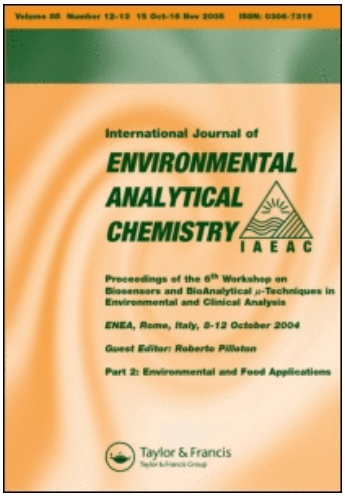

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Degradation of Isoproturon in Soil in Relation to Changes of Microbial Biomass and Activity in Small-Scale Laboratory and Outdoor Studies

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DEGRADATION OF ISOPROTURON IN SOIL IN RELATION TO CHANGES OF MICROBIAL BIOMASS AND ACTIVITY IN SMALL-SCALE LABORATORY AND OUTDOOR STUDIES

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The soil degradation of isoproturon under standardized laboratory conditions was compared to that carried out in an outdoor experiment using lysimeters. After application of ^{14}C-labelled formulated isoproturon (1.5 kg A.I./ha), radiochemical analysis, as well as microbial investigations, were performed to relate changes in soil microbial biomass to its capability of degrading isoproturon. The results showed that the microbial biomass, as well as its dehydrogenase activity, varied under field conditions due to fluctuations of temperature and soil moisture. In the laboratory experiment the microbial biomass decreased during the 100 day experimental period, this reduction being the result of the experimental conditions. Consequently, the herbicide was degraded more quickly in the outdoor experiment where leaching, formation of $^{14}C0_2$, uptake by plants, and photolysis also took place, than in the laboratory experiment. Further microbiological investigation in the laboratory experiment showed that specific populations known to be responsible for the metabolic degradation of the compound were enhanced in the isoproturon-treated soil.

KEY WORDS: Isoproturon, laboratory experiment, lysimeter, degradation, soil microorganisms.

INTRODUCTION

The degradation behaviour of pesticides in soil is often investigated using small-scale laboratory experiments. These experiments are easy to carry out, they provide reproducible results, the advantages of the ^{14}C-labelling technique can be used, and good recoveries are obtained. The results can be used for a comparison of different pesticides. However, in view of the actual field situation it should be remembered that results from standardized laboratory experiments can not be transferred to the field situation without restrictions[1]. These are mainly due to the standardized laboratory conditions (controlled temperature and moisture) themselves which may influence the degradation rate of active ingredients in soil compared with, e.g. varying temperatures[2,3]. Thus not only the air temperature itself, but also its normal fluctuation under field conditions, influence the fate of pesticides. Since it is well known that microbial degradation of the compound applied plays an important role[4], the microbial biomass of soils as well as its activity may be influenced by standardized laboratory experiments.

To investigate that influence in detail, a comparative degradation study was carried out using outdoor lysimeters with undisturbed soil profiles where ^{14}C-labelled pesticides[5]

and erlenmeyer flasks could be used in the laboratory. The compound used was the herbicide active ingredient isoproturon because it is known to be degraded in soils by co-metabolism[4,6,7]. Since it is also known that metabolic degradation may play an important role concerning the fate of the compound in soil, single microbial populations were investigated in the laboratory experiment to examine possible changes in the composition of the soil microbial biomass. The degradation rates of isoproturon in the outdoor and standardized lab experiment were compared and related to the additional microbial investigation.

MATERIAL AND METHODS

Soil

A loamy sand (74% sand, 19% silt, 7% clay) with a pH($CaCl_2$) of 6.9 and and organic matter content of 0.7% was used for all experiments.

Chemical

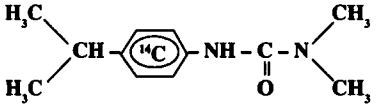
Chemical and physical data for isoproturon are given in Table 1.

Lysimeter studies

Two lysimeters with a surface area of 0.8 m^2 containing an undisturbed soil core with a depth of 1.2 m which were embedded in the ground[5], were used for the outdoor studies (Figure 1).

