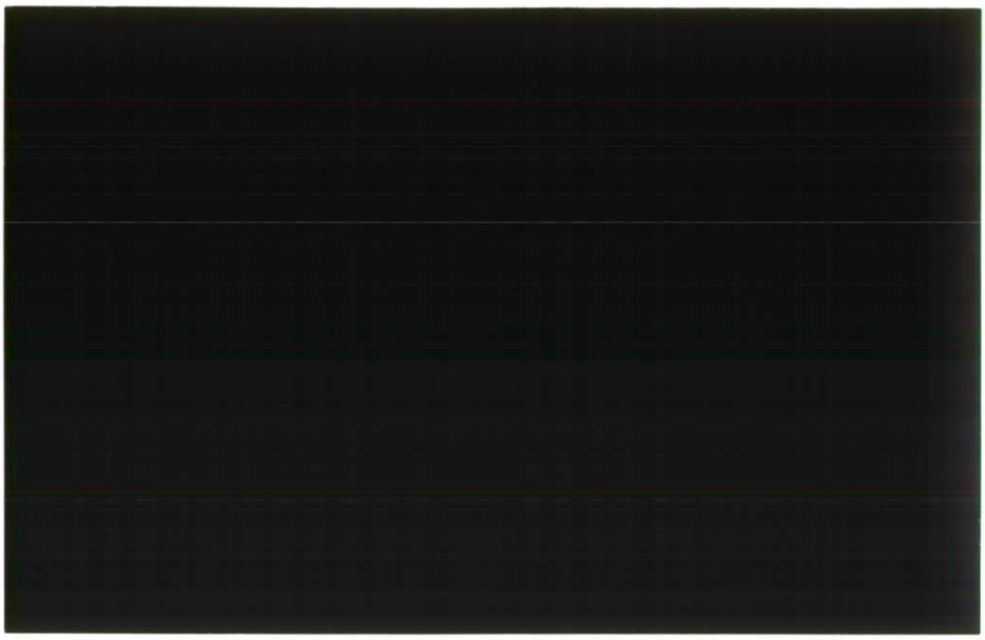


Natural
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The Biodegradation of pesticides in the unsaturated and saturated zones of major UK aquifers

2nd Annual Report

Report to the Ministry of Agriculture, Fisheries and Food

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Contents

1. Introduction

The objective of the project is to study the potential for degradation of isoproturon, mecoprop and atrazine in the Chalk, Permo-Triassic Sandstone and Jurassic Limestone aquifer environments in the UK (Fig. 1a). In the first annual report a discussion of methods and the literature was made. In the absence of a large, long time-scale field monitoring project, using field samples for laboratory microcosm studies was deemed the most appropriate technique to begin studying this subject. A large number of experiments have now been completed on material from Chalk and Sandstone field sites. These experiments addressed the following questions:

1. **Does a potential exist for the degradation of isoproturon, atrazine and mecoprop in; (a) soil; (b) unsaturated zone; (c) the saturated zone.**

2. **If a degradation potential exists, how does it vary; (a) spatially (across the field site); (b) temporally (sampled at different times over the year).**

3. **Can a degradation potential for isoproturon be demonstrated in the unsaturated zone, when unsaturated conditions are mimicked?**

4. **Can a degradation potential for pesticides be demonstrated when low, environmental concentrations are used?**

5. **Can the metabolites generated from groundwater degradation of herbicides be identified?**

Technical Challenges

Due to the low numbers of bacteria, and low metabolic activity, the microcosm experiments have to be incubated for periods of 200-300 d in order to detect any degradation potential. This is a long time for microcosm experiments, therefore, great care must be taken to maintain aseptic conditions, and to ensure that the controls do not become contaminated during sampling. Some experiments have had to be curtailed prematurely due to contamination of the controls.

As reviewed in the first Annual Report to MAFF, so far there has been little research into the pesticide degradation capacity of the deep unsaturated zone. However, increasing evidence obtained from the NERC funded field work at Wonston has shown that the primary water movement mechanism in an Upper Chalk site is through the matrix. Thus, pesticide which escapes through the soil may take tens of years to reach the water table. It may be that the pesticide we see now in groundwater may have been applied in 1975-78, and therefore, today's application may not arrive until 2018! At these travel rates even very slow degradation rates would have a major impact on the concentration which eventually arrives. Therefore, it is important that some of the degradation experiments carried out on the unsaturated zone should resemble the natural unsaturated conditions. For this project two separate new techniques have been developed to study the pesticide degradation potential of this zone.

2. Materials and methods

2.1 LOCATION OF CHALK SITE

Sampling was undertaken at a location of Upper Chalk outcrop near Winchester in Hampshire, UK, at site WON (Fig. 1a). The site is described more fully in the first Annual Report. The Upper Chalk begins from 40-60 cm below the soil surface. The WON field site has been farmed as part of a 3 year rotation of winter cereal to grass production. Isoproturon had been applied at site WON in 1988, 1994, and 1995. The borehole from which groundwater samples were taken are shown in Fig. 1b. The drilling of WON 7 was funded by this project and the chalk material obtained used in some of the microcosm experiments.

2.2 LOCATION OF SANDSTONE SITE

In order to study the Triassic Sandstone aquifer environment a site was selected on land farmed by ADAS Gleadthorpe near Mansfield in Nottinghamshire (Fig. 1a). The site was considered to be in an area which is typical and representative of this type of aquifer. At present around 10% of the drinking water requirement for England and Wales comes from this type of aquifer. At the field site itself, mecoprop and isoproturon had been applied previously but not atrazine. The soil is a typical brownsand (Newport series) and can be found to a depth of 0.9 m. At the lower edge of the field (headland) where the borehole (BH 1) was drilled, the water table was found at 7.0 mbs. Two other boreholes were present at the field site, which had been drilled by the British Geological Survey (BGS) (BH 2 and 3), which were located 10 and 120 m away with water tables 7.9 and 12.5 mbs respectively (Fig. 1c).

2.3 COLLECTION OF CORE SAMPLES AND GROUNDWATER

Drilling was undertaken at site WON in September 1996, and at Gleadthorpe in September 1997. The dry percussion technique was used at both sites and is fully described in the first Annual Report, and Johnson *et al.* (1998).

Groundwater was collected from WON 7 and the other observation boreholes, WON 4, 5, and 6 using a small submersible electric pump. This was also done at all three of the boreholes at Gleadthorpe. Five borehole volumes were pumped out and discarded before collecting samples in sterile bottles. Groundwater was stored at 4°C prior to use. Groundwater hydrochemistry measurements were undertaken by BGS. To assess the number of viable bacteria present in the solid material and groundwater samples a viable count technique was used. This involved taking the samples through a series of dilution's in ¼ Ringers solution before plating out on 0.3% tryptone soya agar (Johnson *et al.*, 1998).

