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BIOREMEDIATION OF ORGANIC AND INORGANIC CONTAMINANTS

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Practical on-farm bioremediation systems to limit point source pesticide pollution

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ABSTRACT

Activities involving the handling, use and disposal of pesticides, which generally take place on hardstandings in farmyards, can represent pollution sources. Impermeable surfaces with rapid runoff limit opportunities for *in situ* pesticide degradation. More permeable surfaces allow water and residues to infiltrate into the substrate where opportunities exist for degradation processes to take place. Specifically designed bioremediation systems, including biobeds (mixture of straw, topsoil and peat-free compost), provide enhanced conditions for microbial degradation. A research project in the UK has investigated the performance of three different pesticide handling and washdown areas, linked to bioremediation systems, and designed to minimise the risk of surface water contamination whilst taking account of the local groundwater vulnerability. Monitoring of the quality and quantity of water entering and leaving the systems was undertaken. Close management of the water within these systems was extremely important to their pesticide removal efficiency. Artificial applications of a suite of pesticides, with variable physico-chemical properties, were made to the sites to simulate severe contamination incidents. Pesticide concentrations greater than 100,000 µg/litre were measured entering the bioremediation systems. All three systems had an extremely effective pesticide removal performance throughout the monitoring period. Pesticide concentrations in the leachate discharging from the treatment systems generally remained below 0.5 µg/litre.

INTRODUCTION

Groundwater and surface water is at risk of contamination from the use of some agricultural pesticides. Pollution of surface waters from agricultural pesticides can have a detrimental effect on water quality and aquatic ecosystems. The presence of pesticides in surface waters may lead to the requirement to treat the water downstream if it is abstracted for use in the potable water supply system. However, this treatment using activated carbon to remove unwanted pesticide residues is very expensive. The cost is, in part, passed onto the consumer. There are many stakeholders involved in the use of pesticides for plant protection and the quality of water resources in the UK (e.g. agrochemical companies, Environment Agencies, water supply companies, farming bodies, conservation bodies, Government). There is also a range of relevant EU and national legislation, codes of practice and advisory information concerning pesticide handling, together with the disposal of associated washings and other materials.

In many circumstances pesticide contamination of water resources is more likely to result from point sources than from diffuse sources following approved application to crops in the field. Such point sources include areas on farms where pesticides are handled, filled into sprayers or where sprayers are washed down.

Monitoring projects in the UK and other countries (Kreuger, 1998; Mason *et al.*, 1999; Bach, 2003) have identified that point sources of pesticides can be responsible for a significant proportion of the total amount of pesticide loading in water and can account for the peak concentrations detected. Point sources can contribute 20-70% of the total load in a surface water catchment, depending on catchment characteristics. The farmyard characteristics, operating practices and local conditions vary but all researchers report similar reasons for the origin of the point source contamination.

Point source pollution incidents in farmyards are largely attributable to operator error or bad practice, machinery faults and the physical characteristics of the handling/mixing area. It is generally considered that it should be possible to control these point sources more easily than diffuse sources. Better operator awareness and training, good machinery maintenance, with undercover storage, are all considered to be fundamental to minimising the risk of pollution from many point sources. Another important consideration is the design and operation of pesticide handling and washdown areas on farms. Traditionally in the UK these areas have been mainly on concrete pads, close to farm buildings where there is ready access to a mains water supply and the pesticide store. Often these concrete pads drain to yard sumps which then connect directly to a soakaway or even to the nearest watercourse. As a result, substantial and rapid contamination of water resources can arise from these pesticide handling and washdown operations.

In 2000 a major research study commenced in the UK to investigate the design of pesticide handling and washdown areas. During late 2001 three new full-scale on-farm pesticide handling and washdown areas were specifically designed to reduce the risk of contamination of water resources. These areas were all linked, either directly or indirectly to bioremediation systems for the *in situ* retention and/or degradation of pesticides. Two of the test bioremediation systems were based on the biobed concept (a mixture of topsoil, straw and peat-free compost) that originated from Sweden (Torstensson & Castillo, 1997). This biomix provides enhanced conditions for microbial degradation of pesticides. The third test system was based on a loamy topsoil as a biological treatment system, as used by Liaghat *et al.* (1996). All these systems had been proved to work very effectively during some earlier tank studies in 2000 (Rose *et al.*, 2001).

