



site 1



site 2



site 3

## Roadside Verges



site 4



LEAD IN THE ECOLOGY OF ROADSIDE VERGES

by

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A thesis submitted in candidature for the  
degree of Doctor of Philosophy at the  
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For my parents

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## General Introduction

Roadside verges are a prominent feature of the British landscape, for invariably, where there is a non-urban road, there is some form of verge. Way (1969) gives details of the structure, function and maintenance of verges. A wide variety of forms will be seen around Britain, but the type studied here (see frontispiece) is perhaps the most common form, and the type closest to the ideal recommended by the Ministry of Transport (Way, op. cit.). This consists of a piece of managed grassland, that is usually flat, of variable width, and bordered by a drainage ditch and hedgerow, separating it from the surrounding land. The road, of course, acts as the other boundary. This clear demarcation of the verge, and its 'wasteland' vegetation leads Way (op. cit.) to suggest that they may be potential 'wildlife reserves' in contrast to the lack of diversity of the surrounding agricultural land. Few attempts have been made to assess any such capacity, though the distinctive form of the verge and its apparent isolation from the surrounding land make it a simple ecological unit and potentially easily studied. This, coupled with their ubiquity, could make them very useful as a comparative ecological unit.

Verges are subject to various disturbances and management procedures. They may be completely destroyed during road repair operations, or simply transgressed by vehicles. The

management programme for verges is discussed by Way (op. cit.), but on established verges consists simply of one or more mowing operations at various times of the year. This prevents the tall-standing grasses (such as Arrhenatherum elatius) from dominating the verge, and thus encourages a wider range of plants.

Since the arrival of motorised transport, verges have been subject to some form of pollution. The heavy metals emitted by the internal combustion engine are only one component of this pollution; lead is the major component of these heavy metals. Assessment of the effects of lead on the ecology of the verge requires examination of the response to lead alone, rather than a compound effect with the other pollutants.

The history of the toxicity of lead is, however, much longer than that of the motor car. The earliest known description of the symptoms of 'plumbism' or lead poisoning is by the greek, Nicander, over 2,000 years ago (Hammond et al, 1972). The typical symptoms were diarrhoea, anaemia, mental disturbance and a blue line around the gums, - a sufficient dose leading to death. Lead is now known to interfere with the synthesis of Heme and the utilization of iron in the body: it impairs the structure and function of mitochondria, particularly in the kidney; it may affect the function of the thyroid, and is known to inhibit certain classes of enzymes. In recent years, lead has been shown to affect the calcium metabolism of nervous tissue.



Paint used to be a common cause of lead poisoning by ingestion, in both humans and farm animals, though most domestic paints no longer contain large amounts of lead (Chow, 1973). Workers in the lead smelting industry were under a more direct threat, as were those involved in the manufacture of lead batteries (Hammond et al, op. cit.). Today, the burning of such batteries is recognised as a serious health hazard, and an intermittent cause of plumbism (Hammond et al, op. cit.). Even more serious risks occur in the production of organic lead compounds used as petrol additives, notably tetra-ethyl and tetra-methyl lead. These compounds are easily absorbed by the skin and have a particular affinity for nervous tissue. For this reason, there are strict safeguards in their manufacture (Chow, op. cit.). They are used to raise the effective octane of petrol, allowing complete combustion in high-compression engines (Haley, 1969). The burning of 'leaded' petrol is now recognised as the foremost cause of atmospheric lead pollution.

Lead levels in urban environments are known to be rising (Hammond et al, op. cit.; Day, Hart and Robinson, 1975) and the evidence is that blood levels are also increasing (Hammond et al, op. cit.). This has recently been directly attributed to automotive emissions (Waldron, 1975), but the sub-clinical effects of this are not known.

Nearly all the toxicological appraisals of lead are written purely in terms of its effects on man (Kehoe, 1969; Chisholm, 1971), or his animals (for eg. Allcroft and Blaxter, 1950). In these cases, lethal doses of lead are rare (and have not been attributed to automobile exhausts), resulting in the belief that the lead problem has little significance to humans.

This has been taken to follow for wildlife, and only very recently has this aspect (other than for a few species of birds) begun to be properly appraised.

From the work that has been done, an association between lead toxicity and calcium metabolism occurs as an underlying implication in nearly all the reports. Venugopal and Luckey (1975) state that there is an association between lead and the alkali metals, particularly calcium; possibly as a reflection of its outer electron shell structure, the lead atom behaves chemically like these metals.

Several workers on vertebrates (eg. Barry and Mossman, 1970; Allcroft and Blaxter, 1950; Golter and Michaelson, 1975; Hammond and Aronson, 1964; Hatch and Funnel, 1969; Schroeder, Vinton and Balassa, 1963) indicate that where there is a greater metabolic need for calcium, lead is more toxic. Lead poisoning is widely reported in young mammals, which obviously require calcium for bone formation (Clegg and Rylands, 1966; Harbourne, McCrea and Watkinson, 1968; Hemingway, Inglis and Brown, 1964; Kostial, Simónović and Pišćić, 1971) and also in birds, in addition requiring calcium for eggshell development (Gullvåg, Ophus and Eskeland, 1975; Bagley, Locke and Nightingale, 1967; Bagley and Locke, 1967).

Experiments with nervous tissue reflect this association further (Kober and Cooper, 1976), and indeed, much of the speculation about lead toxicity is concerned with the brain of the young mammal (Jacobson and Snowden, 1976) with the possibility of lead-induced mental disturbance. Kober et al (op. cit.) have established that the calcium metabolism of nervous tissue is markedly affected by the presence of lead.

More generally, Shields and Mitchell (1941) and Six and Goyer (1970) showed that when rats were fed with a food with

with low levels of calcium and phosphorus, high lead uptake resulted. In fish, Jones (1938) found that lead in substantial quantities caused coagulation of mucus, but that this was prevented by the presence of abundant calcium.

One of the earliest mentions of the effects of lead on invertebrates is by Jones (1940; 1948) who studied the fauna of the River Melindwr in Wales, which drains lead-rich spoil heaps. He noted that molluscs and crustaceans were missing from the most highly polluted stretches of the river, whereas plecopterans and trichopterans (normally the most sensitive of invertebrates to river pollution) were present. Jones did not discuss this at any great length, but it is notable that both molluscs and crustaceans have a high calcium requirement.

Williamson and Evans (1972) from their survey of roadside verges in Co. Durham, reported that animals with a calcareous exoskeleton exhibited higher lead levels than other invertebrates. These workers also added lead to the soil (Williamson and Evans, 1973), but found no effects on the faunal community. They suggest that soil invertebrates may have a high tolerance to lead, since natural soil levels vary to a large degree.

Martin, Coughtrey and Young (1976) assessed the ability of the woodlice Oniscus asellus to accumulate lead (amongst other heavy metals) and Coughtrey and Martin (1977) considered Helix aspersa, the common garden snail, as a monitor of pollution by several heavy metals. Nevertheless, they could not show a simple relationship between lead content of the wet tissues of the snail and lead in its food.

Reports of work on the effects of lead on invertebrate communities are sparse: to date, there have been only two relevant published papers (Price, Ratchke and Gentry, 1974 and

Giles, Middleton and Grau, 1973). Both attempted to show the concentration of lead up a simple insect food chain, but with the latter study, the data is too sparse to provide clear-cut conclusions. The former work divides a sample of roadside insects into those which 'suck plant juices', those that 'chew plant material' (live) and those which are insect predators; again this very rough division of trophic levels and low sample numbers make the apparent concentration of lead up the food chain doubtful. The problem is confounded by the breathing of lead-rich air by the animals, as well as their consuming lead-rich food, and this must be allowed for when estimating lead assimilation from food.