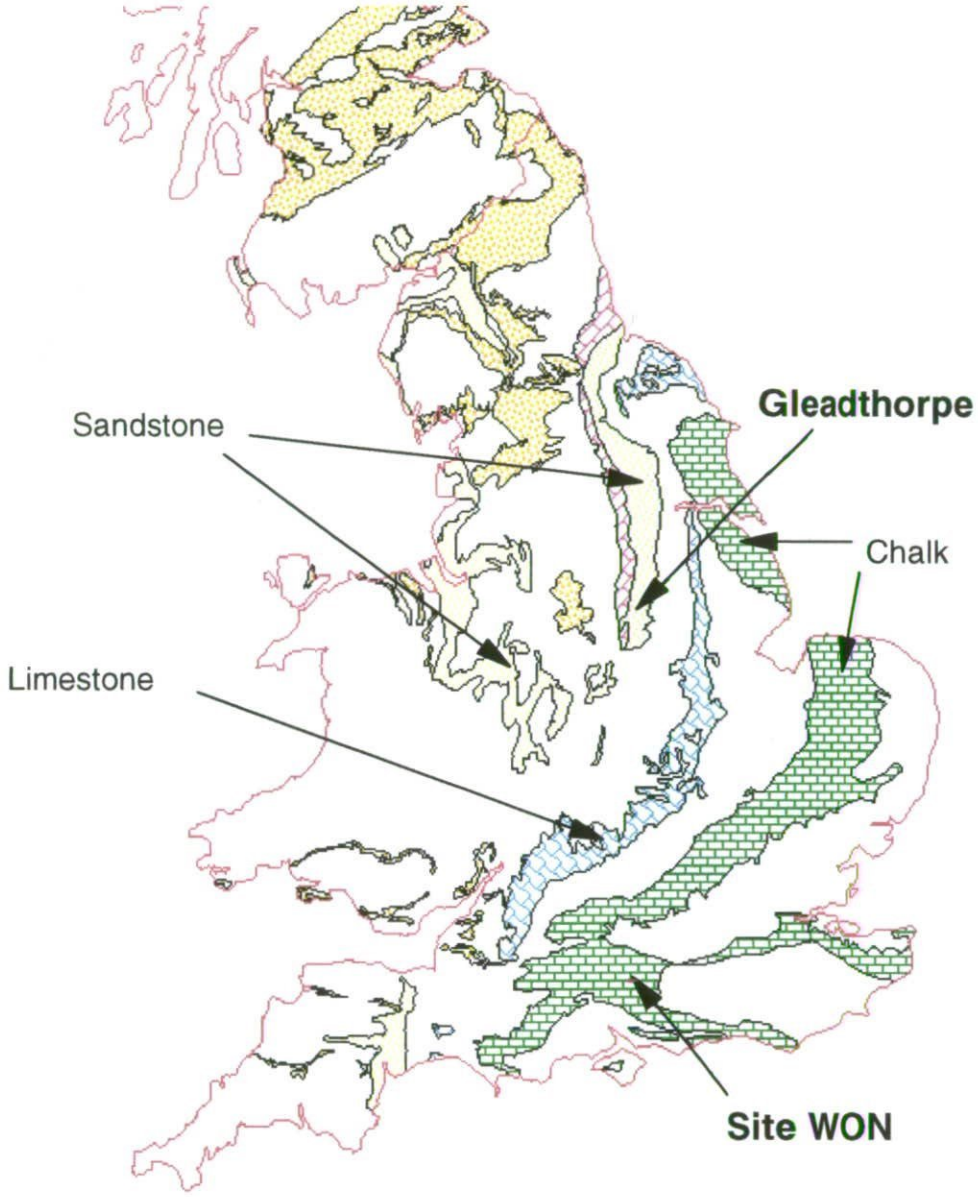

Figure 1a. *Outcrops of aquifer rocks in England and Wales*

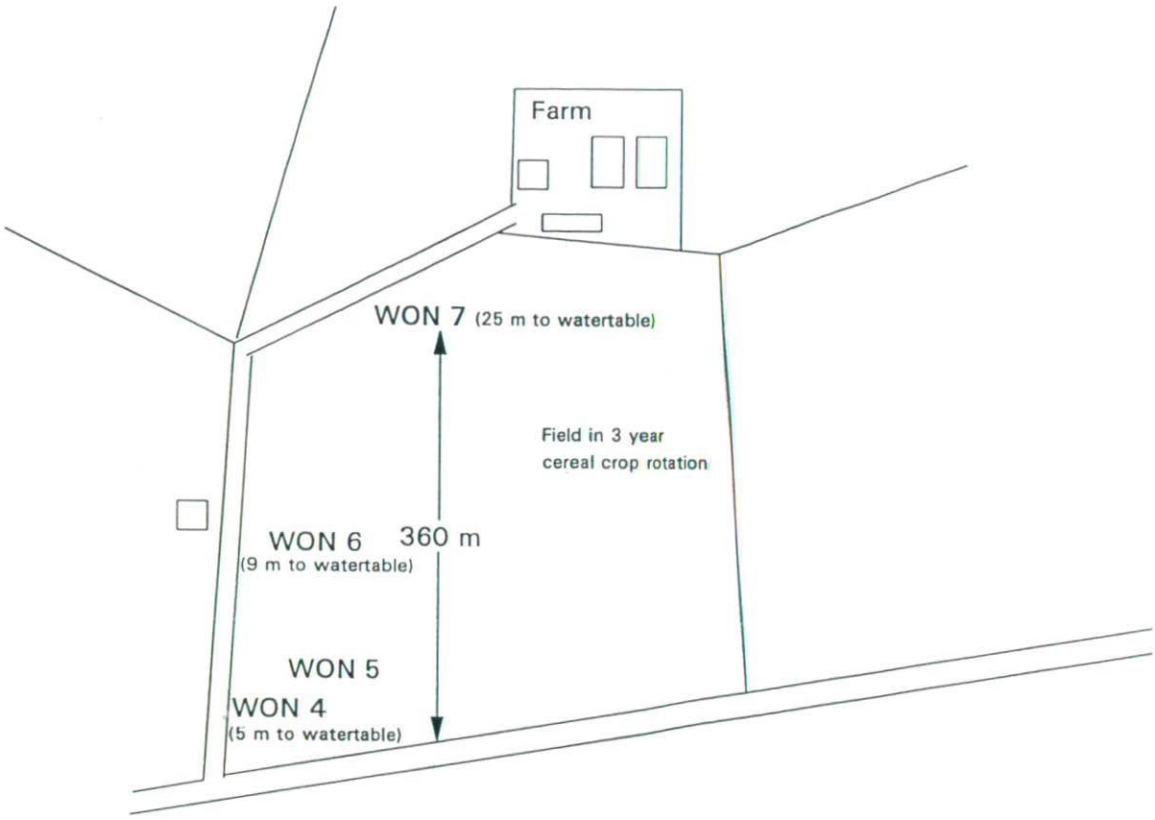

Figure 1b. *Site plan of the WON fieldsite showing location of the boreholes*

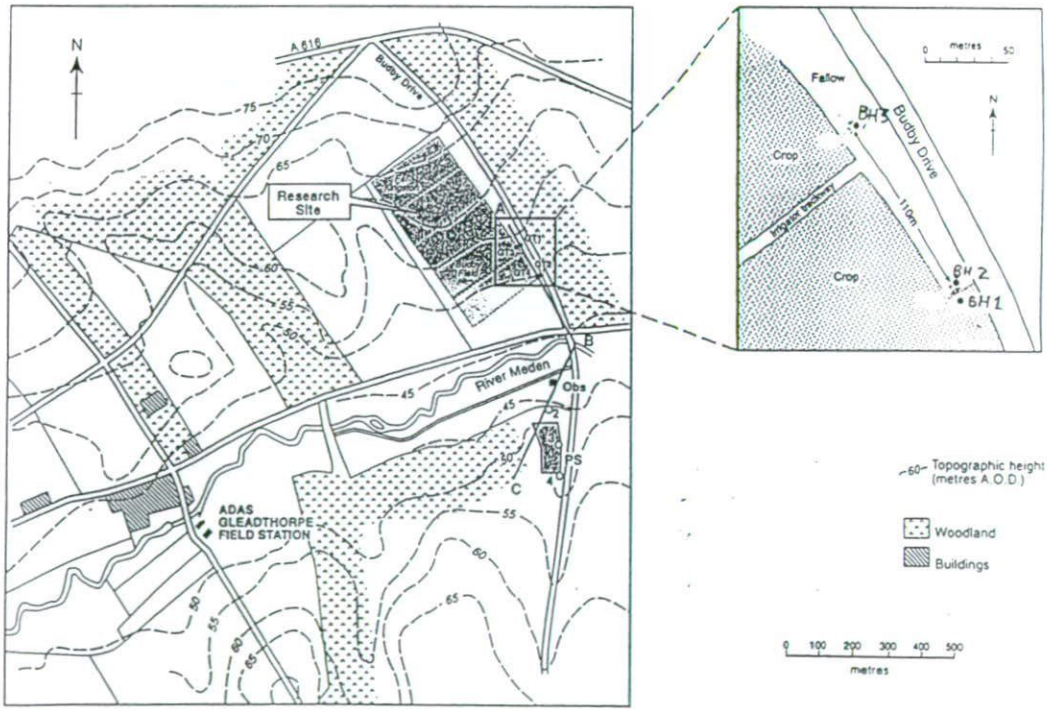

Figure 1c. *Site plan of the Gleadthorpe field site with position of boreholes*

2.4 DEGRADATION STUDIES

2.4.1 Screening experiment

To study the degradation potential of isoproturon, atrazine and mecoprop in chalk, samples were obtained from chalk cores taken from the unsaturated (10.75 mbs) and saturated zones (19.35 mbs). At Gleadthorpe borehole 1 (BH 1) the equivalent samples were taken from the unsaturated 5.29 mbs and saturated 7.35 mbs zones. After carefully removing the caps, the exposed outer end of the core was removed and discarded before the sample was taken from the centre of the core with a presept-sterilised spatula. 10 g of sample was added to triplicate pre-sterilised 100 ml containers (Sterilin). 30 ml of groundwater (collected 3 days previously) was then added aseptically to the containers. For each herbicide 6 mg L^{-1} stocks were made up in water and filtered through 0.45 µm PTFE filters (Gelman). From these stocks 0.5 ml was added to the appropriate containers to give a final concentration of 100 µg L^{-1}. The combinations of herbicides, sterile and non-sterile chalk and groundwater are given below in Table 1.

Table 1. *Treatments of chalk, groundwater and herbicide used in the screening experiment. Each letter refers to the code for that treatment used in the experiment.*

	Isoproturon 100 µg L^{-1}	Atrazine 100 µg L^{-1}	Mecoprop 100 µg L^{-1}
Sterile chalk, sterile groundwater	A	A	A
Sterile chalk, non-sterile groundwater	B	E	H
Non-sterile unsaturated zone chalk, sterile groundwater	C	F	I
Non-sterile saturated zone chalk, sterile groundwater	D	G	J

For treatments requiring sterile chalk or groundwater, these were prepared by autoclaving (121°C, 15psi for 15 min). The containers were not shaken but maintained under aerobic conditions in the dark at 20°C for 203 days. Samples of 1.5 ml were removed from the containers by sterile disposable pastettes and added to 2 ml syringes. The samples were then filtered through 0.45 µm PTFE filters prior to analysis by high performance liquid chromatography (HPLC). This experiment with site WON material started on 28,11,96, and with Gleadthorpe material on 22,10,97.

2.4.2 Isoproturon degradation comparative borehole experiment

Previous research conducted on degradation potentials of isoproturon (Johnson *et al.*, 1998) has shown that organisms in groundwater can degrade the compound provided that a solid matrix is present. On the same date (13,1,97) groundwater samples were taken from four boreholes (WON 4-7) located within 100 m of each other. This was also done for the three boreholes at Gleadthorpe (BH 1-3) on 2,10,97. From each borehole sample, 30 ml sub-samples were added to triplicate 100 ml sterile containers (Sterilin). These containers also held 5 g sterile chalk (sterile sandstone for Gleadthorpe). Isoproturon was added to each container to a final concentration of 100 µg L^{-1}, as described above. Three controls

contained sterile chalk, or sandstone with sterile groundwater. Samples for isoproturon analysis were taken at weekly intervals over 141 days. This experiment was repeated with site WON groundwater on 21,5,97 and 5,3,98, and with Gleadthorpe groundwater again on 5,6,98.

2.4.3 Degradation potential of the local soil for the herbicides isoproturon, atrazine and mecoprop

These experiments were set up in a similar way to the groundwater/aquifer material experiments to act as positive controls. Illustrating the capacity of the topsoil to degrade the hebicides and allowing comparisons with the deeper unsaturated and saturated layers to be made. Fresh topsoil was collected by scraping 500-1000 g into bags from the top 5 cm of soil near the boreholes at site WON and Gleadthorpe. Samples of 5 g of soil were added to the 100 ml Sterilin containers and mixed with 30 ml of 10 mM $CaCl_2$. Sterile controls were autoclaved for 30 min. The herbicides were added from the same stocks described above, to give final concentrations of 100 µg/L. Each treatment had 3 replicates, incubation conditions were the same as for the groundwater experiments.

2.4.4 Examination of the potential of soil bacteria to degrade isoproturon in the groundwater environment

Approximately 500 g of soil was collected from the top 5 cm at the WON fieldsite on 4,6,97. After the sample was mixed 3 g was taken and added to a 100 ml Sterilin container and mixed with 3 g sterile chalk and 30 ml sterile groundwater, thus making a 10^{-1} dilution. After vigorous shaking 3 ml was transferred to another container and mixed with 3 g sterile chalk and 27 ml sterile groundwater, thus representing a 10^{-2} dilution of the original soil. In this manner additional dilution's were made to give 10^{-3}, 10^{-5}, and 10^{-7} of the original soil sample. A control contained sterilised soil. Isoproturon was added from a stock solution to give a final concentration of 100 µg L^{-1}, as described previously. The containers were incubated in the dark at 20°C and sampled fortnightly for the presence of isoproturon. The soil moisture content was calculated to be 6.7%.

2.4.5 Low isoproturon concentration experiment

On 22,5,97 groundwater was collected in a series of sterile 1 L bottles from borehole WON 4. The number of viable heterotrophic bacteria present in the groundwater were counted using TSA plates, as described previously. 2 L quantities were dispensed into sterile 2.5 L Quickfit round-bottomed flasks containing gas-flushing type heads. To give a 1:12 ratio of sterile chalk to groundwater, each flask contained 167 g of <4 mm autoclaved chalk. Three flasks contained non-sterile groundwater and a further three contained sterile groundwater (controls). From a 4.74 mg L^{-1} aqueous stock solution of isoproturon, 0.211 ml was added to each flask to give a final concentration of 0.5 µg L^{-1}. The flasks were then shaken manually to disperse the isoproturon before being allowed to settle for 3 h. The flasks were sampled by attaching peristaltic tubing to one of the L-shaped glass tubes in the flask head. This tube opened directly into the groundwater within the flask. Prior to use the peristaltic tubing was cleaned by pumping through 50 ml methanol, followed by ultra-pure water. The peristaltic pump withdrew 200 ml from the solution for analysis.

Before analysis the sample was concentrated with a solid phase extraction cartridge. The extraction cartridge (Varian mega bond elut C18) was then prepared by eluting one bed volume (4.5 ml) of acetone, one of methanol before cleaning with 2 x 4.5 ml ultra-pure water. 5 ml of the experimental solution was then passed through the cartridge. The cartridge was then air dried for 10 mins by inserting into the bung of a Buchner flask, and passing air through with a vacuum pump. The products were then eluted with 2 ml methanol. The flasks were then incubated in the dark in a constant temperature room 18°C (+/-2°C). Sampling was at monthly intervals.

This experiment was repeated with groundwater from BH 1 at Gleadthorpe on 5,6,98.

2.4.6 Unsaturated zone flow-through column experiment

The cores were obtained from the unsaturated zone of WON 7 in September 1996 by dry percussive coring. With this technique, the cores could be removed with the minimum of disturbance and contamination. The cores were recovered in 0.1 m diameter, 0.45 m long PVC liners. On recovery the cores were capped and sealed and returned to the laboratory the same day. On arrival at the laboratory, cores were stored vertically at 4°C. Cores B (6.85-7.07 m), C (7.95-8.18 m) and D (8.18-8.40 m) all contained solid chalk. Core A (6.62-6.85 m) contained a large flint at the top and the consistency of the chalk was less solid than in the other three cores.

2.4.6.1 Setting-up and running the columns

The four 0.225 m long, 0.1 m diameter cores were set up to simulate conditions in the unsaturated zone of the Chalk aquifer. Nylon netting (1 mm mesh) was tied around the base of each of the cores to prevent chalk material falling out during column preparation. The cores were secured vertically in Buchner funnels and orientated as in the subsurface. A slight dip was created in the centre of the top of each column by scraping away a small amount of chalk. A thin layer of acid-washed sand was placed in this depression to encourage the leachate to disperse evenly over the central part of the surface of each column. The tops of the columns were capped with a plastic lid and the cap / core join was sealed with PVC electrical tape to reduce evaporation and pesticide volatilisation. Similarly the gap between the core and the Buchner funnel was sealed with PVC electrical tape.

2.4.6.2 Simulating conditions in the unsaturated zone

Unsaturated zone conditions were simulated by applying a suction of 1 kPa (0.1 m H_2O) to the base of each the columns. The suction in each of the flasks was monitored using a water filled manometer. A suction of 1 kPa (0.1 m H_2O) would be much lower than normally occurs in the Chalk unsaturated zone. A suction of 1 kPa (0.1 m H_2O) would empty pores greater than 300 μm in diameter. Field research conducted close to WON 4 shows that the average matric potential, during the 1995-96 season, at 3 m mbs is about 5 kPa (0.5 m H_2O). At the same site during the 1995-96 season the lowest matric potential at 3 m was 2 kPa (0.2 m H_2O). The cores used in this study were removed from between 6.62-8.40 m mbs in WON 6. The water table in WON 6 is normally around 9 m (+/-1) mbs. Therefore an applied suction of 1 kPa (0.1 m H_2O) should represent relatively low suction conditions within the Chalk at site WON.

2.4.6.3 Eluting the columns

The columns were eluted with groundwater pumped from WON 5. The groundwater was sterilised in an autoclave before use. It has been reported that strong interactions may occur between dissolved organic carbon in groundwater and pesticides, these interactions may influence pesticide mobility (Huber *et al.*, 1992). Therefore groundwater was used in place

of 2 mM $CaCl_2$. The columns were irrigated via a hypodermic needle which was inserted in the centre of the plastic lid. Columns B, C, and D were eluted for 105 days while column A was eluted for only 89 days, due to pump failure at the start of the experiment. All columns were eluted at a rate of 11 ml day^{-1} using Watson Marlow 101U/R peristaltic pumps (Watson Marlow Ltd, Falmouth, UK) controlled remotely by a Campbell data logger (Campbell Scientific Inc., USA). This flow rate corresponds to a velocity through the core of approximately 1.25 m $year^{-1}$. This velocity is within the upper range of that expected in the unsaturated zone of the Chalk aquifer. An average recharge velocity within the Chalk unsaturated zone may be around 0.9 m $year^{-1}$ (Wellings & Bell, 1980, and Wellings, 1984). It should be remembered however, that recharge to the Chalk aquifer, through the unsaturated zone, normally takes place for only 3-5 months of the year, therefore the actual velocity during the months in which recharge is occurring will be greater than 1.25 m $year^{-1}$. The flow rate used in these columns may therefore be lower than occurs in the field. The columns were continuously monitored throughout the duration of the experiment. The eluent and leachate totals agreed well.