MATERIALS AND METHODS

In early 2002 the full-scale pesticide handing and washdown areas were constructed on a large farming enterprise in Lincolnshire, UK, which undertook spraying operations from three existing farmyards. To allow for the experimental monitoring programme all the existing farmyards were modified to include the bioremediation systems and monitoring equipment. The bioremediation systems were fully enclosed within impermeable liners and single pipe outlets. These were located at the lowest point in the liner, thereby permitting free drainage. Work by Fogg (2001) had reported that unsaturated conditions were needed in the biomix to retain the pesticide removal performance of biobeds. The three test systems constructed were:

- Bunded concrete intercept area draining to a biobed (with turf cover)
- Drive-over biobed (with turf cover)
- Bunded concrete intercept draining to biologically active loamy soil area (with turf cover)

The biomix used in the two biobeds (each 1m deep) was a mixture of straw (50% by volume), local topsoil - silty clay loam (25% by volume) and a peat-free compost (25% by volume). This was left to mature and compost in the farmyard for 4 weeks prior to being loaded into the biobed liners. By the time the biobeds were commissioned for use the biomix was 9 weeks old. The soil that was loaded into the soil liner (1m deep) was a silty clay loam topsoil derived locally. Each bioremediation system had a turf cover to assist with the water management of the matrix. The two bioremediation systems linked to a bunded concrete intercept area were 5m x 4m in size.

The drive-over biobed option differed from the other two designs in that it did not have any bunded concrete intercept area. All liquid inputs (rainfall and pesticides) into the biobed fell directly through the metal grid (with 100mm x 40mm mesh size) that covered the entire area, and any extraneous clean water was excluded. Due to the requirement for the biobed to be lined the removable metal grid had to be designed to span the entire biobed without the need for any supporting pillars in order to take the weight of a fully loaded sprayer (c. 9.5 tonnes). For this reason the drive-over grid was 8m x 5.5m in area.

The bunded concrete intercept areas were 7m x 5m in area to allow the self-propelled spray machinery to fully stand on the area, with spray booms folded, and still allow the operator safe amount of space around it to undertake all the necessary pesticide handling and washdown activities. The presence of the 100 mm high bund meant that extraneous clean water from other parts of the farmyards was excluded from the systems.

Water flow from the bunded concrete intercept areas and leachate flow from the biobed/soil areas was measured by a tipping bucket flowmeter system linked to a dedicated data logger. Automatic water samplers were set up to sample all these waters. From the flowmeters the water was directed into temporary storage tanks within the systems prior to the application, via drip irrigation, to either the biobed/soil area or to the designated disposal area. The use of drip irrigation over the surface of the biobed/soil area allowed the pesticide laden runoff water to be distributed evenly across the entire surface area of the treatment system, thereby maximising the potential for pesticide retention and/or degradation processes to take place. Leachate water from the bioremediation systems was discharged to a land disposal area under an Environment Agency Groundwater Authorisation. A local record of the rainfall and pesticide handling and washdown activities at the three sites was also maintained. Normal commercial use of the systems, following good practice, commenced in April 2002.

In June and September 2002 artificial pesticide applications were made to each pesticide handling and washdown area to simulate the potential maximum pesticide contamination arising from 16 individual tank mixes on one day. A set of controlled mixtures were formulated with known pesticide concentrations and volumes to represent four possible contamination sources, namely: i) dropped foil seals from pesticide packaging (spray concentrate); ii) faulty valves/nozzles/hoses (spray suspension); iii) sump rinsate; and iv) washdown liquid. This artificial application exercise (excluding a major spill of pesticide concentrate) therefore represented a very severe test of the bioremediation systems, which was unlikely to occur during normal spray operations. Pesticides with a range of physico-chemical

properties were chosen for the artificial applications, namely: isoproturon (herbicide), pendimethalin (herbicide), chlorothalonil (fungicide), epoxiconazole (fungicide), dimethoate (insecticide) and chlorpyrifos (insecticide).

The individual pesticide concentrations and volumes applied for the above contamination sources was based on the findings from the Cherwell study on isoproturon (Mason *et al.*, 1999), and adjusted according to the particular product formulation and product label rates for the other pesticides in the suite. These mixtures were applied to specific locations on each of the pesticide handling and washdown areas where it would have been expected that the particular contamination source would fall had the sprayer been in place.

All three bioremediation systems were monitored for a three month period after each artificial pesticide application to evaluate their ability to retain and/or degrade the pesticides prior to disposal to the environment.