The active ingredient was formulated in a soluble concentrate-formulation with a specific radioactivity of 2000 kBq/mg isoproturon and was sprayed onto the lysimeters, planted with summer wheat (3 – 4 leaf stage) with an amount of 1.5 kg isoproturon/ha and 400 l water/ha. Details of the application procedure and the determination of application losses are given by Kubiak[5].

Table 1 Chemical and physical data for isoproturon[8].

Structure	:	
IUPAC name	:	N-(4-isopropyl-phenyl)-'N,'N-dimethylurea
Sum formula	:	$C_{12}H_{18}N_2O$
Molecular weight	:	206,32 g/mol
Water solubility	:	65 mg/l
Vapour pressure	:	$3{,}3 \times 10^{-8}$ hPA at 20 °C
K_{oc}	:	124,8

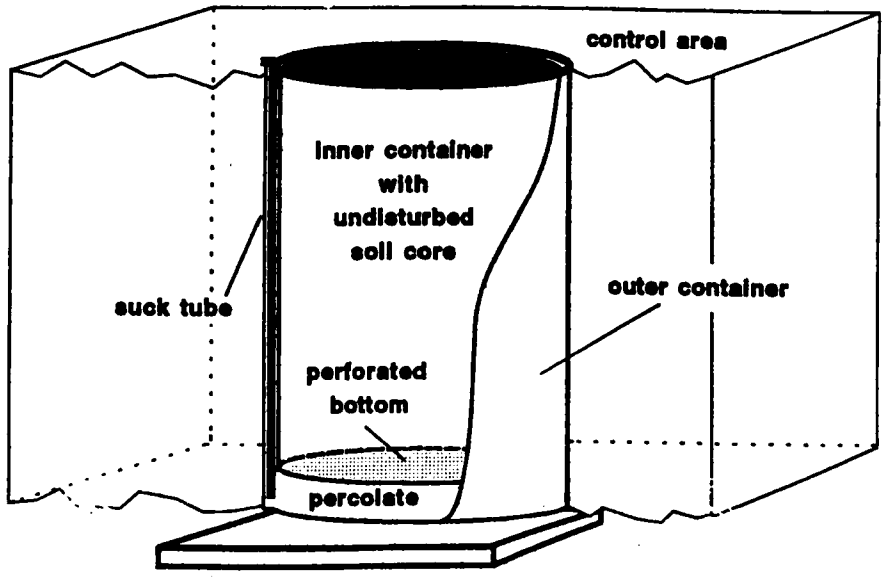


Figure 1 Lysimeter with an undisturbed soil core under field conditions.

Laboratory studies

The degradation experiment in the laboratory was carried out using erlenmeyer flasks with four replicates for the treated and untreated soil and for each sampling date (0, 2, 4, 8, 16, 32, 64 and 100 days after application) containing 100 g of dry soil each in combination with a trap system[9] described in Figure 2. Soil moisture was 50% of the water holding capacity (35 g H_2O/100 g soil) and temperature was 20°C during the whole experimental duration.

The same herbicide amounts as applied in the lysimeter experiment were used for the treated soil in the laboratory. The amounts were calculated on the basis of a soil weight of 1.5 g/m^3 for the upper 0 – 5 cm soil layer and were mixed in the 100 g soil of each erlenmeyer flask.

Sampling and analysis

Three randomized soil samples were taken from the upper 0 - 40 cm of the lysimeter soil with the aid of an auger (3.5 cm i.d.) fractionated every 10 cm of depth. The soil samples of each soil layer were combined to form mixed samples. For investigation of the microbial biomass and activity in untreated control soil under outdoor conditions weekly sampling and fractionating of the upper 0 - 20 cm was carried out as already described. The whole 100 g soil sample of the lab experiment was used for investigation, and the four replicates available for each sampling date were worked up separately.