2.4.6.4 Applying the solutes and collecting the leachate

Once the columns had reached steady state, a pulse of isoproturon, mecoprop and bromide was applied to the top of each column. The pumps were switched off 1 hour before and after the isoproturon and bromide applications to ensure that ponding did not occur on the top of the columns. 1 ml of 1500 mg L^{-1} isoproturon, 1 ml of 1500 mg L^{-1} mecoprop (1.5 mg total) and 1 ml of 100 g L^{-1} potassium bromide solution (100 mg total) were applied evenly over the sand on the top of each column. After the solutes were applied the columns were immediately capped and sealed to reduce volatilisation losses.

The column leachate was collected in pre-sterilised boiling tubes positioned under the Buchner funnels in the Buchner flasks. The leachate was sampled every 2-3 days by carefully lifting the Buchner funnel and column off the Buchner flask and replacing the collecting tube. The volume of eluent was recorded. Immediately after collection the samples were filtered through 0.45 µm PTFE filters. Samples for herbicide analysis were stored in PTFE capped HPLC vials at 4°C. Samples for bromide analysis were stored in 30 ml disposable bottles (Sterilin) again at 4°C.

2.4.6.5 Extracting the bromide and herbicides

The cores were sectioned in order to assess the progress of the solutes through the columns and to assess the efficiency of recovery. Once irrigation had ceased, cores B, C, and D were removed from the column apparatus and frozen. Once frozen, the cores were cut horizontally into four equal sections. The chalk material from the four sections was then emptied into separate containers and weighed. The chalk in each container was thoroughly homogenised prior to extraction.

Bromide was extracted using the following method. 50 g samples were removed from each homogenised core quarter and placed in a 250 ml conical flasks with 100 ml of de-ionised water. The flasks were then shaken on a platform shaker for 24 hours. The slurry in the conical flasks was then transferred to 30 ml disposable bottles (Sterilin) and centrifuged at 3000 rpm for 20 minutes. The aqueous phase was then filtered through 0.45 Fm PTFE filters into 30 ml disposable bottles (Sterilin).

The herbicides were extracted using the same method as for bromide except that methanol was used in place of water and samples were shaken in a water bath at 40°C. Also the methanol samples were filtered straight through 0.45 µm PTFE filters in to re-sealing HPLC vials without centrifugation. Both the samples for bromide and herbicide analysis were stored at 4°C prior to analysis.

2.4.7 Unsaturated microcosm method developed to measure degradation potential in unsaturated zone

The unsaturated flow through columns (section 2.4.6) have the advantage of looking at undisturbed material under natural flow conditions. However, to be able to find the breakthrough peak using this method, high concentrations of herbicide have to be added. Thus, a new method was developed in which lower, more realistic herbicide concentrations could be used. Chalk core WON 16/7, 4.42-4.88 mbs was used. The casing was cut at 5 cm intervals with a sterile hacksaw blade. The sections were then broken, rather than cut, to preserve and not smear over the pores. The small core section were then carefully placed onto sterile Buchner funnels and bedded down on sterile silica flour, as described in 2.4.6. The core and Buchner funnel apparatus were placed on top of 2 L Buchner flasks. The same suction of 1 kPa (section 2.4.6) was generated by syphoning off water from the flask into another, lower, container, as described in section 2.4.6. In contrast to the previous method, isoproturon was not added as a spike but perfused through the cores in the eluting solution. This solution was sterile WON 5 groundwater containing 100 µg/L isoproturon, made up by adding 0.16 ml from a 600 mg/L isoproturon stock (in methanol) to 1 L containers of groundwater. For the sterile control a core was perfused with this solution which contained Presept (25 mg/L sodium dichloroisocyanurate). The cores were eluted with these solutions for 7 d at a rate equivalent to 5.7 mm/d (2.2 ml/h). The manometers were read each day to confirm that the cores remained under unsaturated conditions during the perfusion. Thus, the original pore water was replaced by groundwater containing isoproturon but the moisture content remained the same (23-24% by weight). The cores were then taken from the Buchner apparatus and placed in sterile mixing bowls where they were carefully mixed with sterile pestles under aseptic conditions. Sub-samples of 70-100 g were then carefully placed in 150 sterile glass conical flasks. These were then sealed with cellophane and then grease-proof paper and weighed before placing in the 20°C incubator. There was enough material for sacrificial sampling in groups of 4 at t=0, 100,150 and 200 d. This was done by adding 140-200 ml methanol (methanol:chalk ratio of 2:1) to the conical flasks, capping and shaking overnight in a 40°C waterbath. After allowing settling a 50 ml aliquot was taken off for concentration by rotary evaporation prior to analysis by HPLC.

2.5 DETERMINATION OF THE HERBICIDES, BROMIDE AND DISSOLVED ORGANIC CARBON (DOC)

After filtration samples were stored in PTFE capped HPLC vials at 4°C prior to analysis. The samples were stored no longer than one week prior to analysis. Samples were taken into the HPLC via a 150 µl loop. A C18 column Columbus (Phenomenex Ltd, UK) was used (2.1 mm x 25 cm) with a 35% acetonitrile eluent. Detection was made at 240 nm, and peak purity was checked by comparing the absorbance at 220 nm. The detection limit was 10 µg L^{-1}. Isoproturon, isopropyl aniline, isopropyl phenol, mono and di-demethylated isoproturon were used as standards (made up in 50:50 methanol: water).

Bromide concentrations were determined using ion chromatography (DIONEX, Camberley, UK). The eluent used was 1.8 mM sodium carbonate and 1.7 mM sodium bicarbonate. The regenerate used was 25 mM sulphuric acid. Detection was by electrical conductivity.

DOC was measured using a TOCsin II Aqueous Carbon Analyser (Phase Separations Ltd). The sample is pumped through a heated capillary inlet tube and forced into the oxidation furnace. The carbon dioxide formed is then mixed with hydrogen over a nickel catalyst to form methane. The methane, evolved from the original carbon, is measured using a flame ionisation detector.

3. Results and discussion

3.1 CHARACTERISATION OF THE CHALK AND SANDSTONE GROUNDWATER

The key features to note are the oxygen concentration (Table 2), which indicates saturation for the ambient temperature, and the neutral pH. This indicates that both the chalk and Sandstone aquifers are aerobic, as might be expected in an unconfined area. The neutral pH make it unlikely that the chosen herbicides would be subject to chemical hydrolysis (atrazine chemically stable between pH5.5-8.5, A. Walker *pers. comm.*). The low DOC, assuming it is not entirely recalcitrant, would maintain a heterotrophic population in the groundwater. The sandstone groundwater has a higher concentration of DOC than the chalk and higher concentration of ions, giving a greater conductivity.

Table 2. *Groundwater chemistry at the field sites (concentrations in mg L^{-1})*

Site	DOC	pH	SEC (µScm)	DO_2	Ca	Na	K	Mg	Si	HCO_3	SO_4	Cl	NO_3-N
Chalk	1.2	7.2	716	9.1	134	8.7	2.7	2.7	3.2	281	31.1	21.2	17.4
Sandstone	3.0	7.3	1427	10.3	140	56.1	7.8	89.1	4.4	126	88.4	220	49.3

3.2 NUMBER OF VIABLE BACTERIA IN THE GROUNDWATER AND AQUIFER MATERIAL

It is well known that viable counts underestimate the number of bacteria present, however they serve a useful service as a comparative tool (Johnson et al., 1998). The highest counts obtained can be found in the soil with some ten million bacteria per gram. For both the deep unsaturated zones at WON (10.7 m) and at Gleadthorpe (5.3 m) the counts are very low, only a few thousand per gram, but clearly microbes are not absent. The counts were generally higher in the saturated zones, with the Gleadthorpe saturated zone and groundwater having around ten times more bacteria than at site WON. Comparison of counts from the same boreholes at site WON illustrate a fluctuation in the numbers, 4 times less or 7 times greater, depending on the borehole, over a 10 month period.

Table 3. *Comparison of numbers of viable bacteria (cfu/ml) in the in the site WON and Gleadthorpe (BH 1-3) groundwater samples*

Date	WON 4	WON 5	WON 6	WON 7	BH 1	BH 2	BH 3
14.5.97	3.65×10^4	5.4×10^4	2.5×10^4	1.65×10^4	ND*	ND	ND
23.5.97	6.5×10^4	ND	ND	ND	ND	ND	ND
2.10.97	ND	ND	ND	ND	7.12×10^3	3.93×10^3	2.58×10^3
5.3.98	1.62×10^4	1.62×10^4	4.08×10^4	7.25×10^4	ND	ND	ND

*ND not done

Table 4. *Comparison of numbers of viable bacteria (cfu/g) at the site WON (borehole WON 7) and Gleadthorpe (BH 1) in the solid material*

Date	WON soil	WON 10.7 m	WON 19.3 m	Gt soil	Gt 5.3 m	7.35 m
28.11.96	1.75×10^7	2.6×10^3	6.75×10^4	ND	ND	ND
30.9.97	ND	ND	ND	1.04×10^7	4.6×10^3	1.21×10^5

3.3 DEGRADATION STUDIES

3.3.1 Soil herbicide degrading performance

Examination of the performance of the topsoil at site WON (Fig. 2), and Gleadthorpe (Fig. 3) illustrates an ability of the soils to biodegrade all 3 herbicides. The degradation rate of the mecoprop and atrazine, may be seen as rather slow compared to literature field values, however, there is no history of mecoprop use at site WON, or atrazine being applied at either field site. The microcosm experiment was not meant to represent field conditions, but rather act as a positive control.

3.3.2.1 Site WON screening experiment for isoproturon

The screening test was conducted on material extracted from a borehole at the top of the fieldsite, with a depth of 17 mbs to the watertable. Therefore, a large quantity of chalk separated the part of the unsaturated zone (10.7 mbs) and saturated zone (19.3 mbs) from the soil. The concentration of isoproturon in the sterile control (Fig. 4a) did not decline, but showed a slight increase over the course of the experiment. This is likely to be due to some evaporation of the water occurring, which then increases the (non-volatile) solute concentration. Very little change was seen in the concentration of isoproturon in the incubation with the 19.3 mbs chalk, taken from the saturated zone. Some decline in concentration was seen with the 10.7 mbs chalk (unsaturated zone). From the replicates for isoproturon at 20°C this would give half-lives of 139-273 d. Thus, the microorganisms of the unsaturated zone were active when the matrix was saturated. Further experiments into the potential for degradation in the unsaturated zone are described in section 3.4. The groundwater used in the experiment came from another borehole, WON 4, collected on 25.11.96 and did demonstrate the most rapid isoproturon degradation. All three replicates giving a half-life of 111 d. It is interesting to note that rapid degradation only began after 80 d.

3.3.2.2 Site WON screening experiment for atrazine

In contrast to the isoproturon data, the same samples did not degrade atrazine over the time course of the experiment (Fig. 4b). This is in contrast to the soil which did possess the capability to degrade atrazine. This implies atrazine which penetrated the soil layer would persist at this site.

It is perhaps worth noting that the subsurface and groundwater samples **do not have** the same microbial capabilities of the soil. This may be used as evidence to suggest that the subsurface zone has not been contaminated by soil.

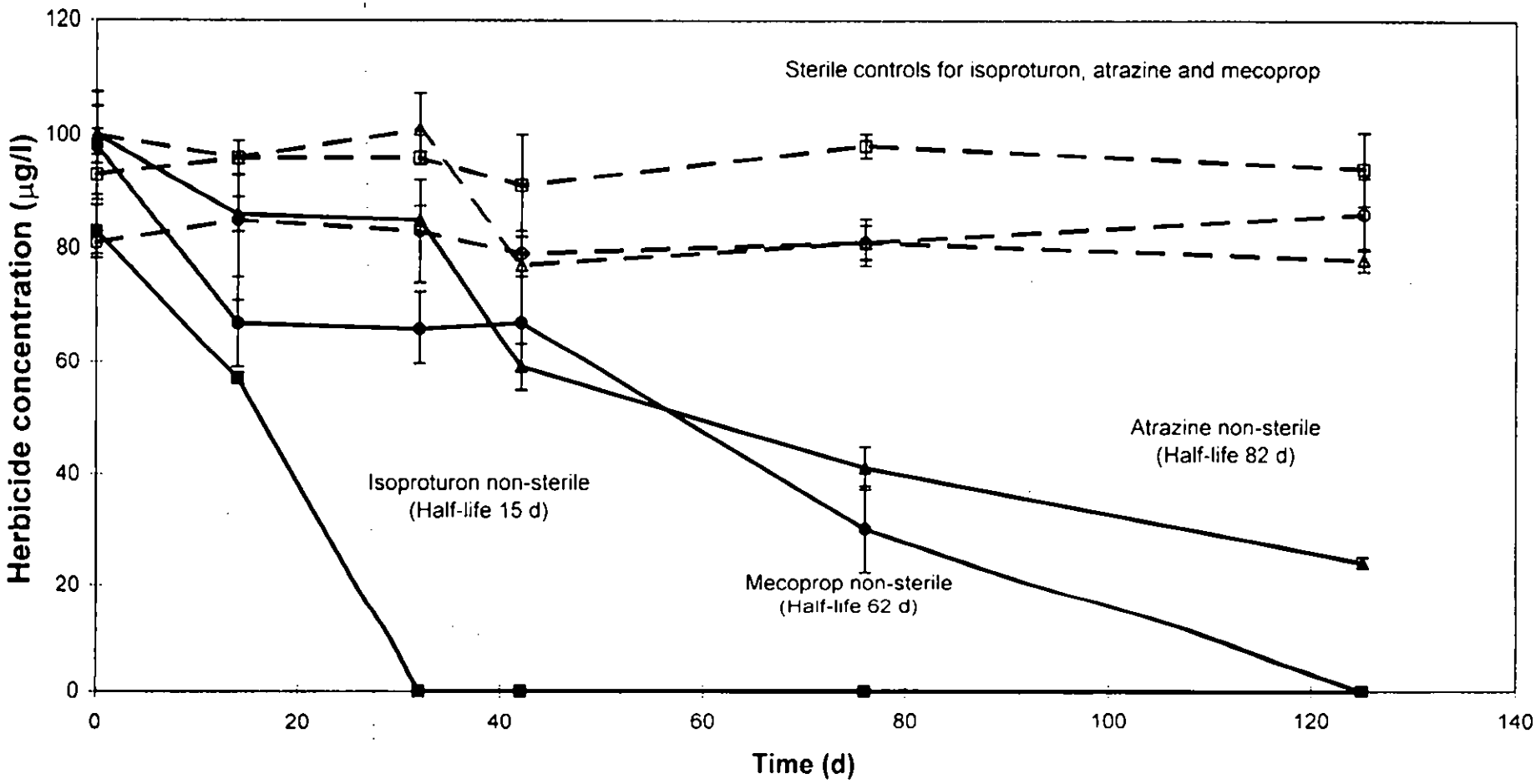

Figure 2. *Ability of topsoil from site WON to degrade isoproturon, atrazine and mecoprop. Microcosm incubations at 20°C (mean of 3 observations with standard deviations).*