In the laboratory the pesticides were extracted from the water samples by shaking them with an immiscible organic solvent dichloromethane. After the immiscible layers had separated, the organic layer was removed and the solvent evaporated to just dryness. The dry extract was then re-dissolved in ethyl acetate. The final determination was by gas chromatography with mass selective detection in selected ion mode. Each analyte was determined by monitoring 3 mass ions (m/z >100) thus precluding the need for further confirmation. The limit of quantification for each individual pesticide was 2 µg/litre for samples derived from the concrete intercept areas and between 0.1 µg/litre and 0.5 µg/litre for the leachate samples derived from the bioremediation systems.

RESULTS

The concentrations of individual pesticides in the artificial mixtures added to each of the sites are shown in Table 1. In general, runoff from the concrete intercept areas was not initiated until the 25 litres of sump rinsate was added. The application of the less concentrated sump rinsate and the washdown liquid acted to dilute the very high concentrations from the spray concentrate and spray suspension.

Table 1. Pesticide application volumes and concentration ranges in the test mixtures

Contamination Source	Volume applied (litres)	Pesticide concentration range (µg/litre)
Spray concentrate	0.4	250,000-4,000,000
Spray suspension	4	62,000-1,000,000
Sump rinsate	25	25,000-400,000
Washdown liquid	150	220-3,600

Both monitoring periods were characterised by wet and dry periods that will have affected the timing and amount of runoff/throughflow, and the speed at which any pesticides were transported through the bioremediation systems. Other environmental factors at the sites will have affected the rate of pesticide degradation and hence the potential for any pesticide transport. These factors include: local meteorological conditions and biobed/soil conditions (e.g. temperature, moisture, organic matter content, biomass activity).

The condition of the turf cover at the sites had a substantial affect on the amount of water that moved through the bioremediation systems. The deep loamy soil based system encouraged a healthy and vigorous grass growth, which was never adversely affected by the high artificial pesticide applications to the base of the grass sward via the drip irrigation tubes. In contrast, the vegetative growth at both biobed sites was impacted by the pesticide applications. This may, in part, due to the fact that the high straw content of the biomix did not encourage good strong root development within the loose matrix.

During each of the three month monitoring periods, following the artificial applications, all the bioremediation systems were extremely effective in retaining and/or degrading test pesticides. In general, all three systems were able to reduce the severe input concentrations by 10,000 to 100,000 folds (Table 2). 87% of the >1100 individual pesticide determinations from the leachate samples discharging from the bioremediation systems had a concentration less than 0.5 µg/litre, the limit of quantification for the laboratory analysis.

Table 2. Maximum pesticide concentrations (µg/litre) in runoff or leachate during second application period (Sept-Nov 2002)

	Concrete intercept to biobed		Drive-over Biobed	Concrete intercept to soil area	
Pesticide	Runoff	Leachate	Leachate	Runoff	Leachate
Dimethoate	44,277	0.9	15.5	24,800	<0.5
Chlorothalonil	96,807	0.3	<0.1	94,600	<0.1
Isoproturon	140,850	<0.5	1.2	55,900	<0.5
Chlorpyrifos	77,646	0.7	0.4	56,300	0.8
Pendimethalin	205,550	2.3	0.5	107,900	0.8
Epoxiconazole	9,108	0.8	0.7	9,450	0.8
Azoxystrobin	2,960	5.8	1.9	6,4100	0.6

DISCUSSION

Specifically designed and managed bioremediation systems can effectively limit point source pesticide pollution originating from farmyards. Over both three month monitoring periods all the three bioremediation systems were able to reduce input concentrations in excess of 100,000 µg/litre to below 0.5 µg/litre, which was the limit of quantification of the laboratory analysis. This represents a substantial improvement in the quality of water entering the environment. If the discharge from these systems is disposed of to suitable soil areas then significant opportunities exist for further pesticide retention and/or degradation processes to take place before the water finally enters water resources.

The results from this project concur with a number of other studies (e.g. Liaghat *et al*, 1996; Henriksen *et al*, 1999; Torstensson, 2000; Fogg, 2001). The biologically active matrix provides conditions for pesticide adsorption followed by pesticide degradation due to a thriving microbial population. Physical and chemical degradation processes can also take place with the organic matrix where aerobic and anaerobic conditions will occur. However, careful water management is needed to maintain these favourable environmental conditions and saturation of the organic matrix for any length of time should be avoided. Further testing

of these systems with a wider range of active substances and even metabolites would provide more comprehensive evidence on their effectiveness.