50 g soil samples (d.w.) were extracted with 200 ml of methanol/water (80/20) twice and one time with 200 ml of methanol for 1 hour each. The solutions were combined and evaporated to the water phase. The water phase was shaken with 100 ml of dichloromethane three times for 20 min. each and the organic phase was concentrated for

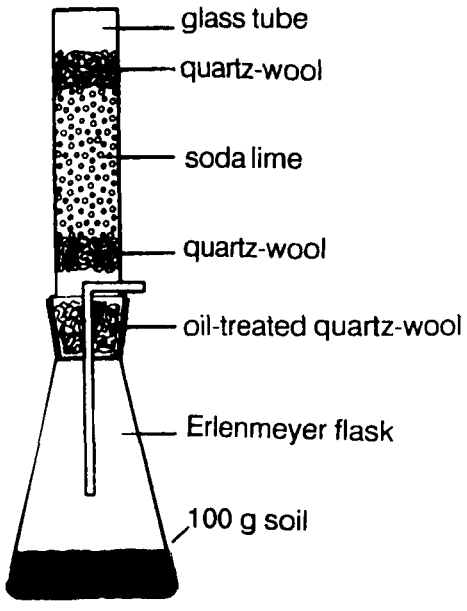


Figure 2 Incubation system for degradation studies in the laboratory.

further analysis. Silica gel 60 F_{254} plates and the solvent systems chloroform/ ethylacetate (1+2) (Rf-value of isoproturon: 0.36) or chloroform/ cyclohexane/ethanol/acetic acid (70+20+5+5) (Rf-value of isoproturon: 0.56) were used for TLC analysis. Radiochromatograms were recorded using a Linear analyzer LB 282 (Berthold). Analysis was focussed on isoproturon. The determination limit was 10 µg/kg, the detection limit was 2 µg/kg.

$^{14}CO_2$ trapped in the laboratory experiment was absorbed in ethanolamine/methanol (3+7) after the amendment of 6 N hydrochloric acid. To investigate the radioactivity in plant (outdoor experiment) and soil, samples were oxidized in a Biological Oxidizer (Packard Tec. 307) and the $C0_2$ was trapped in a mixture of Permafluor and Carbosorb (12 + 9) . Radioactivity of samples was measured using a LSC (LKB, Rackbeta 1219) with automatic quench correction.

Microbological determinations

The potential microbial biomass was investigated using the method of substrate-induced respiration (SIR) described by Anderson and Domsch[10]. The dehydrogenase activity was investigated using the method described by Thalmann[11].

Measurements of soil from the mixed outdoor samples were carried out with two replicates. In the case of the laboratory experiment four replicates were used for investigation at each sampling date.

Besides total bacteria and total fungi, the following populations were isolated from soil samples with and without treatment with isoproturon of the laboratory experiment using commercially available selective media[12]:

Bacteria : actinomycetes, acotobacter
Fungi : fusarium, mucor, cladosporium, penicillium, trichoderma, aspergillus, and monilia

Ten replicates for each of the four erlenmeyer flasks available for analysis were investigated so total amount of replicates amounted to 40 for the treated as well as for the untreated soil.

RESULTS AND DISCUSSION

Figure 3 shows the microbial biomass and the dehydrogenase activity in the 0 – 10 soil layer during a one year period in the outdoor experiment in untreated control soil, thus indicating that the values were fluctuating under the outdoor conditions. These