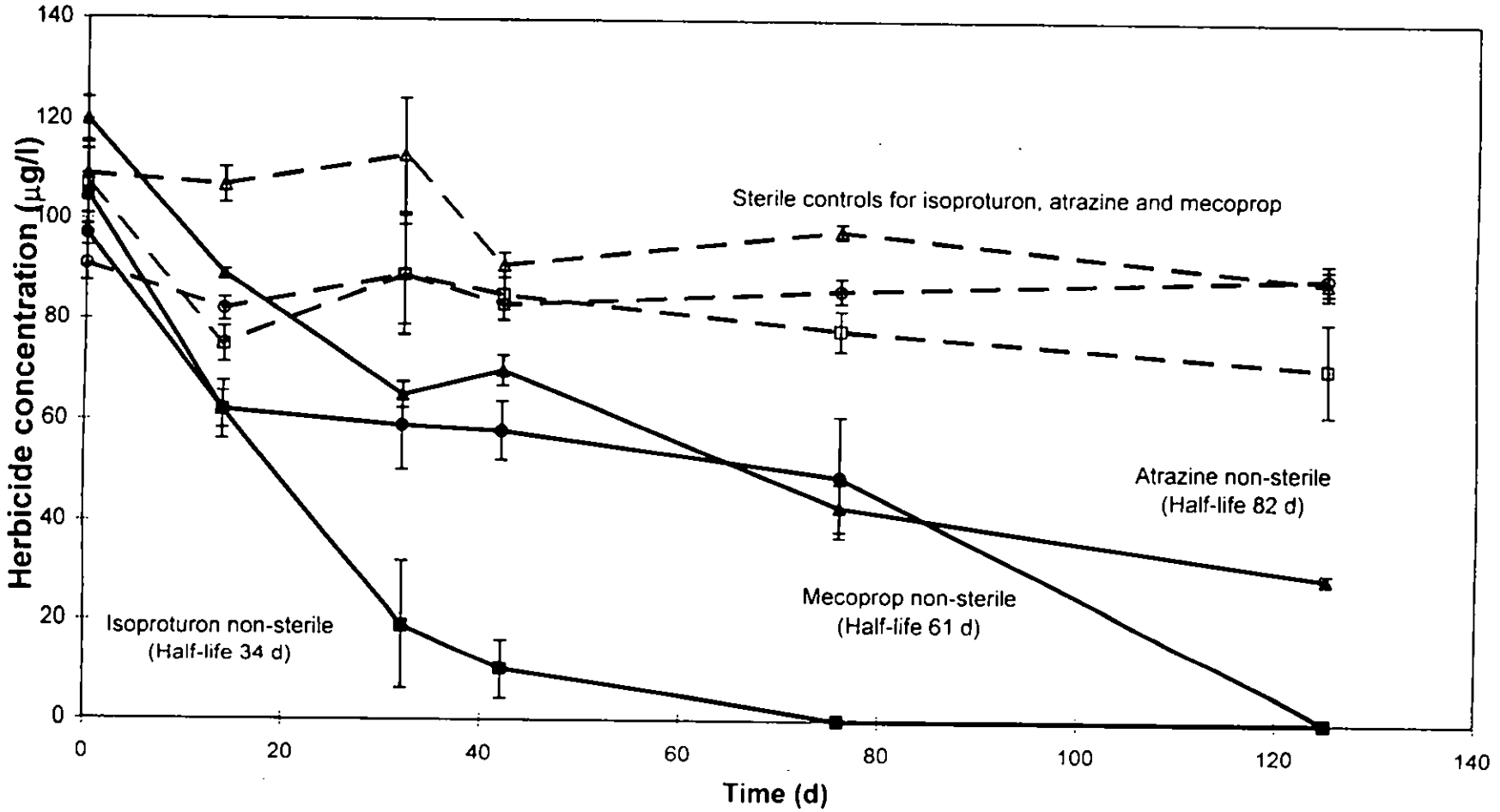

Figure 3. *Ability of topsoil from Gleadthorpe to degrade isoproturon, atrazine and mecoprop. Microcosm incubations at 20°C (mean of 3 observations with standard deviations).*

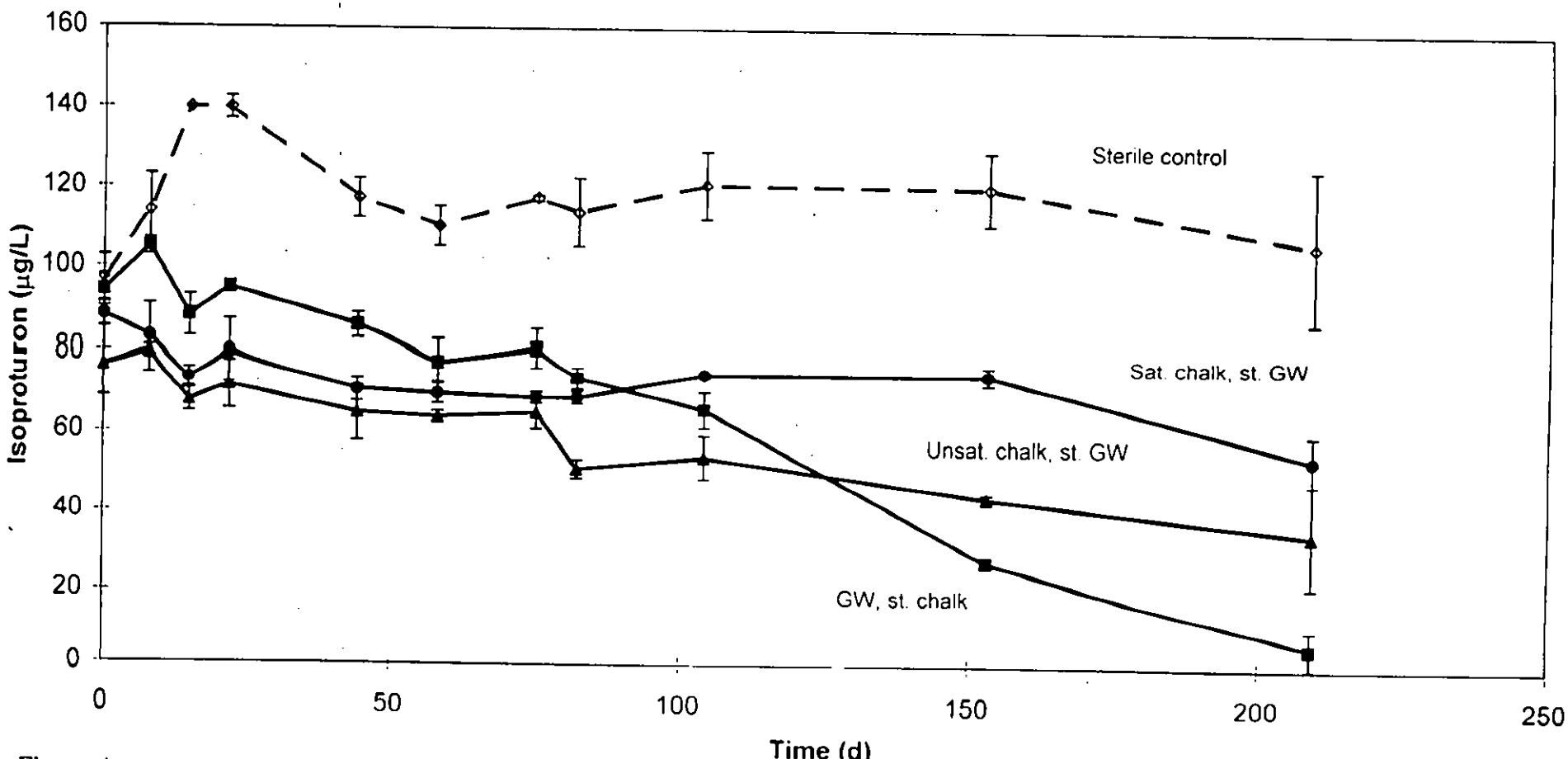

Figure 4a. *Ability of non-sterile groundwater (WON 4) in the presence of sterile chalk (GW st. chalk), non-sterile chalk (10.7 mbs) from the unsaturated zone (Unsat. Chalk st. GW), and non-sterile chalk (19.3 mbs) from the saturated zone to degrade* ***isoproturon*** *(mean of 3 observations with standard deviations).*

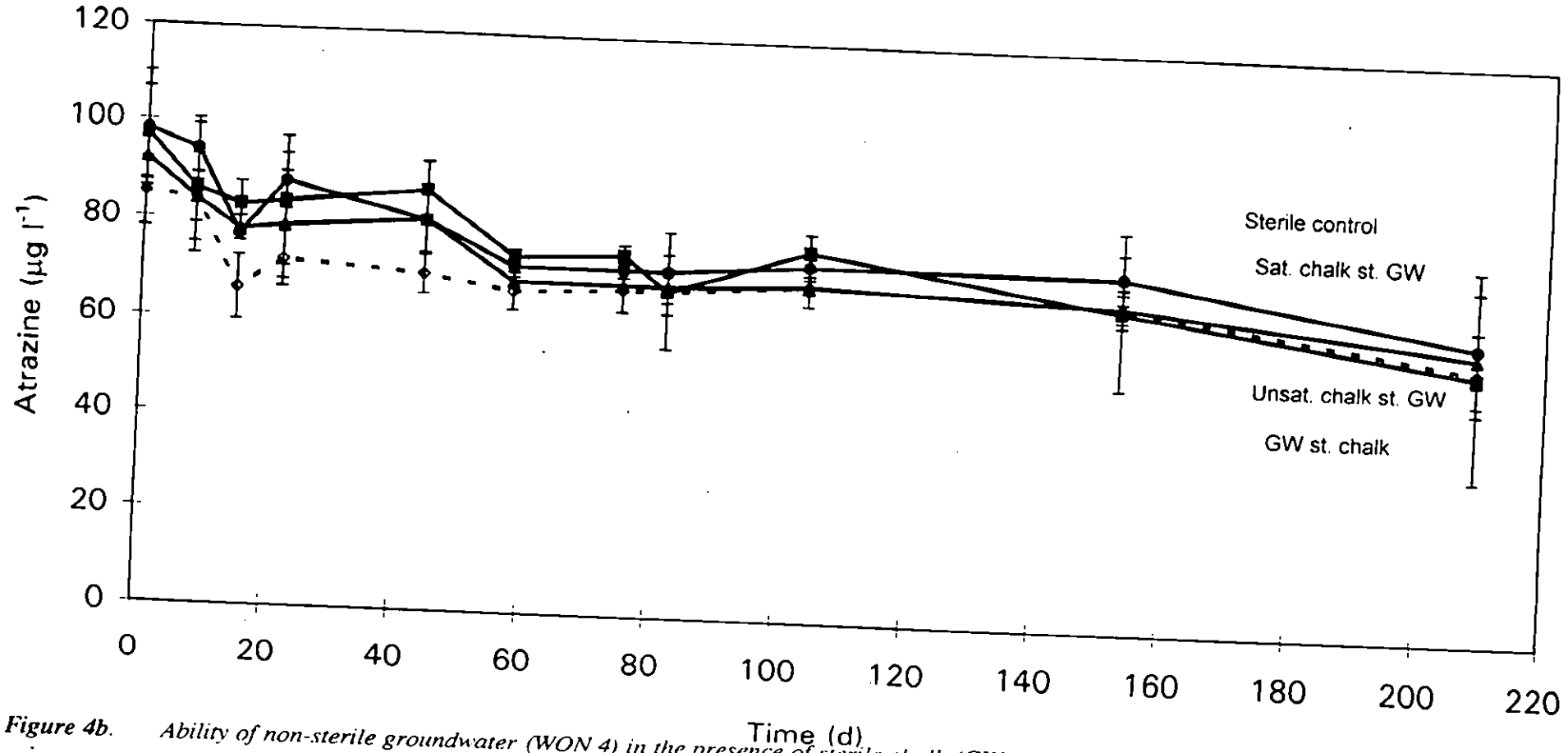

Figure 4b. *Ability of non-sterile groundwater (WON 4) in the presence of sterile chalk (GW st. chalk), non-sterile chalk (10.7 mbs) from the unsaturated zone (Unsat. Chalk st. GW), and non-sterile chalk (19.3 mbs) from the saturated zone to degrade* ***atrazine*** *(mean of 3 observations with standard deviations).*

3.3.2.3 Site WON Screening experiment for mecoprop

The experiment could not proceed beyond 160 d due to problems with the controls (Fig. 4c). However, as with atrazine, there was no evidence of mecoprop degradation in any of the subsurface samples. This appears to contrast with previous groundwater experiments in sandy material (Heron and Christensen, 1992 and Klint *et al.*, 1993) and chalk material (Hughes *et al.*, 1995) where mecoprop degradation was observed.