Bioremediation systems will require a certain amount of management to retain their effectiveness over time. The natural composting process that takes place within the biomix will necessitate an annual topping-up operation. Evidence from Swedish work (Torstensson, 2000) suggests that the biomix may only remain fully active for a 5-7 year period, after which the entire biomix will need to be replaced. Composting of this spent biomix for a further year was shown to remove all the remaining pesticide residues. However, this has yet to be confirmed in UK environmental conditions and the longevity of the soil based system is completely unknown. The disposal options for spent biomix still require further consideration in the UK, within the framework of current environmental regulations.

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Biodegradation of simazine in olive groves under laboratory and field conditions

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ABSTRACT

The use of pre-emergence herbicides, mainly simazine at 2 kg a.i./ha is a common practice in Spanish agriculture to control weeds and to get profitable olive yields. Losses of simazine efficacy due to enhanced rates of biodegradation have been documented after years of continued herbicide application. It is generally accepted that this is due to proliferation of soil micro- organisms that use this pesticide as a carbon and/or nitrogen source for growth. We have studied the biodegradation of simazine in the olive groves and also in the laboratory using soil and soil-inoculated liquid minimum media. In the field, and under natural conditions, 6% of simazine residues are found after 127 days of the herbicide application. In the laboratory, however, the same amount was found after 18 days when the same soil was analyzed. In the liquid medium tests only 0.05% of simazine is found after 10 days of incubation. We have isolated some of the microorganisms responsible for such degradation. These micro organisms are capable of using simazine as the sole nitrogen and carbon source for growth. We present data showing how the addition of various nitrogen (fertilizers) or carbon sources (glucose) influence the rate of degradation of simazine.

INTRODUCTION

Olive plantations are found over most of the Mediterranean region, the greatest concentration of olive production in the world is found in two Spanish provinces, Jaén and Córdoba (Andalucía, south of Spain). Weed control is considering necessary to prevent them from competing with olive crop, particularly for moisture during the late spring and summer, and to obtain profitable yields (Guerrero A, 1997). The most widespread types of herbicide are pre-emergences, such as simazine, which is residual and require only one or two applications per year. However, the effectiveness of annual weed control by use of autumn-applied simazine decreased due to enhanced rates of biodegradation that have been documented after years of continued herbicide application (Racke *et al.*, 1990; Saavedra *et al.*, 1996). Furthermore, the use of simazine has recently been banned in Europe, since several works report about its high potential to leach into ground water and runn-off into surface waters and its persistence and accumulation in soils (López-Flores *et al.*, 2003).

Herbicide and soil interactions involve several processes such as herbicide diffusion, adsorption and chemical and microbial degradation (Barriuso *et al.*, 1994). Microbial degradation of herbicides is the most important route of detoxification in the soil (Benoit *et al.*, 1999). Simazine is used by certain soil microorganisms as a source of energy for growth (Kaufman *et al.*, 1968; Cook, 1987; Martin-Montalvo, 1997). A number of papers have reported that bacteria and fungi isolated or as a consortium are capable of degrading simazine (Behki *et al.*, 1994; Ernest, 1995; Kontchou *et al.*, 1999; Kodama *et al.*, 2001).

The aims of this study were:

(i) To compare the biodegradation of simazine in soil under field and laboratory conditions, and in soil-inoculated liquid media.

(ii) To compare the rates of simazine biodegradation using additional nitrogen and carbon sources in liquid media.

MATERIALS AND METHODS

For the field experiments under natural conditions, simazine (agrisimazine) was applied at 3 kg/ha and 4 kg/ha in the autumn of 2002 and 2003, respectively, in three olive groves located at Cabra (Córdoba). These fields were previously applied with simazine for 4 years at a dose of 3 kg/ha. Soil samples were collected from the 0-20 cm depth, air dried, sieved through a 2 mm mesh, and stored frozen at -20 °C until further analysis. The physical and chemical properties of the soil are given in Table 1.