No work has yet been reported on the lethality of lead to invertebrates in the field. Ecologically, sub-lethal effects are more important, and these are more likely to go unnoticed. For this reason, the present study has concentrated on sub-lethal effects. The invertebrates are the main form of animal life on the verge, and the least studied in respect of lead toxicology. The initial assumption was that a rich invertebrate fauna was to be found on the verge because of the variety of habitats, including the ditch, the hedgerow, the occasional tree and so on. They were thus the obvious place to examine the effects of lead on invertebrate communities.

An essential part of any such study is the quantification of the lead deposition on the roadside vegetation. A 'standard' pattern of the decline of lead in the vegetation with distance from the road, has been established (Motto et al, 1970), but little work has been done on its subsequent availability to animals. Further, there are few reports of seasonal changes in the degree of contamination (Rains, 1971).

The absolute amounts of lead arriving per unit area of the verge has not previously been considered, even though this enables estimation of the addition to the soil.

Initial analysis showed the importance of distinguishing between live and dead grass, and experiments in the laboratory measured the capacity of each to bind lead. The ability of various extractant solutions to remove this lead was used as a measure of how tightly it is held by grass, and its potential availability to herbivores and detritivores.

Variations in the lead content of the vegetation with distance from the road were examined, and related to the physical and climatic characteristics of the sites.

Seasonal changes in both the lead concentration of the vegetation and absolute amounts of lead arriving per unit area were monitored. In this latter respect, comparison was made between mown and unmown sites. Variations in the soil lead level, both with distance from the road, and amongst sites, were related to position and traffic frequency.

In parallel with this study, the invertebrate communities of five verge sites were sampled and related to the degree of lead contamination. The detritivorous woodlice, amongst the invertebrates most likely to be affected by a high lead diet, were collected from several sites, and seasonal changes in their lead and calcium levels noted. They were also compared to herbivorous caterpillars from the same sites.

The possible interaction between lead and calcium uptake was tested in the laboratory with the woodlouse, Porcellio scaber. This animal was also used to test the effects of lead on oxygen consumption and fecundity. Lead/calcium interaction was also examined in juvenile Helix aspersa, the garden snail. The distribution of lead and calcium was measured in the tissues

of adults of H. aspersa.

The intention has been to present an integrated approach to lead in the ecology of roadside verges. Previous work has only dealt with particular aspects of the problem, and the major gap in our knowledge of roadside lead contamination is its effects on the verge ecology. In many respects, this can only be a preliminary study, though it does provide indications for future possible work.

I. Grass sampling

Two methods of sampling the roadside grass were used:

- i. Random crops of approximately 50g from points  $\frac{1}{2}$ m from the road margin. This was then sorted into live and dead components, and was used to determine the seasonal fluctuation in the Pb level of live and dead grass. They were taken with shears and transported in clean polythene bags. This same technique of random sampling and sorting was used to determine the Pb content of the grass at various distances from the road.
- ii. Cropping of all standing vegetation from a sequence of  $\frac{1}{2}$ m<sup>2</sup> quadrats,  $\frac{1}{2}$ m and 7m from the road. These were taken from a carefully chosen uniform length of verge, at two sites, and were left unsorted. The sequence of cropping is shown below, and was to ensure that no area was cropped twice :

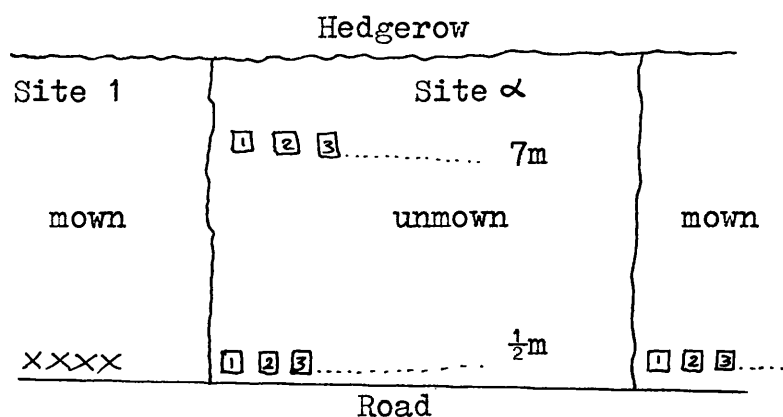


Fig. 1 Grass sampling pattern at sites 1 and  $\alpha$ .

The squares represent the position and order of cutting of these unit area samples, and the crosses show the position of the other, sorted samples. The quadrat series allowed proper estimation of the weight of the standing crop throughout the year.

II. Production of *Dactylis glomerata* as a standard food and



### experimental material

Dactylis glomerata was grown in large seed trays of John Innes No. 2 compost until a thick sward developed. The grass was then sprayed with 'Weedol', a paraquat/diquat weedkiller, and allowed to bleach for three weeks. The weedkiller should have decomposed after this time (Boon, 1967). The grass was then cropped and washed in a strong detergent (10% Decon 90) to remove any surface residues. This was followed by several rinses in deionised water, and drying in an oven at 60°C, until totally dry. The leaf material had lost its chlorophyll and was virtually identical with the dead leaves of roadside grass in winter.

### III. Maintenance of animal cultures

#### i. Porcellio scaber

The laboratory culture of Porcellio scaber was derived from a suburban compost heap, and was kept at laboratory temperatures in a perspex aquarium tank, with soil and turfs from the compost. This follows roughly the technique given by Sutton (1972). The animals were fed on fresh slices of carrot, and water was periodically sprayed into the chamber to maintain the humidity.

During experiments, P. scaber was kept in petri-dishes with damp filter-papers (Sutton, op. cit.) and the appropriate food. All such cultures were placed in a second aquarium tank lined with moistened paper. Faeces were removed every two days to prevent the woodlice consuming them (Wieser, 1968).

#### ii. Helix aspersa

H. aspersa were again collected from a suburban garden and kept in a perspex aquarium tank at 18°C. About 20cm of sterilised soil was placed in the bottom of the tank to facilitate

egg-laying (Boycott, 1934). The adults were fed on cabbage leaves and the newly-emerged young on rolled oats. The adults were induced to aestivate by reducing the temperature to 4°C.

Eggs were collected from the soil and placed in petri-dishes exactly as described for P. scaber. After feeding on rolled oats for two weeks, they were transferred to fresh dishes with the experimental food. Throughout this time they were kept at 18°C.

#### IV. Food dosing techniques

##### i. Solution dosing of potato

This is a development of a technique used by Hassall and Jennings (1976) to feed Philoscia muscorum a high carbohydrate diet.

Peeled potatoes were boiled in deionised water until soft; they were then drained and passed through a sieve to ensure an even consistency. 20g portions were placed in clean glass beakers, to which were added 10cc of  $\text{Pb}(\text{NO}_3)_2$ ,  $\text{Ca}(\text{NO}_3)_2$  or deionised water according to dose. The resultant suspension was stirred for several minutes before placing in an oven at 60°C for one hour to drive off excess moisture. Two samples of experimental food produced in this way were weighed, dried and reweighed, to estimate the mean percentage water content. Each sample was then extracted and analysed for Pb and Ca (cf. section V.i).

The woodlice were then allowed to feed on the dosed potato for a given length of time, after which the food was dried and reweighed. This dry weight was compared to the initial, calculated dry weight (ie. the initial wet weight corrected for mean percentage water-content), and the difference taken to be the dry weight of the food consumed.

### ii. Solution dosing of grass

10g of dried, dead D. glomerata were placed in 100cc of either deionised water (control), 1mM  $\text{Pb}(\text{NO}_3)_2$ , or 10mM  $\text{Pb}(\text{NO}_3)_2$  (low and high load respectively), solution and agitated intermittently for three hours. The grass was then thoroughly rinsed in deionised water,<sup>dried,</sup> and two samples taken for analysis (cf. section V.i). Weighed portions of the grass were presented to individual woodlice (P. scaber) in the fecundity experiment, and also in early trial experiments with Oniscus asellus.