The stability of all the herbicides in the sterile groundwater/chalk controls suggests these compounds were stable in these pH conditions.

3.3.3 Gleadthorpe Screening experiment

This incubation is still continuing, the only evidence so far (100 d) for the existence of a biodegradation potential is for isoproturon with the Gleadthorpe groundwater (Fig. 5). As with site WON, the groundwater with sterile matrix and isoproturon gave the best example of biodegradation.

3.3.4 Site WON groundwater isoproturon degradation comparison experiment

This experiment was set up to examine the differences between isoproturon degradation in groundwater across a single field (1 ha). The groundwater was collected from boreholes 12-100 m apart on the same day (13,1,97). Compared to the sterile control, there appeared to be little change in isoproturon concentration with the samples from boreholes WON 4 and 6 (Fig. 6). However, there was a decline in isoproturon concentration with groundwater from boreholes WON 5 and 7 with a concomitant rise in mono-demethylated isoproturon. For WON 5, from the replicates for isoproturon at 20°C this would give half-lives of 141-640 d, from WON 7 better agreement was obtained between the replicates giving half-lives of 138-227 d. Previous field data from the NERC project conducted at this site had shown the groundwater from boreholes WON 4-6 to be contaminated with low concentrations of isoproturon, however, no pesticides were present in WON 7. Therefore, the level of isoproturon contamination found at a borehole is no predictor of the isoproturon degradation potential which may be found there. Another surprising feature is that the groundwater collected from borehole WON 4 on 25,11,96 did degrade isoproturon (Fig. 4a), but that collected on 13,1,97 did not (Fig. 6). As seen with the previous experiment (Fig. 4a), a lag phase of at least 70 d was required before significant isoproturon degradation occurred at 20°C.

This experiment was repeated with groundwater collected from the same boreholes on 14,5,97, however, the experiment was run for a shorter period (98 d) due to problems with the controls. Over this short period only WON 5 gave evidence of isoproturon degradation, which was confirmed by the appearance of the mono-demethylated metabolite. A fresh experiment was set up on 5,3,98. A comparison of the isoproturon degradation performance from the boreholes on the same site are shown in Table 5. This includes the data collected from the previous NERC project.

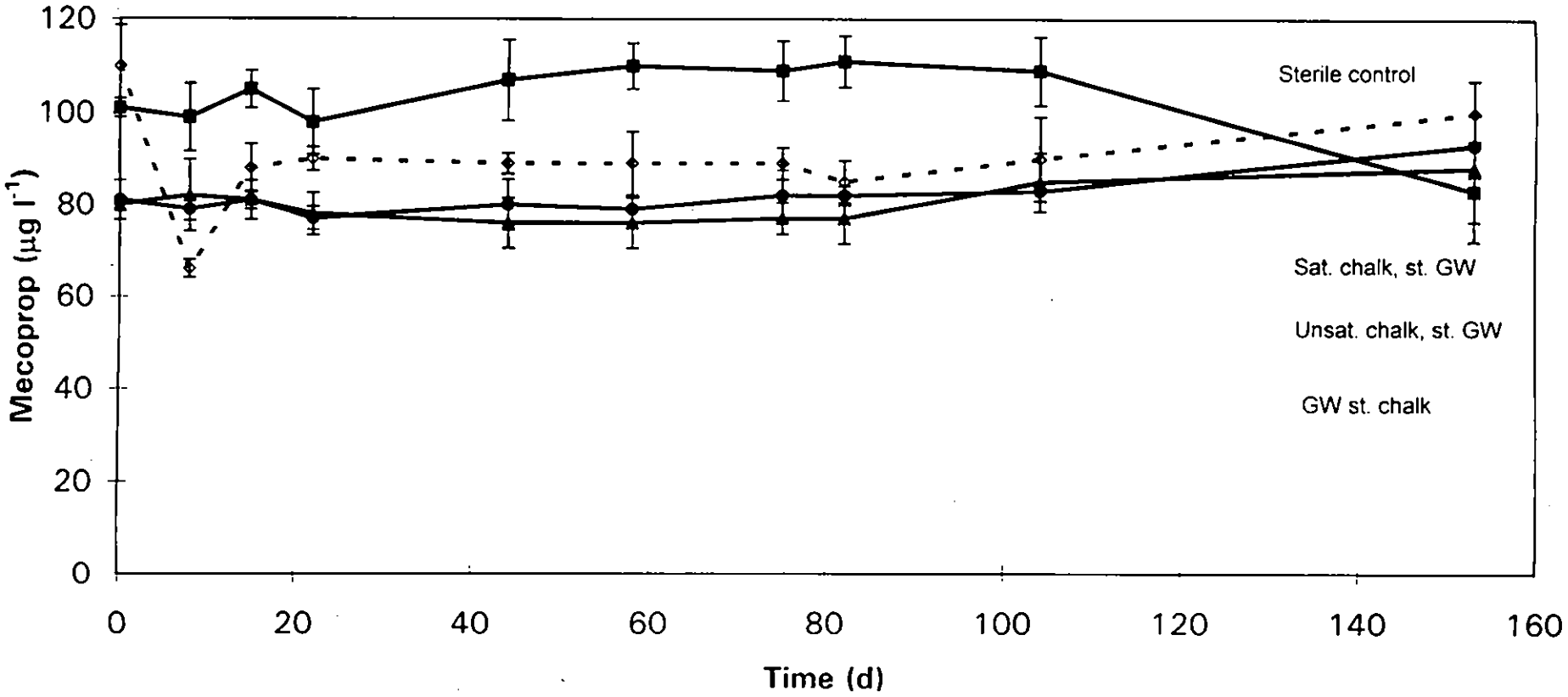

Figure 4c. *Ability of non-sterile groundwater (WON 4) in the presence of sterile chalk (GW st. chalk), non-sterile chalk (10.7 mbs) from the unsaturated zone (Unsat. Chalk st. GW), and non-sterile chalk (19.3 mbs) from the saturated zone to degrade* ***mecoprop*** *(mean of 3 observations with standard deviations).*

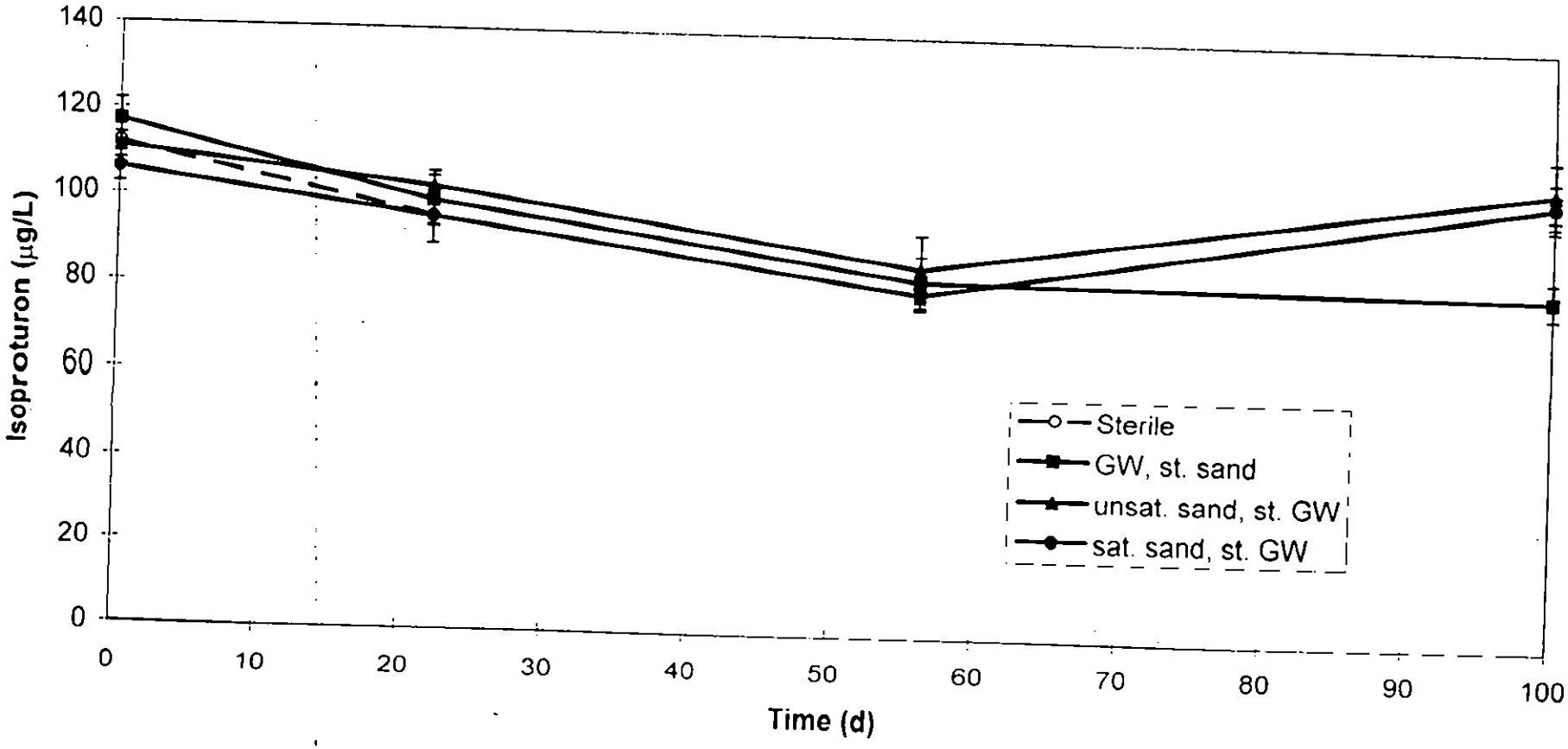

Figure 5. *Ability of non-sterile groundwater (BH 1) in the presence of sterile sandstone (GW st. sand), non-sterile sandstone (5.3 mbs) from the unsaturated zone (Unsat. sand st. GW), and non-sterile sandstone (7.3 mbs) from the saturated zone from Gleadthorpe to degrade* **isoproturon** *(mean of 3 observations with standard deviations).*

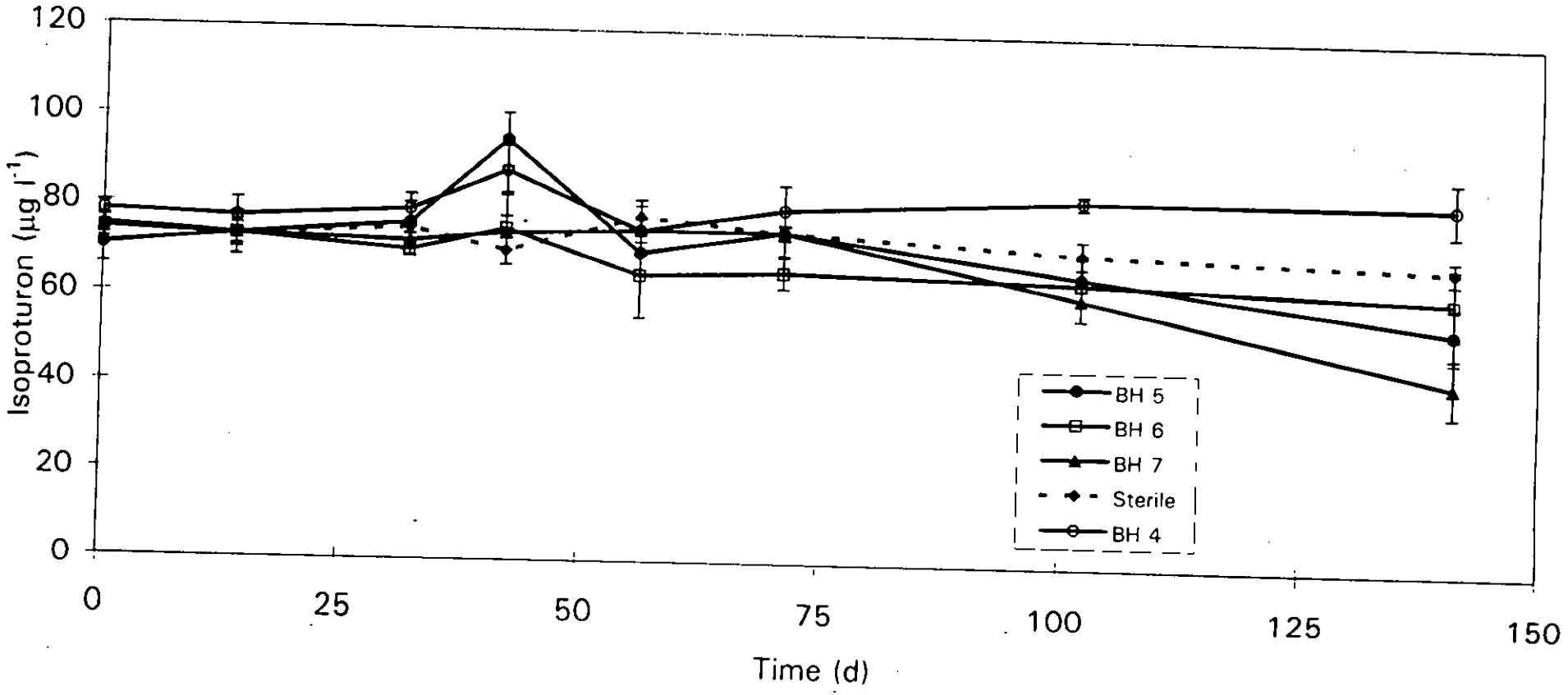

Figure 6. *Comparison of the* ***isoproturon*** *degrading ability of groundwater collected from 4 boreholes from site WON on the 13,1,97 (mean of 3 observations plus standard deviations).*