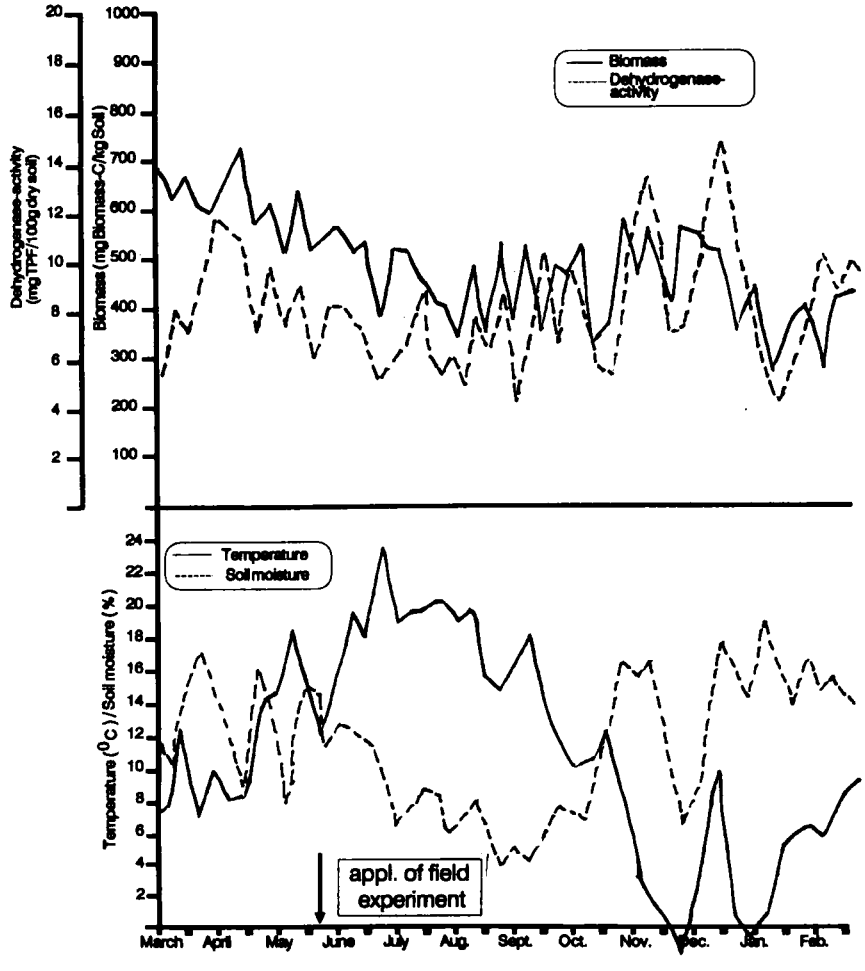


Figure 3 Microbial biomass and dehydrogenase-activity data for untreated outdoor soil (mean values of 2 replicates) compared with temperature and soil moisture. (Standard deviation ≤ 5%)

fluctuations were the results of variations in temperature and soil moisture which are known to be important for the growth of soil microorganisms.

These microbial investigations with untreated soil in the outdoor experiment, beginning with the day of application (June 6, 1989) of ^{14}C labelled formulated isoproturon to the lysimeters, were compared to the results obtained from the corresponding laboratory experiment carried out under standardized conditions with untreated control soil over a 100 day period (Figure 4).

The data showed that dehydrogenase activity as well as the microbial biomass, were lower in the laboratory experiment than during the course of the field experiment. This indicated that the soil microorganisms were influenced by the stabilized temperature and moisture conditions as well as by the physical restraints of the laboratory experiment both of which inhibited substrate transformation in soil. This was confirmed by the microorganism species investigated. Results showed that the acotobacter population disappeared under these conditions indicating that the nitrogen transformation in soil was