### iii. Use of lead-free carrot

Carrot, shown by analysis to be lead-free, was used before and after all dosing experiments with woodlice. The production of red faeces was taken to indicate a gut devoid of Pb-dosed food, thereby ensuring that enhanced Pb levels were not measured in the individuals.

### iv. The lead dosing chamber

This was devised to dose grass with Pb derived from a petrol-engine exhaust, and so simulate conditions on the verge. The basic layout is given in Fig. 2.

Essentially, it consists of a sealed chamber into which are passed the fumes from a small generator (Honda type E300). The exhaust fumes are rotated and mixed by an internal fan near to the engine exhaust, and removed by an extraction fan at the other end of the chamber. Air can be drawn into the chamber by these fans by opening a vent situated beneath the generator. A water-spray, in front of the vent, allows the addition of a fine spray of water to the fumes, all of which are mixed by the turbulence fan. The chamber itself is a Dexion skeleton with blockboard endpieces, covered with polythene sheeting. Velcro strips seal the access flap on one side

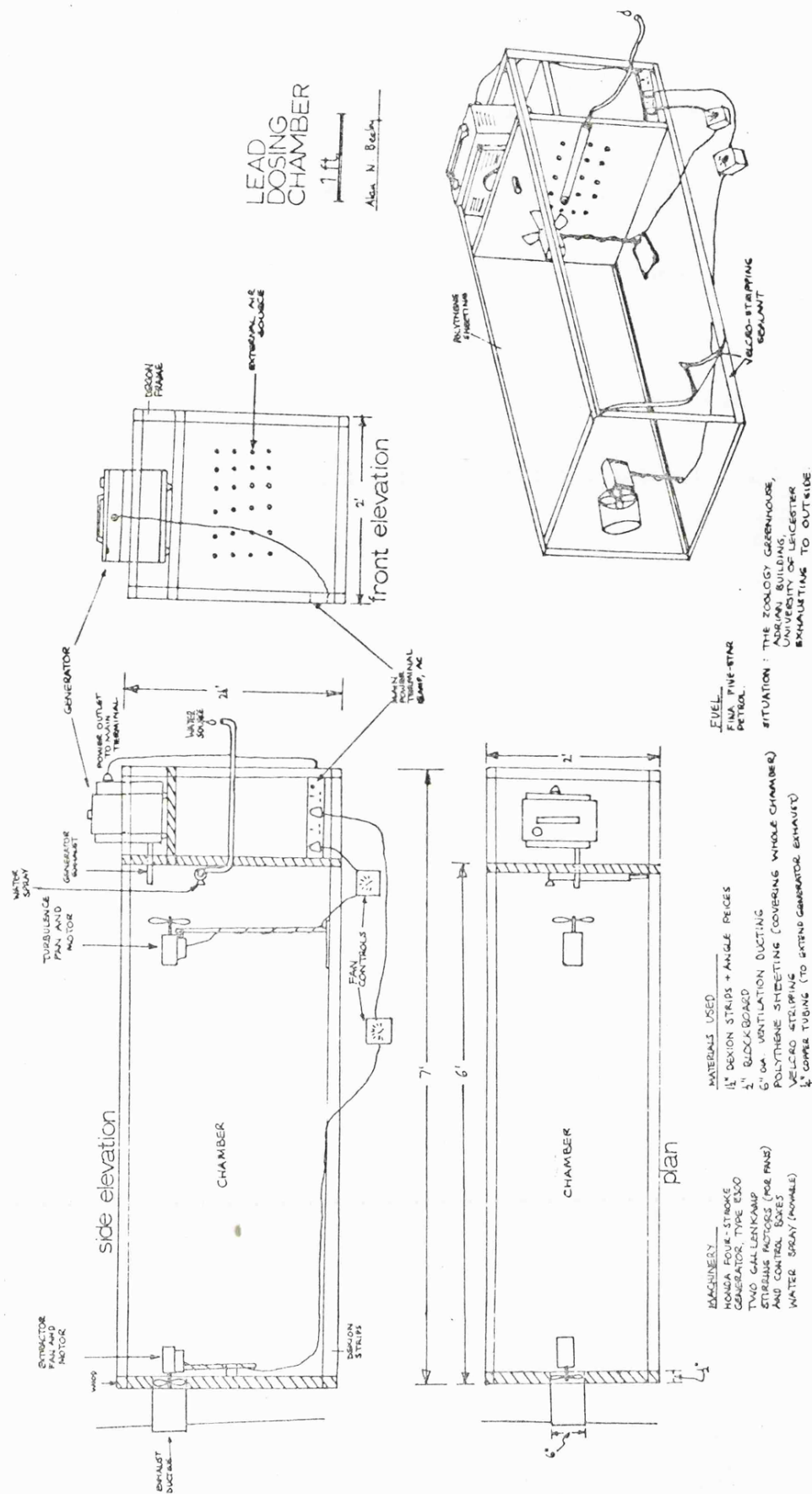


Fig. 2 Plans for the lead dosing chamber.

of the chamber.

Seed trays with D. glomerata were placed in the chamber under various operating conditions and various lengths of time. Initial experiments showed that the best conditions for the greatest deposition of Pb from the exhaust fumes were as follows: heavy moisture content of the air, produced by the spray running continuously, slow removal rate of the fumes (extraction fan), extensive mixing of the internal air (turbulence fan), fast running of the engine, and finally, close proximity to the exhaust inlet. Independent experiments also suggest that a thicker sward of grass improves deposition (Woodings, Undergraduate research project).

Fina 101-octane petrol was always used, since this grade has the highest organic Pb content. Both fans were controlled by resistors with numbered settings, for ease of replication. Uniform deposition did not occur in the chamber, but was at a maximum immediately next to the exhaust inlet. For this reason, a smaller chamber would have been preferable, since more even deposition is likely to occur in a smaller air volume, which may be mixed more effectively. Nevertheless, the larger the chamber the more closely it simulates the situation on the verge, and in this respect it is an extremely useful tool in trying to simulate roadside deposition. Indeed, the possibilities were not fully explored, and more extensive experimentation would have been possible.

#### V. Atomic Absorption Spectrometry (AAS)

The techniques for the analysis of biological material by AAS are fairly standard, particularly with regard to vegetable material. Generally, the extraction methods used here are based on those given in 'Lead: Airborne lead in perspective'

(Hammond et al, 1972). Throughout 'Ultrar' grade  $\text{HNO}_3$  is used, which has extremely low levels of Pb (0.01ppm) and Ca (0.05ppm), and this was particularly necessary for the flameless atomisation techniques used.

#### i. AAS analysis of vegetation and soil

Dried grass or soil was passed through a hammer mill with a 1.5mm sieve. The mill was cleaned between operations with a vacuum cleaner and brush. 1g of the milled vegetation was then digested in 10cc of 25%  $\text{HNO}_3$ , gently boiling for 1 hour. The cooled digest was filtered (Whatman No. 541) and made up to 100cc with distilled, deionised water.

The larger unit area samples and soil from the profiles sampled were milled in an 8" Christy-Norris laboratory mill, which was again cleaned between operations. The lead extraction technique was the same.

Analysis on the spectrophotometer (Varian Techtron AA6) used standards of the appropriate concentration of Pb (as  $\text{Pb}(\text{NO}_3)_2$ ) made up in 2.5%  $\text{HNO}_3$  (the normal background of the samples). The measurements were made using the 217.0nm line, with an air/acetylene flame and a lamp current of 5ma. Concentrations were read-out in milli-molarities, and subsequently converted to parts per million (ppm = mg/l, or  $\mu\text{g}/\text{ml}$ ) where appropriate.

#### ii. AAS analysis of animal material

The problem of matrix interference is one of the main barriers to the straight-forward analysis of animal tissues. This non-atomic absorption interferes with both flame and flameless analytical methods, <sup>as</sup> absorbing matter other than Pb in the light path. It is assumed that comparison of samples by taking readings using a Hydrogen continuum light source

(at the same wavelength) enables estimation of this non-atomic component, and the results presented are so corrected.