Table 5. *Comparison of isoproturon degradation (half-lives in days) from groundwater collected from the site WON boreholes and incubated at 20 °C*

Borehole	May 95	Nov 95	Nov 96	Jan 97	May 97
WON 4	7 d (7-20)	97 d (92-102)	104 (100-104)	None after 149 d	NC
WON 5	ND	362 (57-575)	ND	387 (227-641)	363 (163-540)
WON 6	ND	ND	ND	None after 149 d	NC
WON 7	ND	ND	ND	174 (138-227)	NC

ND Not done
NC Experiment terminated after 98 d due to contamination of controls

3.3.5 Gleadthorpe groundwater isoproturon degradation comparison experiment

Over a 100 d incubation period isoproturon degradation was observed with all three borehole groundwater samples (Fig. 7). This was confirmed by the generation of mono demethylated isoproturon from all non-sterile borehole samples (Fig. 8a, 8b, and 8c). This suggests a more active, or larger isoproturon degradation population exists at Gleadthorpe, than at site WON, something that may have been predicted by the higher indigenous DOC (Table 2) and 10 times higher microbial population (Tables 3 and 4). This experiment is being repeated with fresh groundwater from the Gleadthorpe boreholes collected on 4,6,98.

3.3.6 The performance of site WON soil dilution's in groundwater experiment

The degradation of isoproturon by increasing dilutions of soil is shown in Fig. 9. Since it is effectively a smaller number of the same bacteria in each sample, it can be shown that the lag phase is not related to a delay prior to enzymatic induction, but rather to the need for cell multiplication before an impact on isoproturon concentration is detected. The metabolite generation of the 10^{-1} dilution is shown in figures 11a and 11b. The isoproturon degradation curve of the 10^{-5} soil dilution was similar to some of the raw groundwater samples, and in addition these low number of soil bacteria also generated mono-demethylated isoproturon, like the groundwater samples.

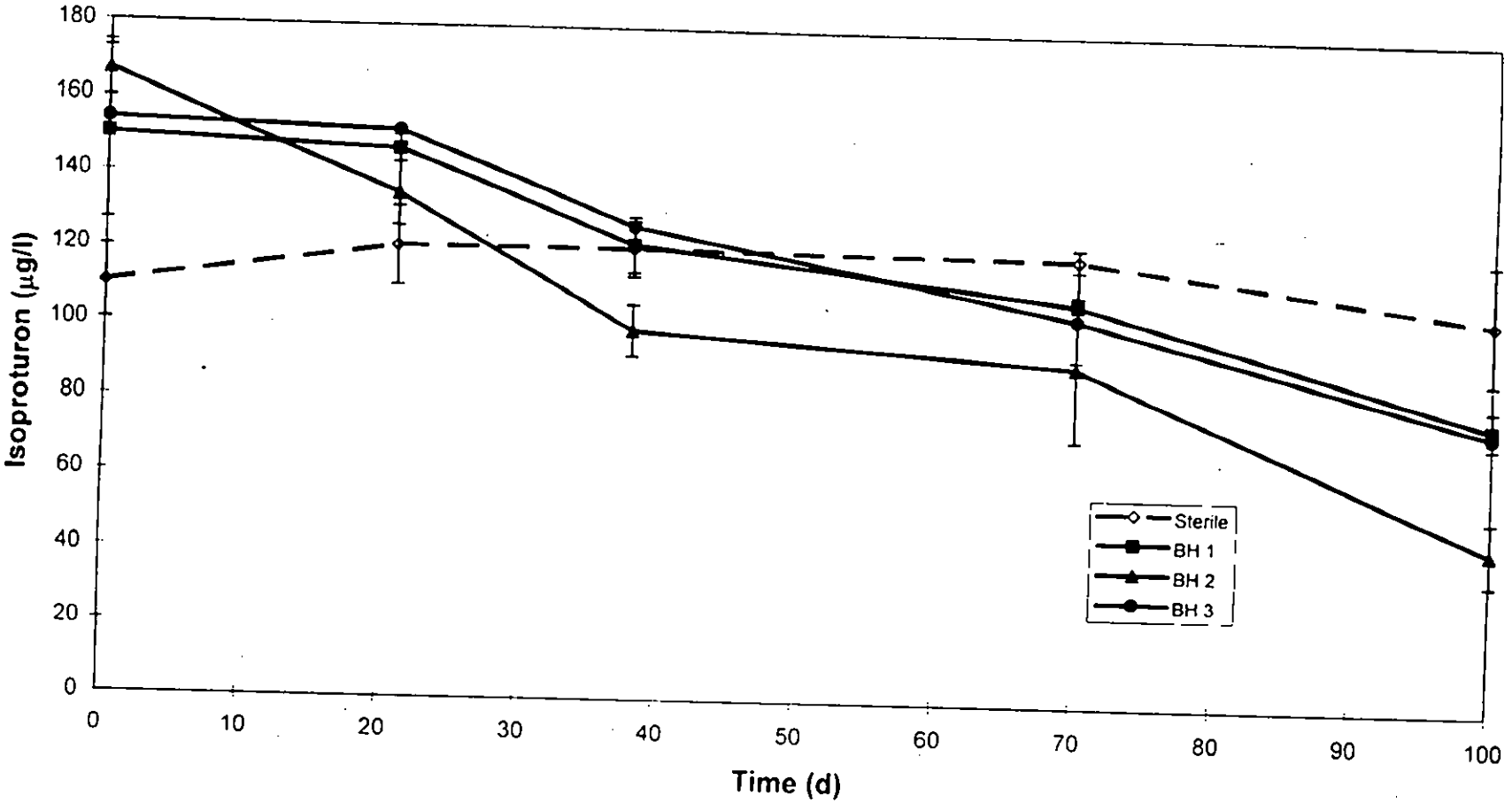

Figure 7. *Comparison of the* ***isoproturon*** *degrading ability of groundwater collected from 3 boreholes from Gleadthorpe on the 2,10,97 (mean of 3 observations plus standard deviations).*

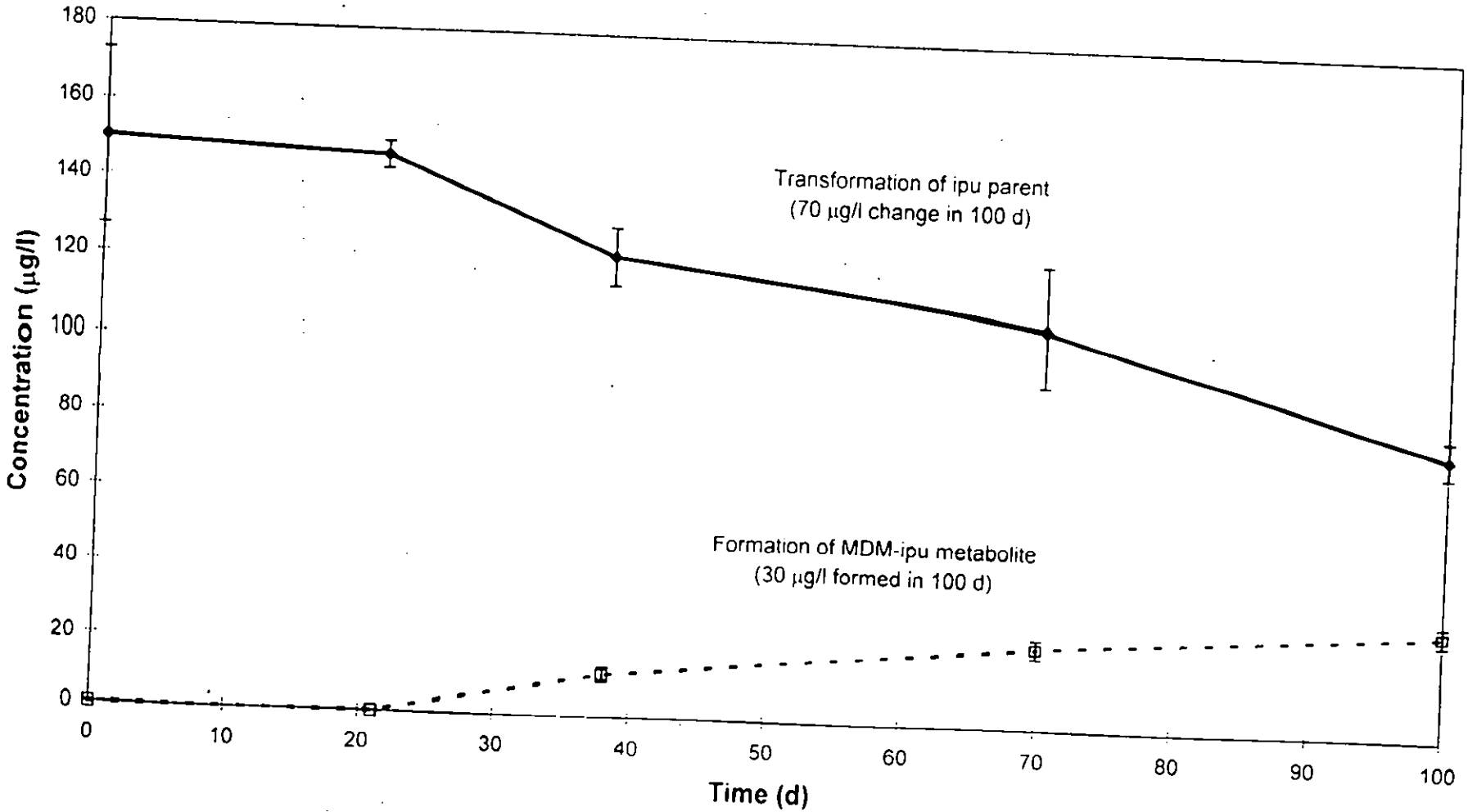

Figure 8a. *Comparison of isoproturon degradation with the generation of the metabolite mono demethylated isoproturon from Gleadthorpe BH 1 (mean of 3 observations plus standard deviations).*

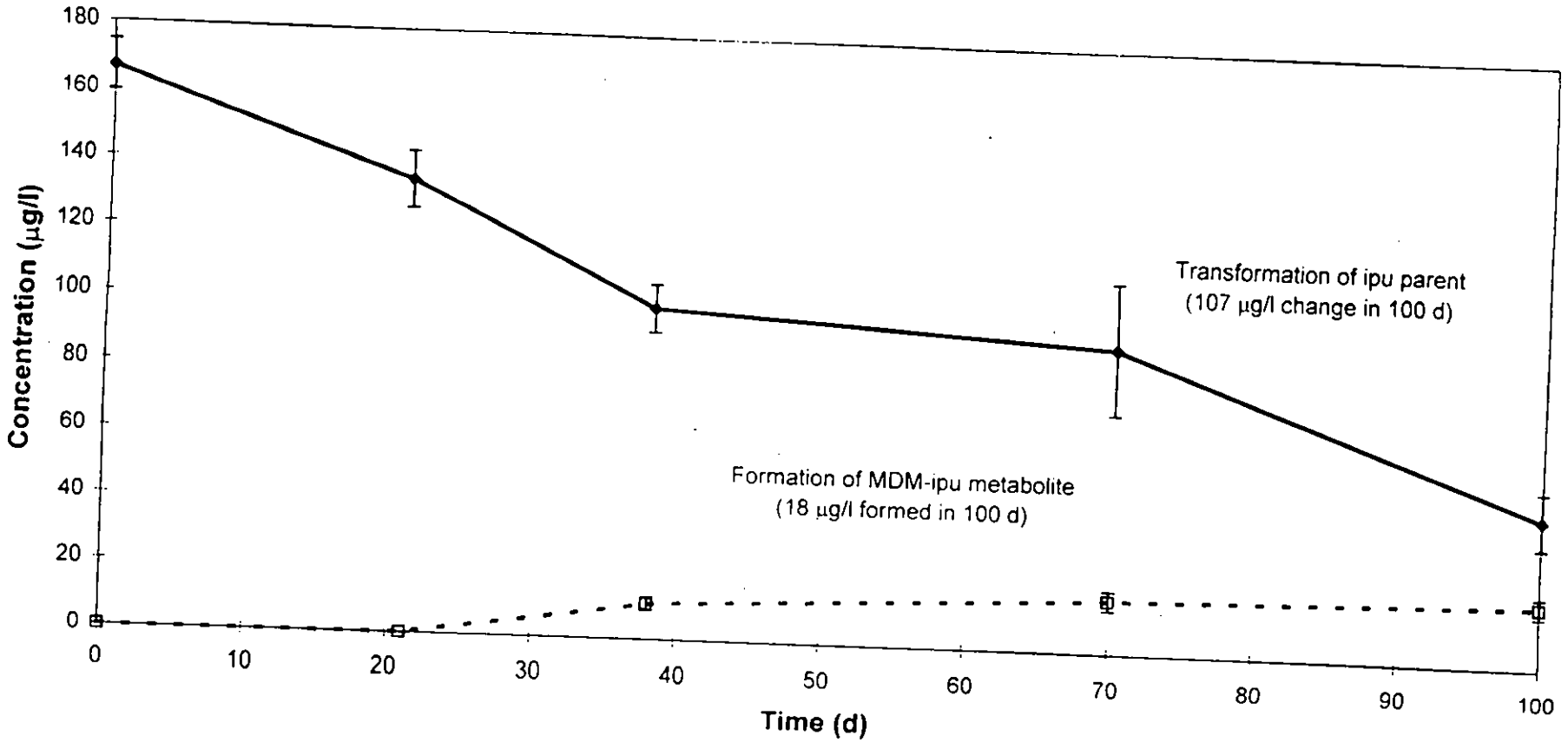

Figure 8b. *Comparison of isoproturon degradation with the generation of the metabolite mono demethylated isoproturon from Gleadthorpe BH 2 (mean of 3 observations plus standard deviations).*