Table 1. Chemical and physical properties of the soil studied

parameter	pH	Conductance ds/m	Sand %	Clay %	Silt %	$CaCO_3$ %	NO_3^- mg/kg	NO_2^- mg/kg	NH_4^+ mg/kg
Soil	8.2	101.8	35.4	12.7	51.9	48. 21	2.27	0.75	4.3

The herbicide was extracted by shaking 25 g soil three times with 50, 25 and 10 ml methanol for 60, 30 and 10 min respectively. Recoveries of simazine were higher than 90%. The herbicide was determined with an HPLC (Beckman, System Gold) at 221 nm using a C18 ultrasphere column (25 x 4.6 mm x 5 µm and 80 Å) with acetonitrile: water (30:70) as the mobile phase. Analyses were carried out at 25 °C with 50 µl samples and a flow rate of 1.8 ml/min. A detection limit of 0.02 mg/kg was achieved.

For the degradation of simazine under the laboratory incubations, duplicate samples (100 g) for each analysis were incubated at 25 °C in 250 ml plastic cups in the dark. Water content was adjusted at 70% of field holding capacity. The herbicide was sprayed using a cabinet track sprayer equipped with an 8001E-VS flat-fan nozzle that delivered a spray volume of 300 L/ha under a pressure of 200 KPa. The final simazine concentration in soil was 10 mg/kg.

For the degradation of simazine in liquid media, soil samples (0.5 g) were added to 20 ml of minimum media containing 30 mg/kg of simazine (99% purity) in a rotary shaker under constant agitation (130 rpm) at 30 °C in the dark. Minimal medium contained 10 mM disodium phosphate and 10 mM potassium phosphate buffer (pH 7) and 200 mg of

$Fe_2SO_4.7H_2O$, 10 mg of $Zn_2SO_4.7H_2O$, 3 mg of $MnCl_2$, 30 mg of H_3BO_3, 24 mg $CoCl_2$, 1 mg of $CuSO_4$, 2 mg of $NiCl_2$, Na_2MoO_4, 50 mg of $Ca(OH)_2$ per liter. After two weeks of continuous incubation, subcultures were done in fresh media. This was repeated five times. Samples were taken and their simazine content analyzed. In some of these samples, simazine was added to the media plus 1 mM sodium nitrate, 1 mM urea, 1 mM ammonium chloride or 10 mM glucose as additional carbon or nitrogen sources.

RESULTS AND DISCUSSION

The degradation kinetics for field, laboratory assays and the soil-inoculated liquid medium are shown in Figure 1.

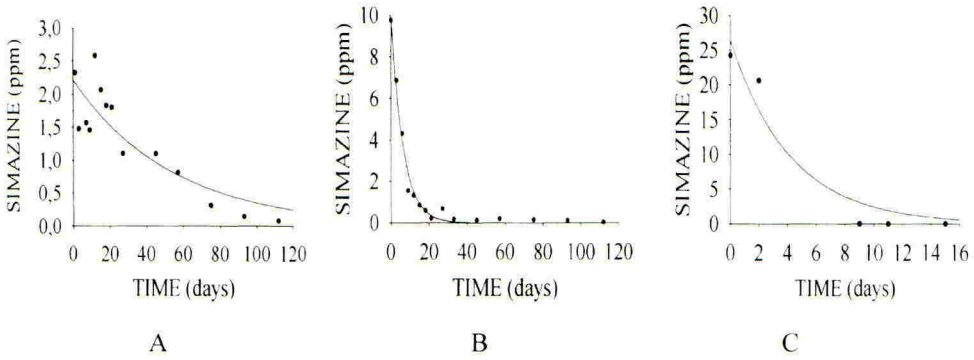

Figure 1. Degradation kinetic curves of simazine. (A) In field; (B) in laboratory conditions; and (C) in soil-inoculated liquid media.

In the three cases, the curves of degradation followed the first-order kinetics. Degradation of simazine was very rapid in the field under natural conditions in comparison with other reports previously described. As far as we know, the degradation half-life values (DT_{50}) are much smaller than those normally found in agricultural soils. This could be due to the fact that these soils were previously treated with s-triazines herbicides (Humburg *et al.*, 1989; Yassir *et al.*, 1999), and to the high temperatures (18-20 °C) and the high rainfalls (Figure 2) occurring in short periods of time in autumn coinciding with the herbicide application. After 127 days of incubation under natural conditions, only 6% of simazine was recovered (Figure 1A).

Under laboratory conditions, however, the rate of degradation was even faster: a recovery of the 6% of the simazine took place after 18 days only (Figure 1B). The higher degradation rate could be due to a higher and constant incubation temperature in the laboratory assays (25 °C). It has been demonstrated that the rate of simazine degradation increases at higher temperatures (Saavedra & Pastor, 1996).