Interference between Pb and Ca in flame atomisation has been reported by Coughtry, Martin and Young (1976). The interference reported however, was for extremely low levels of Pb and very high levels of Ca. Tests performed on the Varian AA6 using standards covering the ranges of Pb and Ca most commonly found in this study, could show no such interference. It is possible that different machines and models will differ in their response to this possible effect, but particular caution is necessary in the choice of analytical reagents when performing such tests.

### iii. AAS analysis of woodlice and young snails

Specimens were dried in an oven at 60°C for 12 hours, weighed and placed in a 25cc combustion tube with 10cc of 50%  $\text{HNO}_3$ . 25 tubes were placed in a drilled-out aluminium block on a hot-plate, and gently boiled for 2 hours. The resultant liquor was poured into a 25cc volumetric flask and made up to volume with deionised water.

Analysis was performed on the Varian Model 63 Carbon Rod atomiser. Optimum settings were found to remove all sources of non-atomic absorption during the ash cycle, without atomisation of Pb. The Pb content of the 5µl sample was measured during the atomise stage, where temperatures were increased to a given limit at a known rate ('ramp atomise'). The highest signal during this time is read-out at the end of the operation. For this to be meaningful, a smooth, rapid rise and fall toward the peak was obtained by adjusting the temperature and ramp rate. The machine was standardised using 200µg/l and 40µg/l Pb standards in the normal background of 20%  $\text{HNO}_3$ . Reagent blanks were ran to determine any background contamin-



ation, and all samples were checked against the hydrogen source for non-atomic absorption. Any sample values above the top standard<sup>were</sup> measured again using flame atomisation, as described above.

One potential source of error with this digestion technique is that it does not necessarily break down all the fats of the animal. To test its efficiency in extracting Pb and Ca, the method was compared to one using perchloric acid/nitric acid with different halves of the same woodlice. Ten replicates were made, and the results corrected for weight differences. In the latter digestion, boiling was continued until fats disappeared, but no significant difference between the two techniques could be found.

All estimates of Ca were made using an air/acetylene flame and the 422.6nm line. Ca analysis always followed Pb analysis since it was necessary to suppress phosphate interference with SrCl (1600ppm in the solution analysed). This gives strong non-atomic absorption and makes Pb analysis on the Carbon Rod impossible.

Ca levels were normally expressed as a percentage of the dry weight of the animal.

#### iv. AAS analysis of adult *Helix aspersa*

The animals were killed in boiled, deionised water, and dissected. The organs were placed in dry, clean and preweighed crucibles, and dried in an oven at 60°C for 12 hours before reweighing. They were then placed in a muffle furnace at 550°C for 1 hour, in order to reduce non-atomic absorption by ashing. Any samples not completely ashed were returned to the furnace until this was completed. The residue was taken up in 50% HNO<sub>3</sub> (10cc) and analysed in the same manner as given for woodlice. Matrix interference from the shell was too large

for accurate measurement of its Pb content.

## 1. General Features of the Sampling Sites

The sites chosen for study are shown in Map 1, and comprise six sites of intensive work (the 'major sites') and three 'minor sites' where only grass, soil, and animal samples were taken.

### 1.1 The Major Sites

Initially, five sites were chosen around the Six Hills junction on the A46, about 16km north of Leicester. Three sites (1 - 3) were actually on the A46, a major dual-carriageway, while sites 4 and 5 were minor roads that ran approximately parallel to it (Map 1). All verges faced eastward on a road that had an approximate north-south axis, and were thus in the lee of the predominant westerly winds. Verges were selected to cover a wide range of hedgerow types, whilst maintaining a fairly uniform verge profile. The exception to this is site 3, which has a strongly sloping profile, and although the hedgerow is the same size as at the other sites, the top is actually below the level of the road surface.

The profile of the verges is shown in Fig. 3. Site  $\alpha$  was subsequently added to the major sites, to estimate the effects of the mowing regime: all verges are mown by the council at least once a year (usually after the first spring growth), but an arrangement was made with the local council authorities to leave a stretch of verge, immediately next to site 1, unmown. This site ( $\alpha$ ) extended for about 30m beyond site 1, after which, the normal mowing programme was resumed. It was left unmown from May 1975 until May 1977.



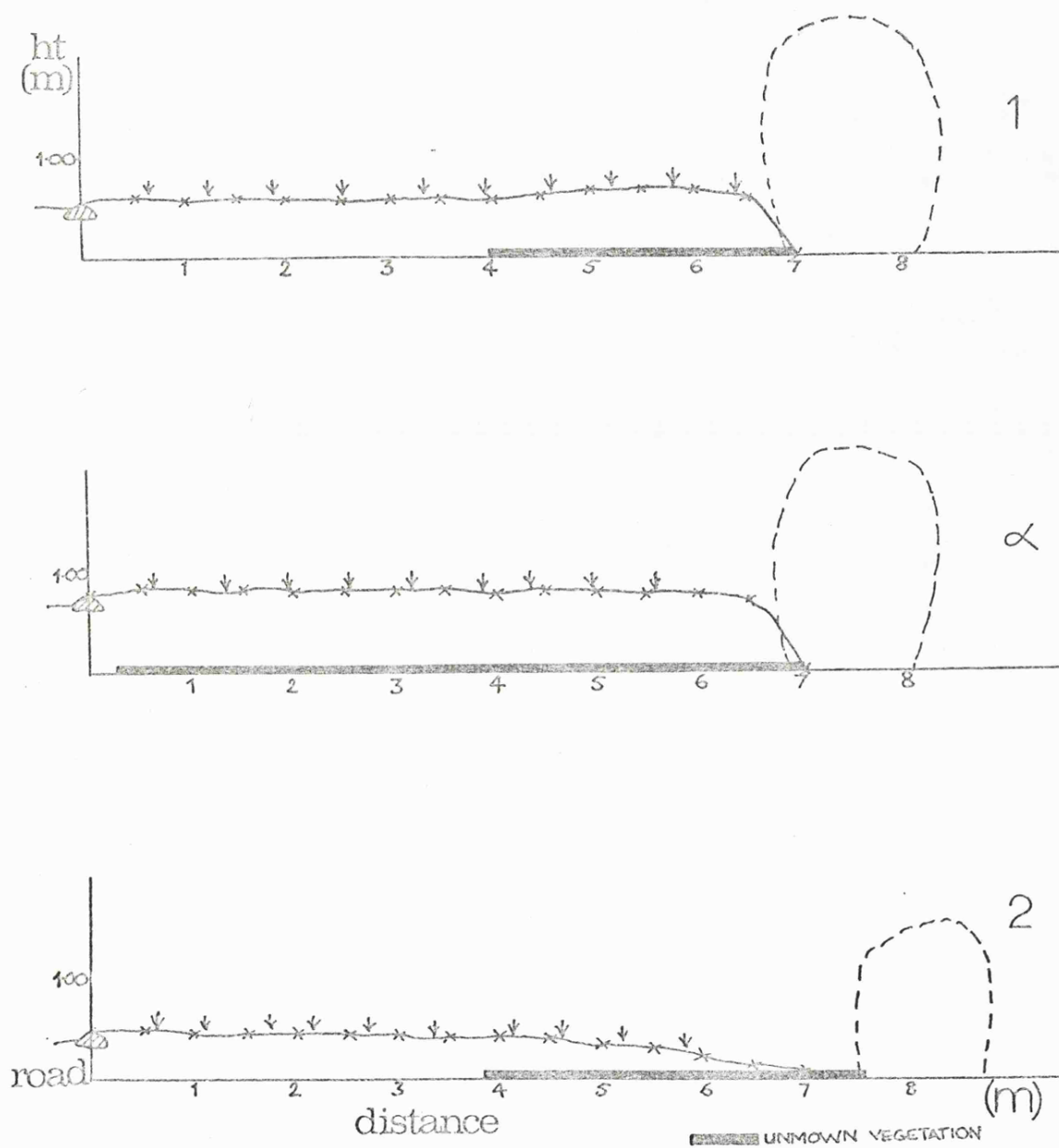


Fig. 3 Profile of the major sites. The crosses denote the position of a levelling, the arrow a pitfall trap. The extent of the unmown vegetation is shown by the thick line, and the hedgerow by the hatched line. (Figure is continued overleaf).