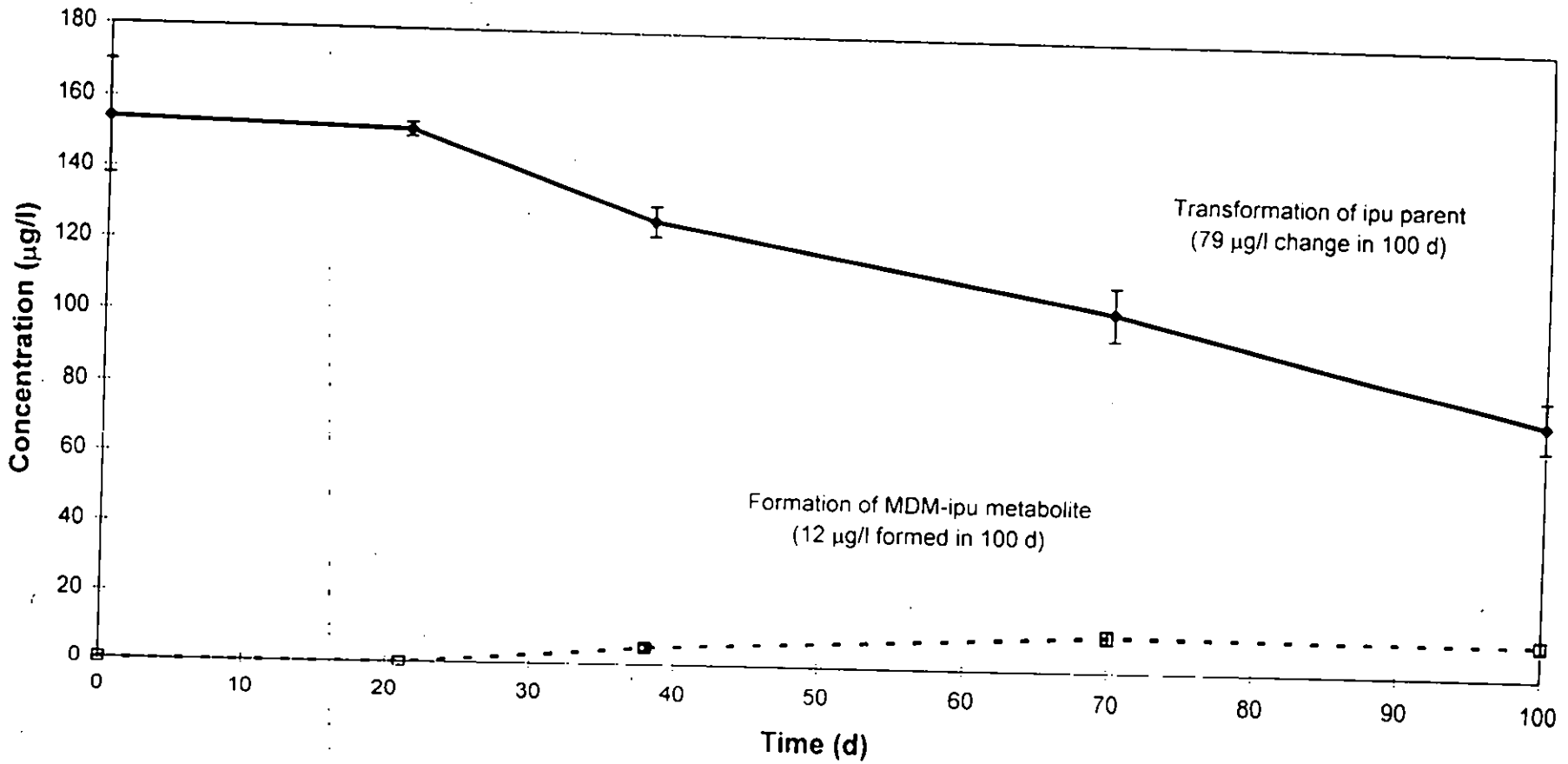

Figure 8c. *Comparison of isoproturon degradation with the generation of the metabolite mono demethylated isoproturon from Gleadthorpe BH 3 (mean of 3 observations plus standard deviations).*

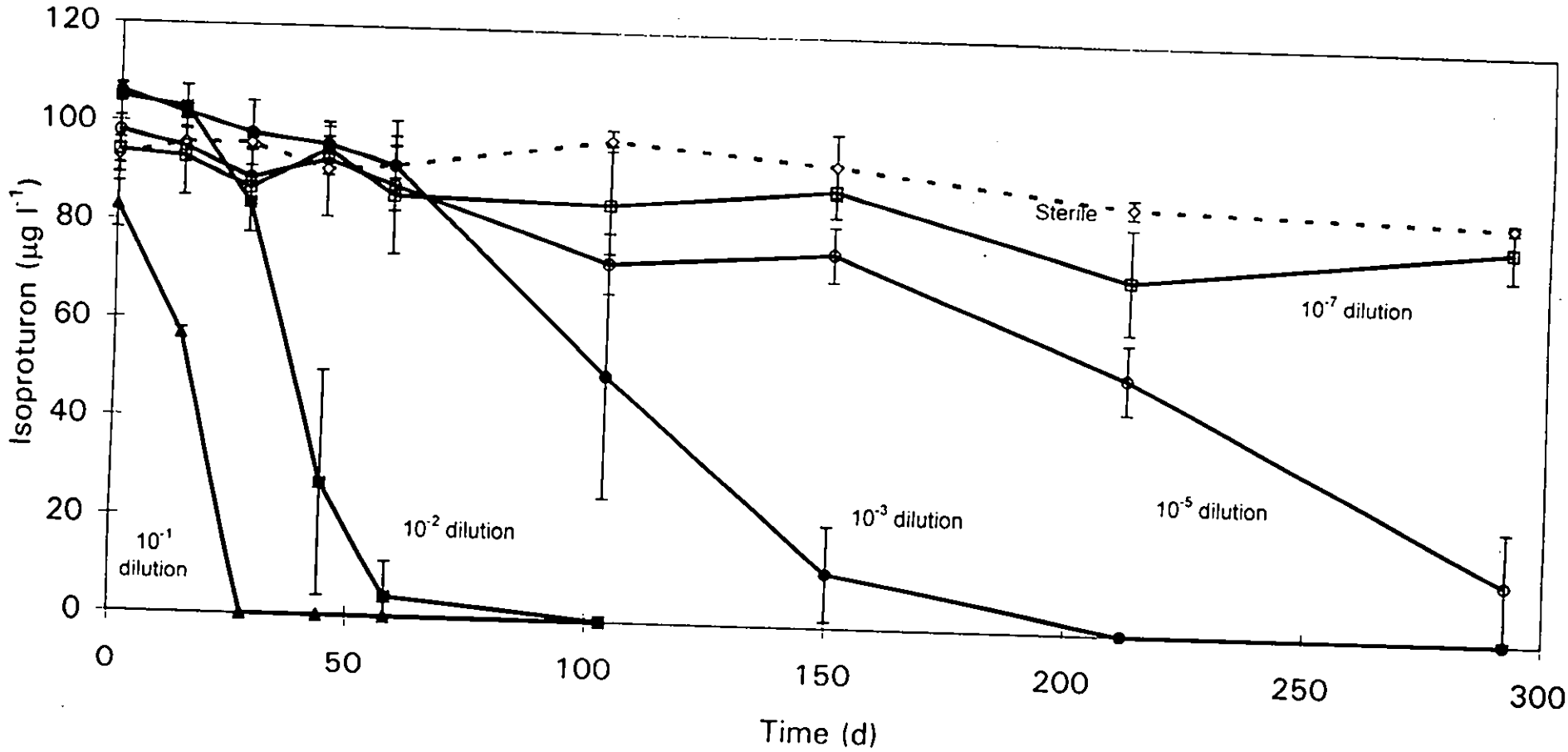

Figure 9. *The ability of increasing dilution's of topsoil from site WON to degrade isoproturon in a chalk/groundwater matrix (mean of 3 observations plus standard deviations).*

3.3.7 Low isoproturon concentration

The experimental set up was successful in being able to monitor low concentrations of isoproturon (Fig. 10). However, there was little evidence of degradation over the 350 d incubation period. This cannot be accepted as a definitive answer to whether pesticides can be degraded at the low natural concentrations, because of the noted variability of different groundwater samples to degrade 100 µg/L isoproturon (Table 5). Clearly this is a key experiment for the whole groundwater question and needs to be repeated, this is being done with Gleadthorpe groundwater.

3.4 UNSATURATED ZONE SPECIALISED EXPERIMENTS

3.4.1 Unsaturated zone column flow-through experiment

This experiment used 20 cm core sections obtained from the unsaturated zone of WON 7. These core sections were spiked with isoproturon, mecoprop and bromide before eluting with sterile or non-sterile groundwater (1.1-1.2 mm/d) from WON 5 for a period of 162 d. Over the experiment 2.3 pore volumes were eluted, the peak concentration of the solutes occurring around 1 pore volume. A retardation factor of around 1.3 for isoproturon with respect to bromide was calculated. A mass balance of the solutes is given in Table 6. The bromide recovery varied from 92-99%. That for mecoprop varied between 82 and over 100%, suggesting that little or no mecoprop was lost through degradation in the columns. However, for isoproturon the recoveries varied from 48-79%. The average of the two columns eluted with sterile groundwater was 72% (SD 9.2) and for non-sterile groundwater was 54% (SD 9.2). This implied, assuming extraction's and analysis were successful, that some isoproturon had been lost through degradation in the unsaturated zone. Although it is difficult to calculate half-lives, for column D, the 162 d period accounted for slightly over 50% of the compound. It may be that the high concentrations used in this experiment (spike 10-20 times normal concentration used in microcosms) stimulated degradation, where the lower concentrations are less effective in promoting degradation.

