the monosaccharide content [3, 4].

It has been found in the post-treatment period that the quantity of carbohydrate in the sown grass decreased from 34.9 to 29.9% (Table 4). A considerable loss of mono- and disaccharides was also observed. The cellulose percentage remained unchanged.

The weed mortality after treatment with the herbicide changed the character of the remaining plants, which in its turn should be reflected with their chlorophyll content. The determination of the sown grass on the experimental plots showed that there were no weeds after the third year of the herbicide treatment. The quantity of both "a" and "b" fractions of chlorophyll increased.

It is possible that the chlorophyll quantity increase after destruction of weeds by means of the herbicide treatment is connected with an intensive synthesis of chlorophyll in plants.

The conclusion from this study is that the 2,4-DB may be used for weed control, but its catabolism in soil should be studied.

REFERENCES

[1] Asjejewa I. V., Gorcharuk L. G., Altuchow M. D., Samochwałow A. N.: Nauczn. Dok. Wis. Szkoli. Biol. Sci. 2, 1973, 87.
[2] Beevers V. F., Peterson D. M.: Plant Physiol. 38, 1963, 6, 675.
[3] Black C. C., Hymphreys T. E.: Plant Physiol. 37, 1962, 1, 66.
[4] Kearney P. C., Kaufman D. D.: Degradation of herbicides, 1969.
[5] Smith E. G., Hamner Ch. L., Carlson R. F.: Plant Physiol. 22, 1947, 1, 56.

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ZASTOSOWANIE ESTRU BUTYLOWEGO 2,4-D DO POLEPSZENIA PASTWISK GÓRSKICH

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Streszczenie

Po zastosowaniu herbicydu 2,4-DB wyraźnie spada zachwaszczenie pastwisk, a zwałszcza silnie reaguje *Veratrum*. Wysokość plonu w roku zastosowania herbicydu spada, zwiększa się jednak procentowa zawartość traw. Zaobserwowano

niewielkie zmiany ilościowe i jakościowe w mikroflorze gleby oraz w jej aktywności enzymatycznej.

Zawartość związków azotowych i chlorofilu w roślinach zwiększa się po zastosowaniu herbicydu, a ilość węglowodanów spada.

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ПРИМЕНЕНИЕ ГЕРБИЦИДА 2,4-Д БУТИЛОВЫЙ ЭФИР ДЛЯ УЛУЧШЕНИЯ ГОРНЫХ ПАСТБИЩ

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Резюме

Сорняки, особенно чемерица зеленая, сильно подавляются в результате обработки гербицидами. В год обработки происходит снижение общего урожая, при увеличении участия злаков. Отмечены небольшие изменения в качественном и количественном составе микроорганизмов и в ферментативной активности почвы.

Содержание азотных соединений и хлорофила в растениях после обработки гербицидом повышается, при снижении количества углеводов.