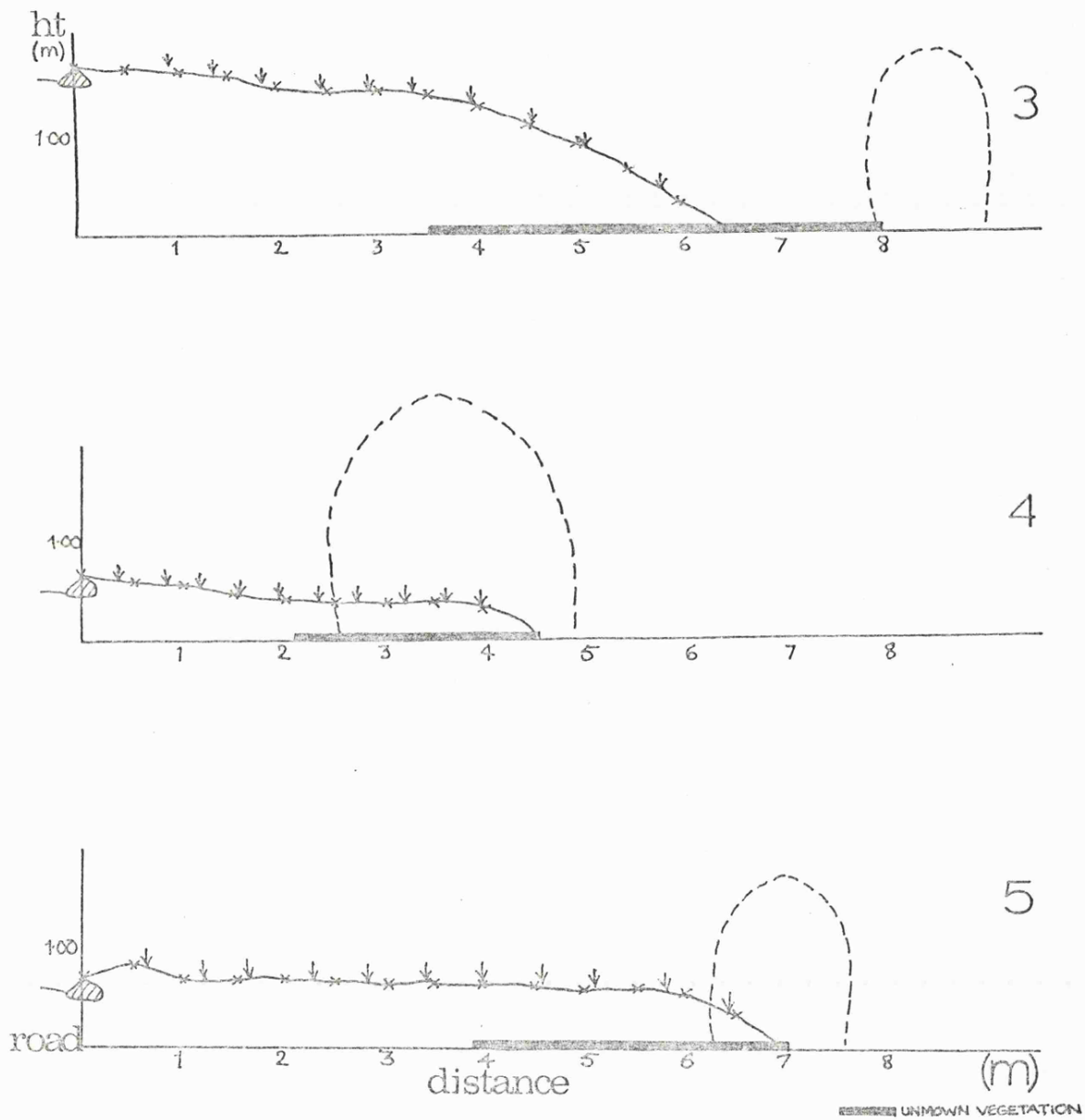


Fig. 3 - continued

Fig. 3 shows the vegetation height across the verge, and the effects of mowing can be seen at all sites. In fact, mowing by the council was stopped at all sites from 1976 to May 1977, and thus Fig.3 shows only the pattern in 1975. Mowing covered only the first three or four metres of verge away from the road, and a large zone at the back of the verge was left untouched.

## 1.2 Traffic data

Sample traffic figures for the major sites are given below. The values for site 1 will be roughly the same for site 2, as there is no major exit between these two sites.

A46	1965 Six Hills	10,564 vehicles/16h day	
	1971 Leics. County boundary	7,565	
	1972 Syston	8,090	
	28/8/75 Site 1	8,410	1/6/77 1 6,752
		3 8,060	3 6,992
		4 112	4 64
		5 512	5 256

All figures prior to 1975 were provided by the County Engineer and Surveyor. The other figures were collected by three observers with tally counters at positions that covered all the major sites.

These figures are obviously in keeping with what was subjectively expected of each road, though the 1975 figures may be slightly inflated by August bank holiday traffic. Otherwise, the numbers are probably representative of an average traffic frequency. In this case, the A46 seems almost constant in its 7,000 vehicles per day over the last six years, though this can only be considered as a rough estimate of its traffic throughout the year.

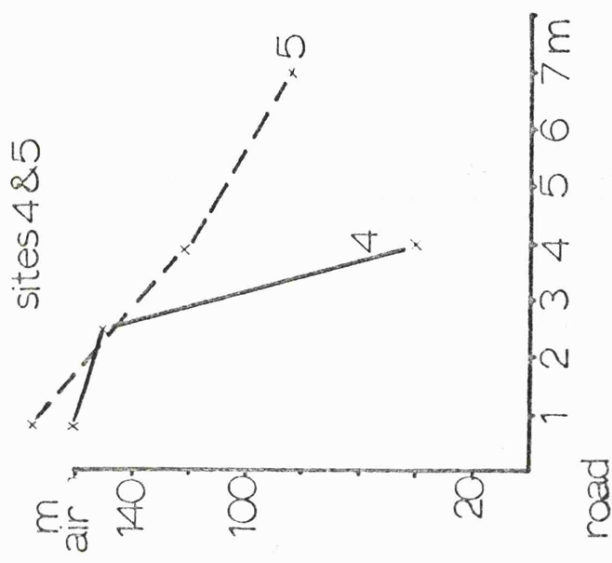
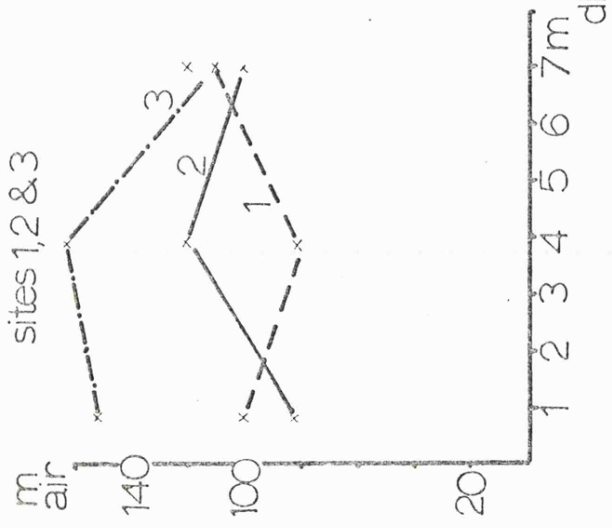
### 1.3 Climatic characteristics of the major sites

Measurements of wind movements at the major sites were made when the wind was blowing in its predominant westerly direction. Two methods were used: vane anemometer readings measured the volume of air moving in one minute of observation, and cup anemometers recorded the highest windspeed during the same period. Both measurements were made at 25cm ('ground level') and 1.5m above the ground, at 1m, 4m and 7m from the road margin. Only two distances could be recorded at site 4, owing to the extent of the hedgerow. The means of ten consecutive readings for each position are presented in Fig. 4. These show a drop in both measurements at ground level toward the hedgerow at all sites, and a similar, if less marked, decline in the readings at 1.5m from the ground. This is almost certainly due to a 'shadowing' effect of the hedgerow, - with the wind blowing from behind the hedge, there would be a zone of 'stagnant' air. (with little net movement) in its lee.