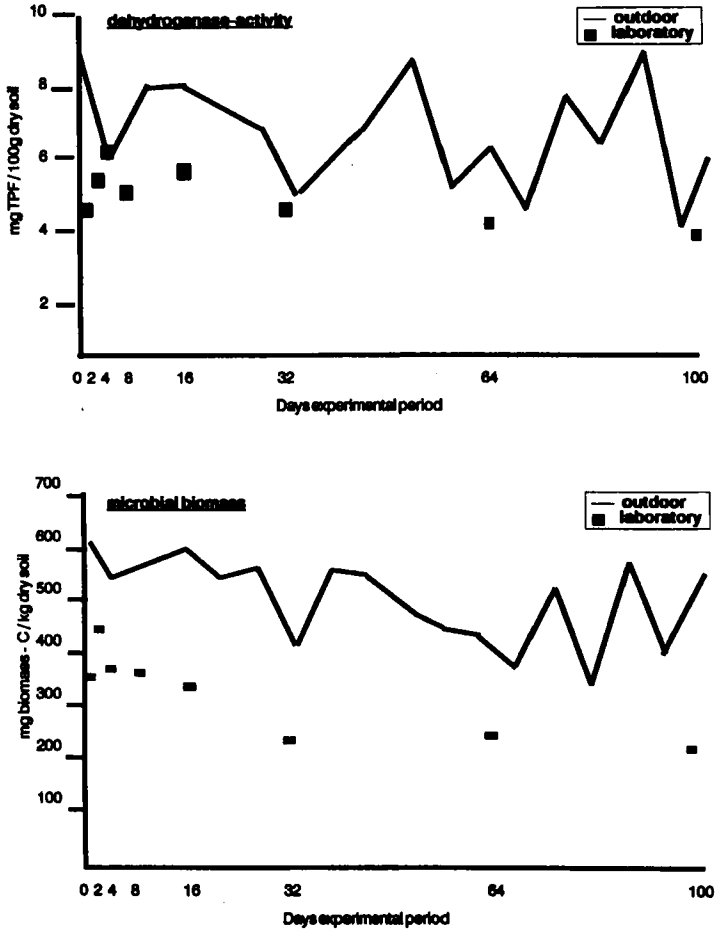


Figure 4 Microbial biomass and dehydrogenase-activity data for outdoor (mean values of 2 replicates) and laboratory conditions (mean values of 4 replicates) in untreated control soil (Standard deviation of outdoor measurements ≤ 5%; Standard deviation of laboratory measuremnts ≤ 4%)

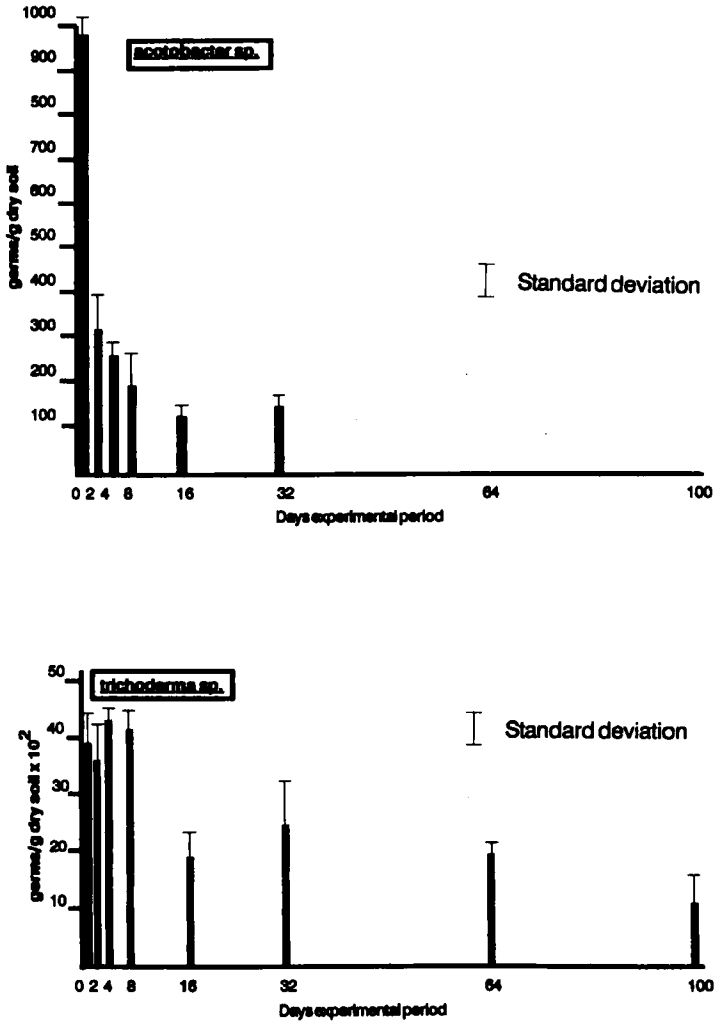


Figure 5 Numbers of bacteria and fungi in untreated control soil under laboratory conditions (Mean values of 40 replicates).

retarded (Figure 5). Other populations like trichoderma were reduced to about one third of their initial value during the experimental period of 100 days (Figure 5).