Table 2. Degradation rates of simazine in field, laboratory incubations and liquid media. K is the first order rate constant per day and DT_{50} is the half life

Parameters	K (day^{-1})	DT_{50} (days)	R^2
Field	-0.015	45.3	0.836
Laboratory incubations	-0.159	4.4	0.992
Liquid media	-0.237	3	0.970

The simazine in liquid media disappeared faster than in the soils incubated in the field or the laboratory (Figure 1C). In this medium, simazine was the sole carbon and nitrogen source for the growth. This can be due to the fact that we previously enriched the culture media with microorganisms able to degrade this herbicide. This was done inoculating minimal media containing simazine as the only source for growth with minor portions of soil samples. It is likely that the media either contained growth factors absent in the soils, or lacked of inhibitors (Ralebitso *et al.*, 2002).

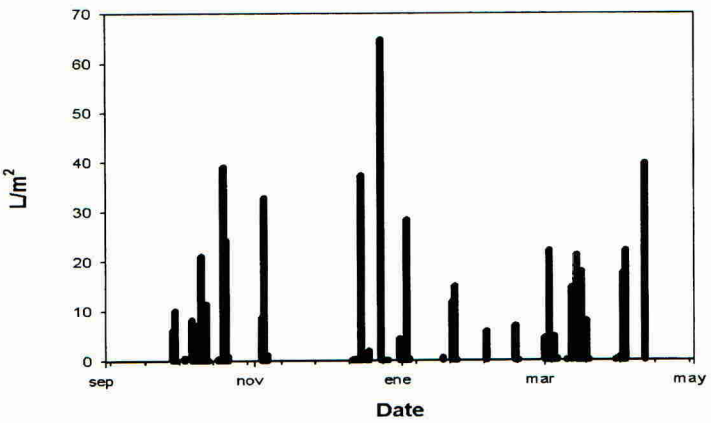

Figure 2. Precipitation during the first experimentation year.

Figure 3 shows the degradation of simazine in the presence of additional nitrogen (nitrate, urea and ammonium) or carbon sources (glucose). The nitrogen sources tested are commonly used as fertilizers. In all cases, degradation of simazine took place at similar rates that the controls lacking these additional compounds. This means that none of them acted either as a catabolic repressor or as inhibitors of the enzymes implied in the degradation of this herbicide. This is noteworthy to mention as it has been described elsewhere that nitrate substantially inhibits degradation of *s*-triazines (Entry, 1999; Gebendinger *et al.*, 1999; Abdehafid *et al.*, 2000).

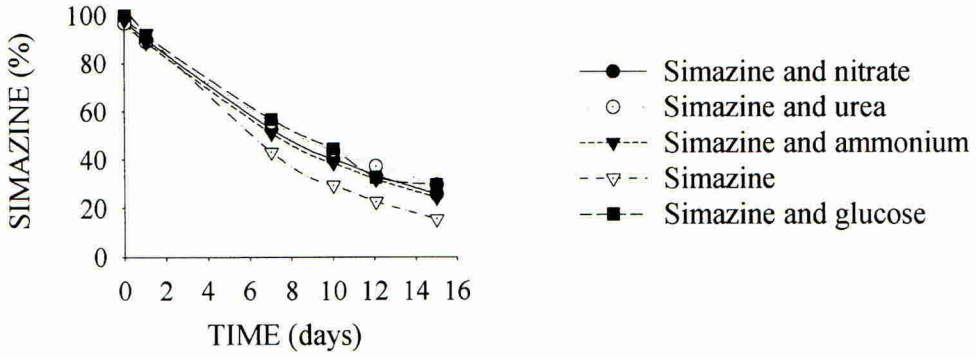

Figure 3. Degradation of simazine in media with additional carbon or nitrogen sources.

CONCLUSIONS

Simazine is rapidly degraded under all conditions tested due mainly to the presence of microorganisms that have developed after the continuous treatment of the olive groves with this herbicide during the last 5 to 10 years. Climatic factors such as temperature and rainfalls play an important role in the rate of biodegradation. At least some of the enzymes implied in the degradation pathway seem to be constitutively expressed in these microorganisms, since this degradation is not substantially influenced in the presence of additional nitrogen and carbon sources such as nitrate, urea, ammonium or glucose.

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