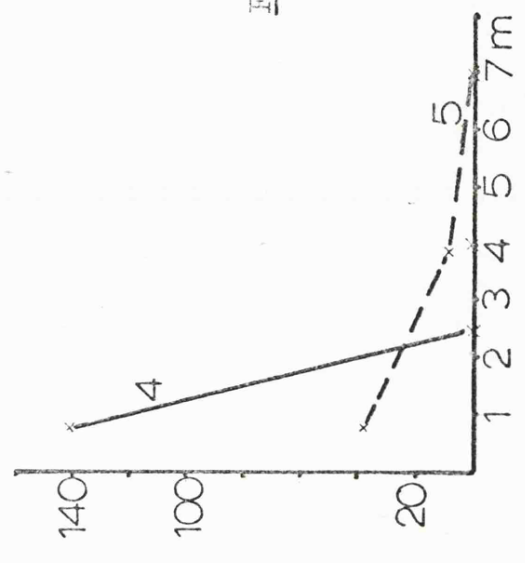
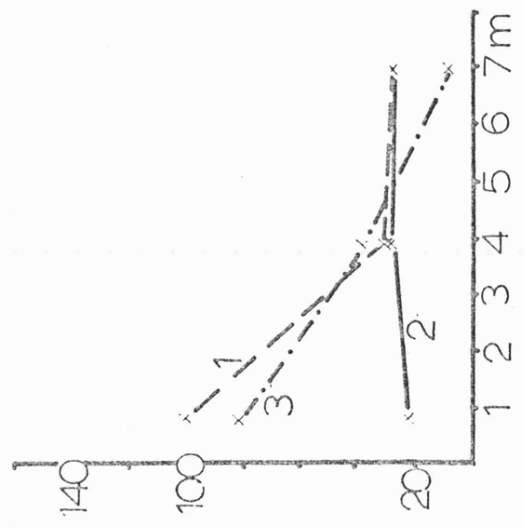
From observation of the anemometers during the measurements, the figures next to the road were enhanced by approximately 15% from turbulence by passing vehicles. This suggests that this zone would be receiving a better supply of lead-rich air: conversely, there would be a lower supply of the lead-enriched air around the base of the hedgerow.

The temperature of the soil at the major sites was measured using thermistor probes connected to an automatic Grant recorder. The tip of the probe was buried to a depth of about 3cm; however, the metal sleeve of the probe would conduct heat from the whole of this depth, therefore, the values presented represent a rough mean of the top 3cm. Initially,





15m  
above  
ground  
level

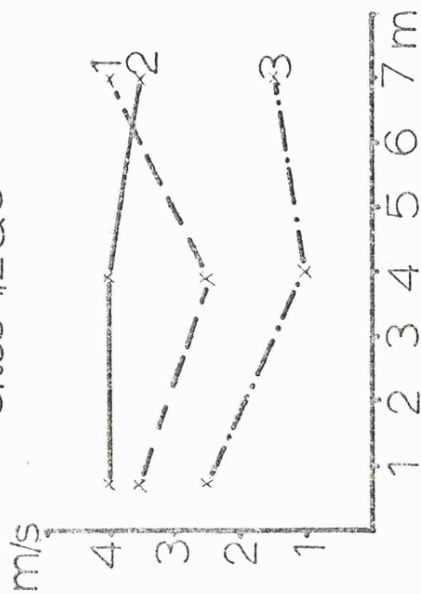


ground  
level

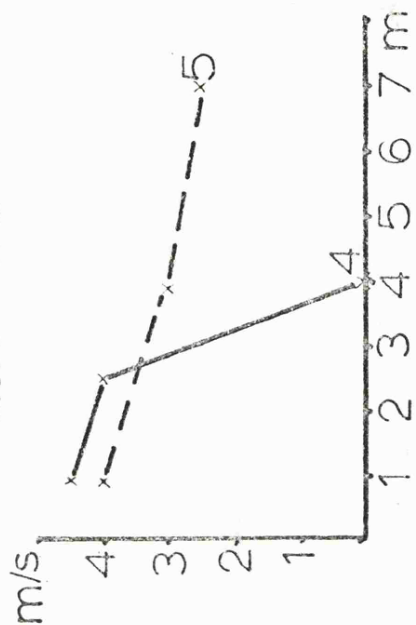
Fig. 4

The wind characteristics  
of the major sites, -  
mean volume of air  
moving per minute at  
two heights, at each site.

sites 1,2 & 3



sites 4 & 5



1.5m  
above  
ground  
level

ground  
level

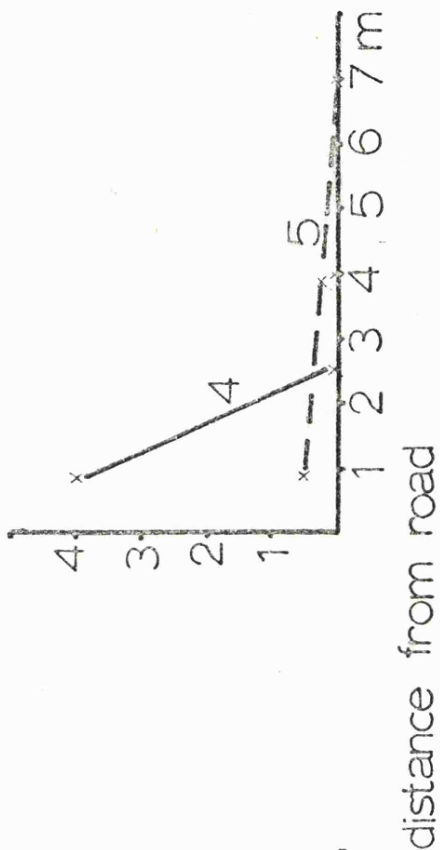
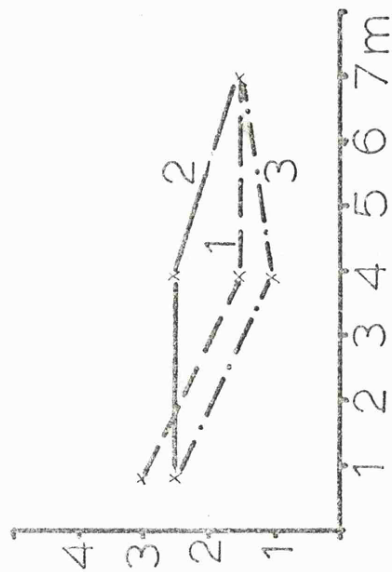


Fig. 4 - continued.  
Mean air speed at  
each site.

readings were taken over a fifteen minute period, around mid-day, at all sites. A characteristic pattern emerged, common to all sites, except site 3. The pattern is illustrated by the results of site 1, given in Fig. 5. Here the highest temperatures are for the bare soil next to the road, which falls rapidly away from the road, rising to a low peak around the 4m distance. This 'thermal hump' occurs in this middle region of every site, except site 3. At this site, the slope reduced the angle of incidence of the sun's rays, considerably reducing the insolation for much of the day.