While microbial biomass and dehydrogenase activity were not influenced by the isoproturon treatment, the investigation of mucor, penicillium, and aspergillus, which are known to be involved in the metabolic degradation of isoproturon metabolites[13], showed differences between untreated and isoproturon-treated soil (Table 2) with respect to the initial values at the beginning of the experiment. Concerning penicillium and aspergillus, an enrichment was observed in the treated soil while these populations were reduced in the untreated soil. Mucor was not influenced in that way.

Since it is known that isoproturon is degraded by cometabolic and metabolic processes in soils[6,7,13], the differences between outdoor conditions and the laboratory

Table 2 Laboratory study under standardized conditions Numbers of microorganisms, known to be involved in the metabolic degradation of isoproturon Mean values of 40 replicates

	Day 0	*% SD*	*Day 100 untreated*	*% SD*	*Day 100 treated*	*% SD*
Mucor	100	± 105.0	65	± 10.0	43	± 28.7
Penicillium	100	± 28.6	83	± 16.8	175	± 11.7
Aspergillus	100	± 17.8	42	± 23.4	210	± 9.3

(% of initial value on day 0; SD = Standard deviation)

concerning the soil microorganisms led to different isoproturon residues in both experiments after 100 days. While in the laboratory experiment 1.3% of the amount applied represented the unchanged herbicide, residues in the outdoor experiment were below the detection limit. Most of the radioactivity in soil was no longer extractable in both types of experiments on the basis of the radioactivity still found in the soil of the laboratory experiment and still found in the 0 – 20 cm soil layer of the outdoor experiment (Figure 6). From additional intermediate investigation, half life times of 10 days could be determined for isoproturon in the lab experiment while the degradation time was significantly shorter in the outdoor experiment.

The radioactive balance of both experiments showed that 10% of $^{14}CO_2$ could be detected in the sodalime trap of the erlenmeyer flasks at the end of the experiment indicating the total mineralization of the labelling position of isoproturon whereas further distribution of radioactivity in plants as well as in deeper soil layers (20 – 40 cm) took place in the outdoor experiment. This radioactivity did not represent isoproturon. About 45% of the applied ^{14}C was unaccounted for and assumed to be the result of

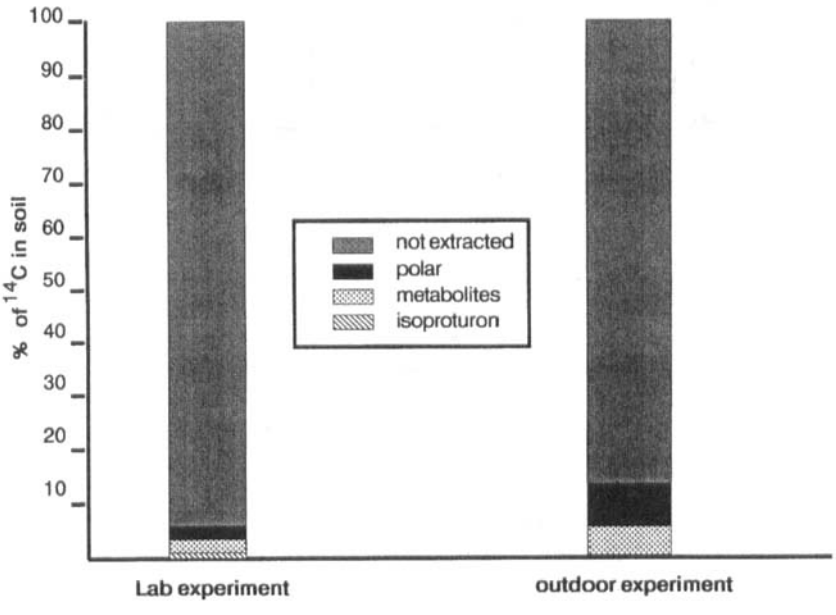


Figure 6 ^{14}C–balance in the soil 100 days after application. Radioactivity in the soil = 100.

