Microbial Decomposition of the Herbicide Bromoxynil

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A knowledge of the mechanisms taking part in the biodegradation of xenobiotics in complex natural environments at various levels of the system, and an adequate experimental complexity, are necessary if man is to be able to modify the actions of these mechanisms. This paper presents some examples of studies concerning the microbial decomposition of the herbicide bromoxynil /3.5-dibromo-4-hydroxybenzonitrile/.

Materials and methods

Samples of chernozem soil, brown acid soil, brown soil and meadow soil were used in laboratory experiments, while grey forest loamy soil was employed in a field experiment. The samples were taken from the top layer /0 to 100 mm/, air dried and sieved to obtain structural aggregates of 2 to 5 mm in diameter that were stored at room temperature. The continuous cultivation of the soil samples was carried out according to MACURA /1961/. In the field experiment, the persistence of bromoxynil added in an amount of 750 $mg.m^{-2}$ was estimated in at a depth of 5 or 40 cm /plots measuring 1.5x1.5 m, moistened twice a week, GOLOVLEVA et al., 1988/.

A culture of Pseudomonas putida 13XF was grown in a mineral medium /TAYLOR, 1951/ with glucose or ribose /1000 ppm/ and/or bromoxynil /25 ppm/ using a reciprocal shaker /110 strokes min^{-1}/ at 28 °C. The samples were removed, centrifuged or filtered, and further analysed.

The number of bacteria in the soil was estimated by the dilution plate method on silica gel by using either a yeast and soil extract/tryptone medium, if copiotrophs were to be detected /TAYLOR, 1951/, or, in the case of oligotrophs, a medium developed by STALEY /1981/ containing 9 or 45 ppm organic carbon. The presence of bacterial decomposers of bromoxynil was determined by using a set of 80 randomly-chosen isolates pregrown in the above-mentioned media, whose ability to grow on a silica gel medium containing bromoxynil, as the only source of carbon and energy, was tested.

Bromoxynil and its degradation products were detected by HPLC after the extraction on a solid phase /SILICA-cart, TESSEK/. The protein fraction was detected using an HPLC-gel filtration column.

Results

Bromoxynil was decomposed in chernozem soil samples and in a pure culture of Pseudomonas putida 13XF gradually yielding 3,5-dibromo-4-hydroxybenzamide and 3.5-dibromo-4-hydroxybenzoic acid as the metabolic products. Thus, the results of SMITH /1971/, COLLINS /1973/ and GOLOVLEVA et al. /1988/ were confirmed.

Bromoxynil could only be degraded to produce the above-mentioned intermediates in the presence of suitable co-substrates, such as ribose, glucose,

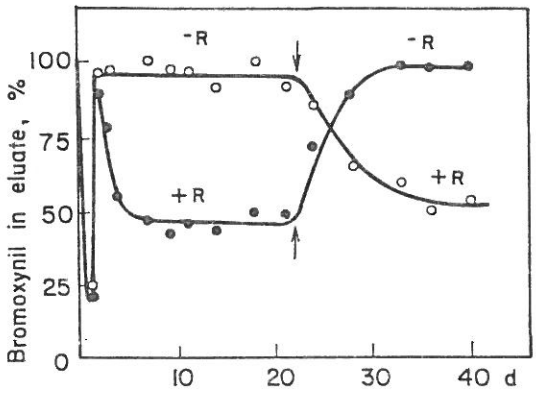

Fig. 1
The effect of simultaneously supplied ribose on the concentration of bromoxynil continuously added in solution after its passage through a column of 30 g of chernozem soil. Bromoxynil and ribose concentrations were 10 and 1000 ppm; flow rate 40 ml/d. +R: ribose present; -R: ribose absent; the arrows show a change in the supply of the cosubstrate

glycerol, etc. both in pure cultures of bacteria and in the soil /GOLOVLEVA et al., 1988/. An example of the stimulating effect of ribose on the degradation of bromoxynil during continuous cultivation can be seen in Fig. 1. These results should be taken into account when assessing the possibility of decomposition in soils containing various amounts of the organic component, after organic fertilizing or in the rhizosphere.

Bromoxynil can be decomposed extracellularly. The herbicide was added at an amount of 22.5 ppm to a 48 h-old Pseudomonas putida 13XF culture filtrate. After the next 24 h, two protein components having molecular weights of 25 000 and 13 500 were detected in the solution, in addition to bromoxynil /17.3 ppm/ and 3.5-dibromo-4-hydroxybenzamide /1.6 ppm/. The presence of the protein components can be attributed to the degradation activity observed. This finding may be of importance with respect to the action of the enzyme in the soil sorption system.

The presence of microorganisms capable of utilizing bromoxynil as their sole source of carbon and energy was demonstrated in samples of chernozem soil, brown soil, brown acid soil and meadow soil. Degradation ability was observed for the first time for the oligotrophic component of the soil microflora. As many as 1.6×10^6 CFU of copiotrophic and 0.2×10^6 CFU oligotrophic microorganisms decomposing bromoxynil were found in 1 g of soil, which represented 12 and 55% of the bacterial population, respectively. Some examples are shown in Table 1. The number of degrading microorganisms

Table 1
Occurrence of microbial decomposers of bromoxynil in soil samples

Decomposers of bromoxynil	Meadow soil non-treated, 1986	Brown acid soil, currently treated 1965	1987
COPIOTROPHS			
%	1	3	30
CFU, $10^{-3}.g^{-1}$	3	49	309
OLIGOTROPHS			
%	0	4	9
CFU, $10^{-3}.g^{-1}$	0	3	58

differed in various types of soil, but in general their numbers were higher in soils that had been treated with the herbicide in the past, and were also higher in samples removed in the period 1984 to 1988 in comparison with those taken between 1965 and 1966. The results suggest that natural communities have a remarkable ability to respond to the introduction of xenobiotics by the creation of a new quality. This fact should be taken into account when evaluating whether inoculation of the soil with a decomposer would be useful.

The decomposition of bromoxynil was also observed under complex natural conditions in a field experiment. The herbicide persisted in the soil for more than three months after the application and, therefore, cannot be recommended for widespread use /GOLOVLEVA et al., 1988/.

Summary

A description is given of results of biodegradation studies on the herbicide bromoxynil at various levels of the system and of experimental complexity. Bromoxynil was metabolized both in the soil and in a pure culture of Pseudomonas putida 13XF via 3,5-dibromo-4-hydroxybenzamide and 3,5-dibromo-4-hydroxybenzoic acid. The presence of a cosubstrate was essential. The degradation of bromoxynil also proceeded extracellularly. Copiotrophic and oligotrophic components of the soil microbial association took part in the degradation process. The number of microbes capable of degradation depended on the history of the soil. In the field experiment, the bromoxynil was detected in grey forest soil after 3 months.

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