The 'hump' may be a result of the gradation in vegetation cover across the width of the verge, occurring where the vegetation is thick enough to insulate the soil against excessive heat loss, but not too thick to prevent effective insolation.

Fig. 6 shows the same information for site 5 over a period of 11 days in November 1975, which included periods of heavy rainfall and sunshine. Here the 'hump' is apparent for most of the period, though it is more a zone of relatively constant temperatures, than one of consistently high temperatures. The parts of the verge nearest the ditch and the edge of the road (where the vegetation is most sparse) have the greatest fluctuations. These data are too sparse to attempt correlation with the faunal samples, or rates of grass growth and decay, particularly as the mowing regime brings about drastic changes. However, in this last respect, it was apparent from all the sites that the greatest turnover in plant biomass was at the edge of the verge: here the spring growth was first seen, and this zone was devoid of upstanding vegetation during the winter months.

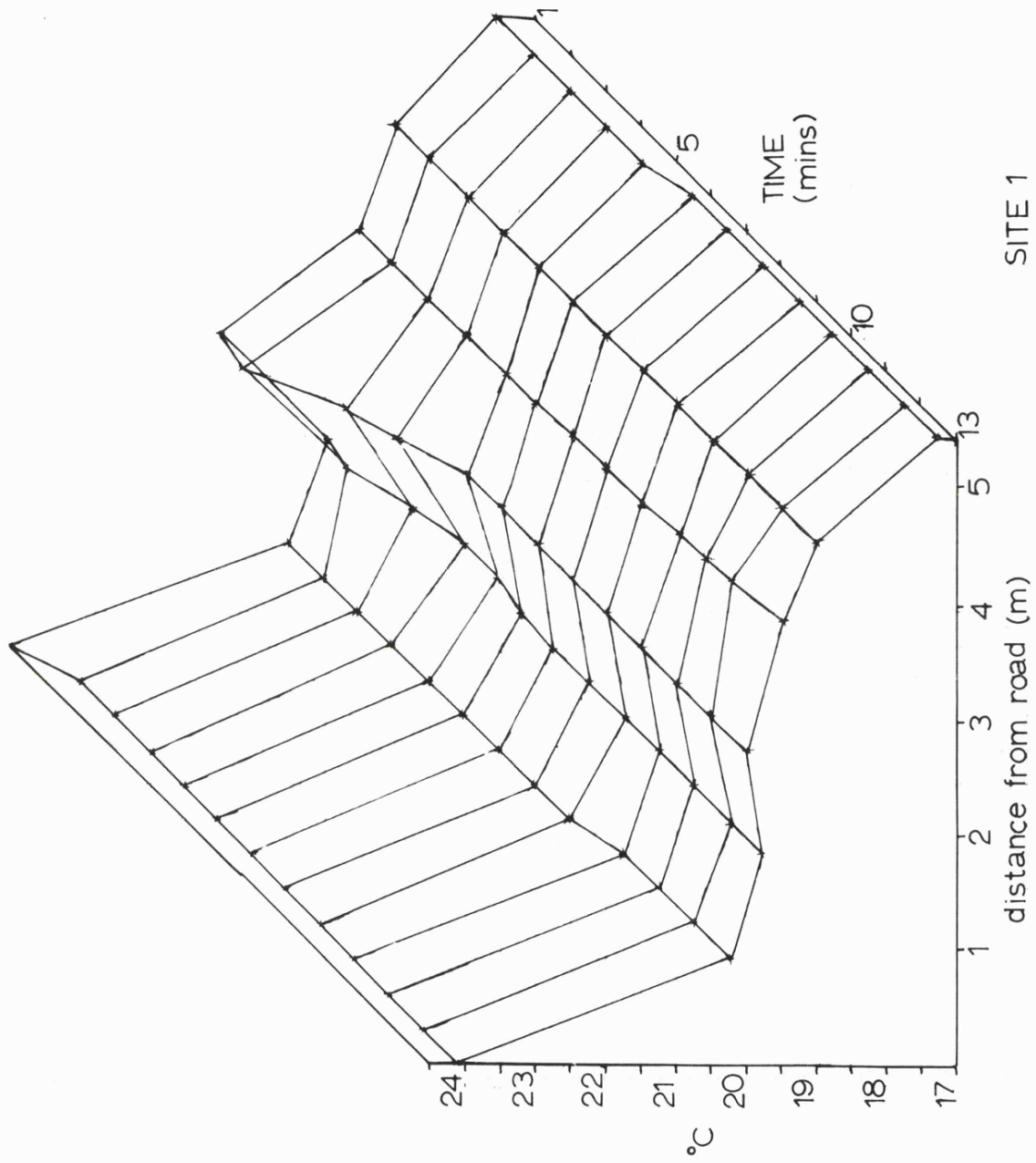
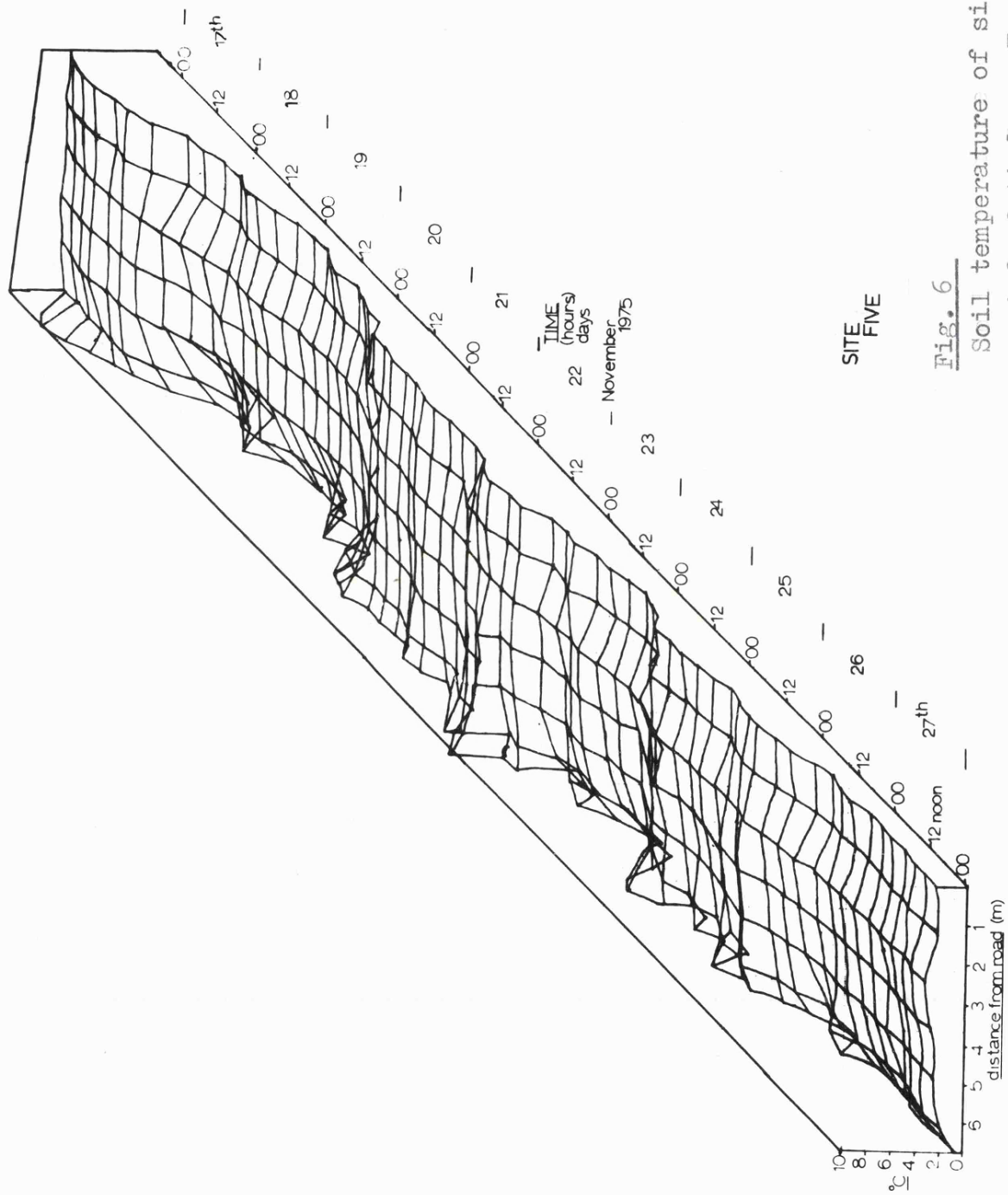


Fig 5  
Soil temperature  
at site 1, around  
midday (28/8/75).  
Details are given  
in the text.