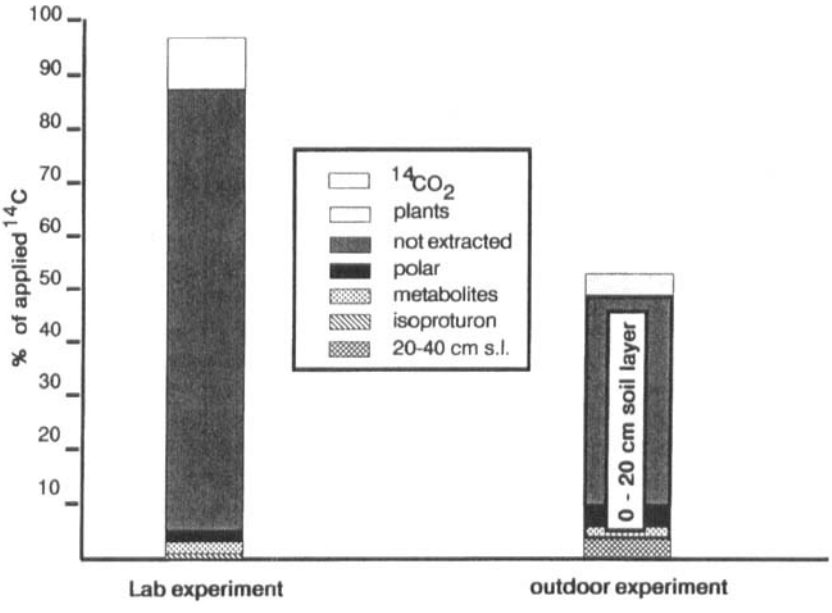


Figure 7 ^{14}C-recovery of both experiments 100 days after application. Radioactivity applied = 100.

mineralization and photolysis (Figure 7) which is also known to play a role concerning the fate of isoproturon under outdoor conditions[14].

Further investigation of deeper soil layers and percolate showed that less than 3% of the ^{14}C applied were found in depths lower than 40 cm and that volatilization of isoproturon was negligible[15].

The results show that metabolic and co-metabolic degradation of isoproturon is influenced not only by biochemical processes, but also by soil microorganism ecology, which may be influenced by side effects when small-scale laboratory experiments are used as an experimental tool.

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References

1. R. Kubiak, F. Führ, W. Mittelstaedt, M. Hansper and W. Steffens, *Weed Science*, **36**, 514–518 (1988).
2. F. Führ and W. Mittelstaedt, *J. Agric. and Food Chem.* **28**, 122–125 (1979).
3. R. Kubiak, *Juel–Report no. 2055*, ISSN 0366–0885 (1986).
4. K. H. Domsch, *Pestizide im Boden*, VCH Verlag (1992).
5. R. Kubiak. In: *Lysimeter Studies of the Fate of Pesticides in the Soil* (F. Führ and R. J. Hance, eds, BCPC, Farnham, 1992) pp. 51–55.
6. P. J. Mudd, R. J. Hance and S. J. L. Wright, *Weed Research*, **23**, 239–246 (1983).
7. G. Soulas and M. A. Reudet, *Z. Pflanzenkrankh. PflSchutz*, **8**, 227–235 (1977).

8. Industrieverband Agrar, *Wirkstoffe in Pflanzenschutz- und Schädlingsbekämpfungsmitteln* (BLV, München, Wien, Zürich, 1990) 2nd ed., 478pp.
9. J. P. E. Anderson, *Z. Pflanzenkrankh. Pflanzenschutz*, **7**, 141–146 (1975).
10. J. P. E. Anderson and K. H. Domsch, *Soil Biol. Biochem*, **10**, 215–221 (1978).
11. A. Thalmann, *Landwirtschaftl. Forschung*, **12**, 249–258 (1968).
12. W. Dunger and H. J. Fiedler, *Methoden der Bodenbiologie* (Gustav Fischer, Stuttgart, New York, 1989) 1st ed., 432pp.
13. J. C. Fournier and G. Catroux, *Chemosphere*, **9**, 33–36 (1980).
14. G. Kulshrestha and S. K. Mukerjee, *Pestic. Sci*, **17**, 489–494 (1986).
15. R. Kubiak, T. Maurer and K. W. Eichhorn, *Proc. of 8th EWRS Symposion, Braunschweig*, 551–558 (1993).