Table 6. *Solute mass balance percentages (both leachate and total residues left in columns) from the 4 columns (A and B columns had sterile groundwater and columns C and D had non-sterile groundwater).*

Solute	A	B	C	D
Bromide	99	98	98	93
Mecoprop	82	94	127	98
Isoproturon	79	67	61	48

3.4.2 Unsaturated microcosm experiment

The concentrations shown in Table 7 for the non-sterile treatments illustrate the consistency which was achieved in perfusing the 4 chalk core samples with isoproturon. A problem occurred with the sterile controls, possibly due to the sterilising chemical reacting with the isoproturon. However, it can be seen that over 150 d, maintained under unsaturated conditions, no isoproturon degradation has occurred in the unsaturated zone chalk. This persistence of isoproturon in the unsaturated zone chalk contrasts with the loss of isoproturon during transit in the 162 d flow-through experiment (Table 6), and from the

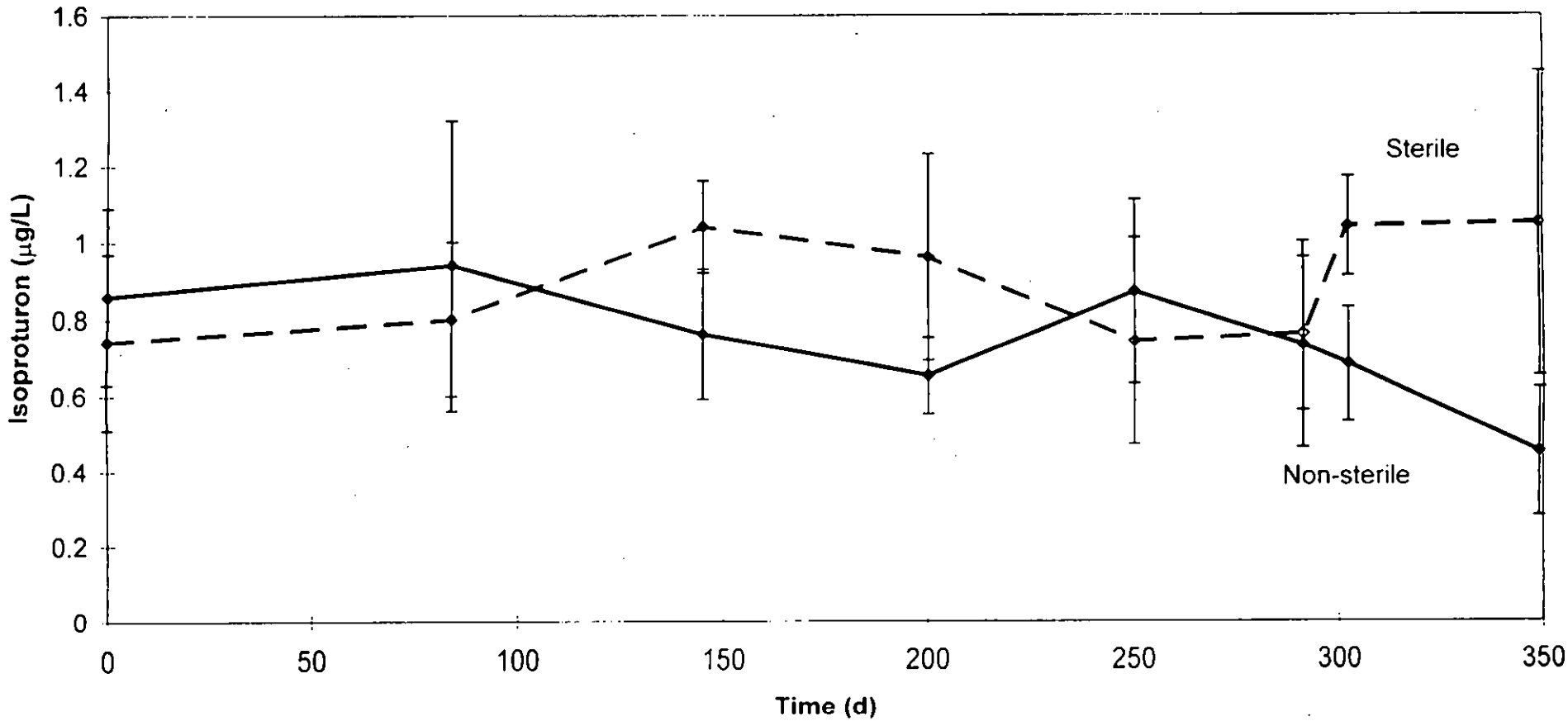

Figure 10. *The ability of groundwater from WON 4 (22.5.97) to degrade a low concentration of isoproturon (mean of 3 observations plus standard deviations).*

initial screening experiment (section 3.3.2.1) where the chalk was saturated. However, in the flow through experiment much higher concentrations of isoproturon were present than in the static microcosm experiment. Although not tested, the high concentrations of isoproturon (a pulse of almost 2 mg/L) may stimulate degradation, whereas lower concentrations (100 µg/L) may not.

Table 7. *Current status of unsaturated microcosm experiment, showing persistence of isoproturon (µg/kg) within the chalk.*

Cores	t=0	104 d	153 d
Non-sterile	15 6 (SD 0.87)	15.2 (SD 1.5)	15.2 (SD 1.2)

3.5 ANALYSIS OF THE METABOLITES FROM THE ISOPROTURON DEGRADATION IN SOIL AND SUB-SURFACE SAMPLES

This work cannot be considered complete due to the limitations of budget, staff time, and the small number of metabolites available to use as standards. In addition, both the soil and groundwater already contain small quantities of metabolites, which are present even before the experiment begins. So far, only 10-15% of the metabolites formed have been identified. A number of metabolites can be observed having been formed from degradation by soil bacteria from site WON (Fig 11a). The traces shown in Fig. 11a and 11b are typical after 14 and 28 d incubation. The main feature is an unknown metabolite which elutes just prior to isoproturon as well as some di-demethylated isoproturon. There is no obvious candidate for this unknown metabolite generated in the soil incubations even when this trace is compared with comprehensive analysis of isoproturon metabolites (Lehr *et al.*, 1996). This is not seen with the groundwater samples. The most consistent new metabolite generated by subsurface degradation was mono-demethylated isoproturon. This can be seen in the groundwater (Fig. 11c) and chalk (Fig 11 d).

With the Gleadthorpe samples mono demethylated isoproturon was also generated from the groundwater samples (Figs 8a-c).

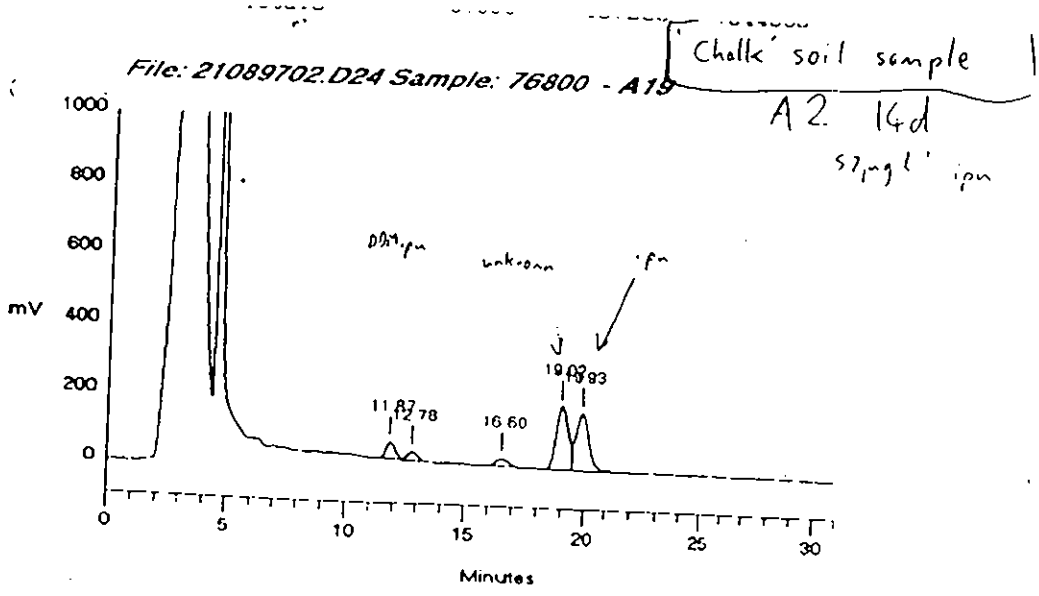

Figure 11a. *HPLC trace, 240 nm for a site WON soil sample, following 14 d incubation.*

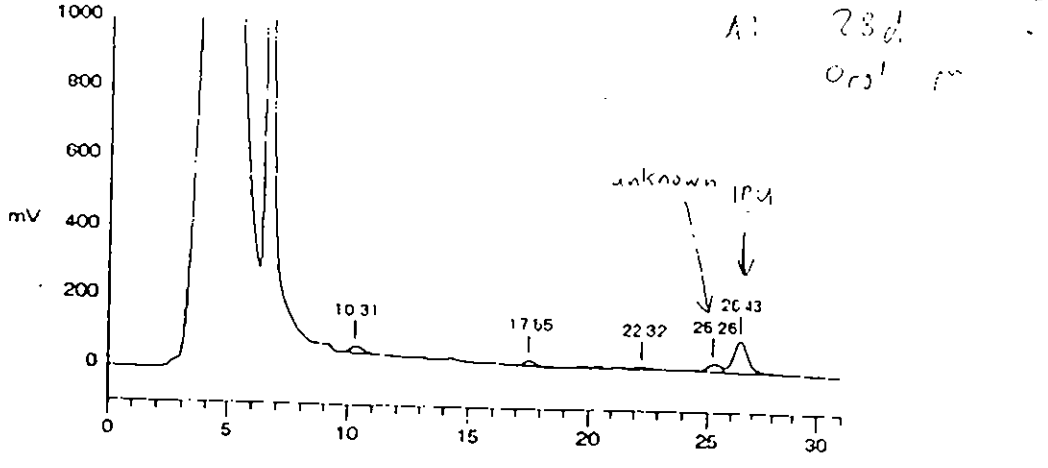

Figure 11b. *HPLC trace, 240 nm for a site WON soil sample, following 28 d incubation*

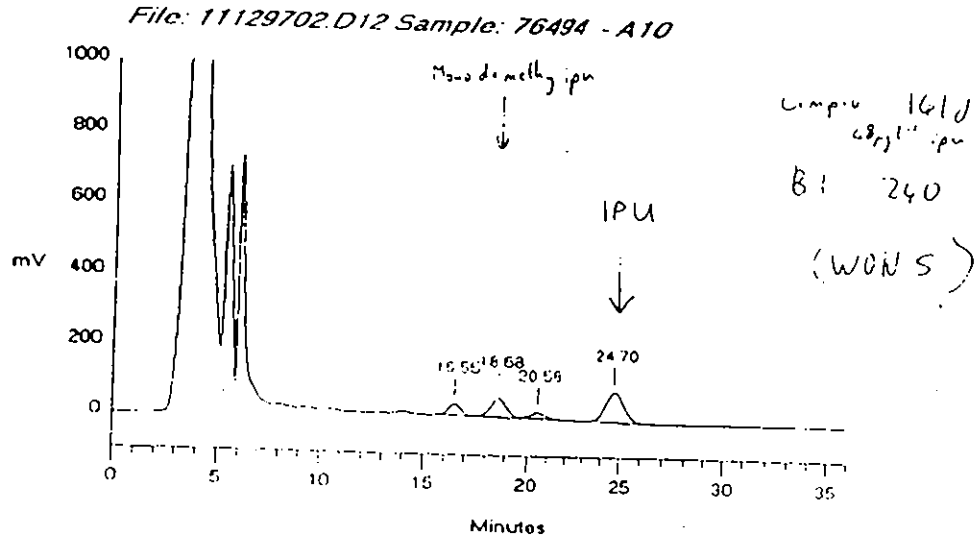

Figure 11c. HPLC trace, 240 nm for WON 5 groundwater , following 141 d incubation

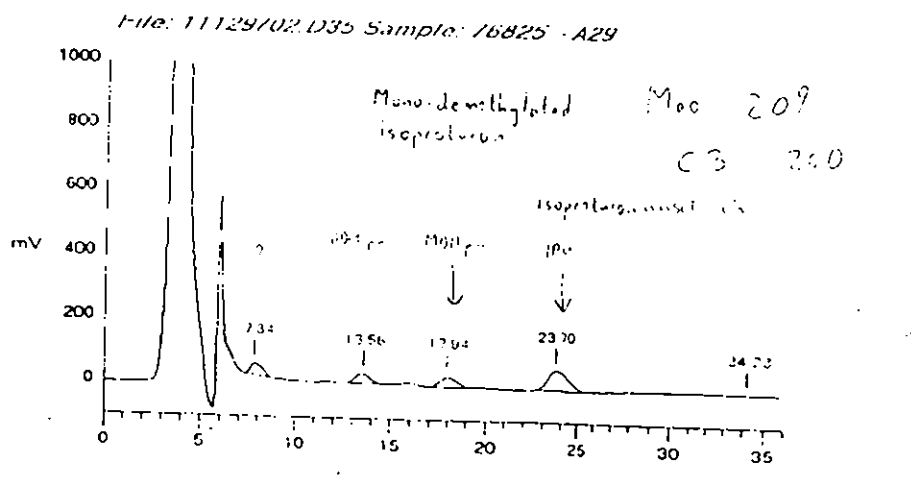

Figure 11d. HPLC trace, 240 nm for WON 7 unsaturated chalk , following 209 d incubation

4. Preliminary conclusions

When approaching the subject of groundwater degradation there are two issues which must be borne in mind: Do the indigenous bacteria possess the competence to degrade the pesticide, and secondly, is degradation likely to occur given the particular environmental conditions present?

With reference to the objectives given in the introduction, with respect to the Chalk site:

1) A degradation potential for isoproturon (100 µg/L at 20°C) was demonstrated in the soil, unsaturated and saturated zones. The soil could degrade atrazine and mecoprop, but this potential was not demonstrated in the subsurface samples.

2) The isoproturon degradation potential is not a constant feature of the groundwater samples (see Table 5).

3) Attempting to replicate the unsaturated conditions of the unsaturated zone again did not reveal a mecoprop degradation potential. For isoproturon the results were less clear in that the flow-through experiment suggested degradation had occurred, but this was not confirmed by the microcosm experiment, which used a lower more realistic concentration.

4) No clear evidence could be seen of the chalk groundwater being capable of degrading low natural isoproturon concentrations (1 µg/L).

5) The study of the metabolites is still in its infancy, with only 10-20% of the metabolites being identified, however, mono-demethylated isoproturon was generated in groundwater.

The assessment of the results from the Sandstone site is still incomplete, however, there does seem to be a parallel with the Chalk site with isoproturon degradation being observed, but not atrazine or mecoprop.

Whilst the extent to which isoproturon degradation might occur throughout an aquifer might be arguable, there is, so far, very little encouraging evidence of the existence of a potential to degrade atrazine or mecoprop at the sites studied in this project.

5. Future work

A new fieldsite is being examined for the Lincolnshire Limestone. The next drilling will take place in October 1998. Similar experiments to that undertaken at the Chalk and Sandstone sites will then be carried out to complete the comparative studies prior to completing the final report.

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