SITE  
FIVE

Fig. 6

Soil temperature of site 5 over a period of 11 days. For details see text.

Rainfall was initially measured at three sites: 1, 3 and 5, but owing to thefts, this was later reduced to 1 and 3, and then to 1 alone. This lack of security meant that no expensive equipment could be left unattended at the sites, and thus only rainfall was continuously monitored. Additional temperature and rainfall data were obtained from the meteorological office (Newtown Linford), and there was good correlation with the results from the rain gauges on the experimental sites. The implications of the rainfall data is discussed in connection with the seasonal changes in the Pb content of the vegetation. It should be remembered, however, that the whole study took place during two exceptional years, 1975 and 1976, both of which had extremely dry summers. This was almost certainly a major cause of the low numbers of woodlice collected at the sites studied.

#### 1.4 Biological characteristics of the major sites

This section is mainly concerned with the floral components of the verges, which are given as a series of lists in Appendix I. No attempt was made to quantify these data, or to compare them mathematically.

It will be apparent from the floristic lists that the verge is essentially a wasteland dominated by grasses. Verges do not fit into any convenient ecological type, but their flora is more likely to be determined by their soil type, rather than any other factor. This is the conclusion reached by Bates (1937) who has also described what might be expected to make up the verge flora.

Arrhenatherum elatius and Dactylis glomerata are the two most common species near the ditch of most of the sites, though at site 4, the hedgerow overhangs much of the verge.

Anthriscus sylvestris is also found in this zone, though at site 5, Rubus fruticosus agg. is present, with mosses beneath this bramble canopy. Urtica dioica dominates at the foot of the slope of site 3.

The edge nearest the road is much less variable from site to site and prostrate species, such as Plantago major, Ranunculus repens or Taraxacum officinale are conspicuous. Changes in the composition of the vegetation with distance from the road were not studied, although this most certainly exists. Plants with a low profile are concentrated where mowing takes place and such species as Arrhenatherum elatius only occur in the region which is never cut. Many other factors also contribute to these differences, such as contamination of the road margin by salt and vehicle pollutants, transgression by vehicles, large inputs of gravel from the road, degree of shelter and so on. A study of these effects would require a verge which had been recently made-up, and where such parameters could be held constant or varied at will. Some work has been done on the effects of road-salting (for eg. Ranwell, et al 1973), but this is outside the scope of the present work.

The composition of the litter layer also changes with distance from the road: the greatest thicknesses being in the unmown zone where tall grasses are allowed to complete their development. Usually, the litter is thinnest at the road margin, though there is rarely a regular increase in litter across the verge.

Biologically, there are five distinct zones of the verge, moving back from the road: a thin (about  $\frac{1}{2}$ m wide) zone of bare soil, overgrown only in the spring, behind which is the mown

area with its low-lying plants, extending about 4m from the road. The remainder of the verge is typically unmown, with a thick vegetation cover, followed by the ditch, and usually, a hawthorn hedgerow.

### 1.5 The Minor Sites

Three other sites were added to the study for comparative purposes, to sample three other main arterial roads to Leicester (Map 1). No detailed measurements were made of their profiles, wind characteristics or vegetation cover.

All were chosen on a relatively flat and straight stretch of road, with a similar profile to that of site 1. The one major deviation from this plan is the A50, which had a rock outcrop in place of a hedgerow. The A46 (South) also had a more extensive ditch than site 1, while the A47 most closely resembles site 1, except that here, the hedgerow is lower (about 1½m).

These sites all have a different aspect from the major sites, and from each other (Map 1). They also differ in their traffic frequencies, sampling data of which are shown below:

A46 (South)	Sutton Hill	Aug. 1976	13,959 vehicles/16h day.
A47	Houghton-on-the-Hill	Aug. 1975	6,982 "
A50	Markfield	Aug. 1974	13,143 "



## 2.0 The Contamination of Roadside Vegetation and Soil with Lead

### 2.1 Introduction

Verges are the land most directly exposed to emissions from motor vehicle exhausts, and consequently their Pb contamination has been extensively studied. This work has declined in recent years, since the pattern of Pb deposition was thought to have been established, but there are still questions to be answered.

The decline of Pb in vegetation with distance from the road is well documented, being described as an 'almost logarithmic' pattern (Cannon and Bowles, 1962; Motto et al, 1970; Chow, 1970; Fidora, 1972; Hopkinson et al, 1972), and Daines et al (1970) showed that this reflected a similar decline in the Pb levels of the air above the verge. They attributed this to predominant wind characteristics, proximity to traffic, and reactions of the lead aerosol with time. The same group of workers (Motto et al, 1970; Daines et al, op. cit) relate the general level of contamination to traffic frequency.

Chow (op. cit) showed conclusively that the anti-knock additives of petrol were the source of Pb on roadside verges. For this, he recorded isotopic Pb deposition on the roadside soil and vegetation, and compared the results with the isotopic composition of Pb added to petrol. On combustion, this organic Pb is converted to lead chlor-bromide ( $\text{PbClBr}$ ) (Hirschler and Gilbert, 1964), but the halides are lost after about 18 hours, leaving lead oxides, carbonates and sulphates (Ter Haar and Bayard, 1971).

The Pb emitted by the automobile exhausts is essentially an aerosol, and the time spent in the air by any one particle depends on its size: the larger particles falling immediately after emission (Hirschler et al, op. cit). Mean particle size

was shown to depend on engine speed and condition, and hard acceleration increases the output of Pb (Hirschler et al, op. cit). The mean residence time for Pb particles in the air is 1 - 4 weeks (Hammond et al, 1972), which although rather vague, allows the Pb ample time to distribute itself away from the roadside (Hammond et al, op. cit). About half of the Pb emitted falls by gravity within a few hundred feet of the road, the rest remaining as an aerosol until removed by precipitation (Hammond et al, op. cit).

Pb arriving at the verge may be deposited on either the vegetation or the soil, though the decline in soil Pb levels with distance from the road is thought to be a reflection of the deposition on the overlying vegetation (Lagerwerff and Specht, 1970; Maclean et al, 1969; Motto et al, op. cit). Olson and Skogerboe (1975) used X-ray powder diffraction to show that about 70% of this Pb is in the form of sulphate, the other large components being PbO and  $\text{PbCO}_3$ . Typically, Pb concentrates in the upper part of the soil profile, and Hildebrand and Blum (1975) show this to be largely due to binding by the organic fraction of the soil. Pb is also effectively held by clay minerals and this Hildebrand and Blum (op. cit) attribute to surface  $\text{SiOH}$ ,  $\text{AlOH}$  and  $\text{AlOH}_2$ -groups. These bonds are 'irreversible' with regard to cation exchange above pH 7, while below this pH Pb may be adsorbed by amorphous oxides of iron. The humus-held Pb only becomes available with very high acidities, (Hildebrand et al, op. cit).

This immobility of Pb in soil is reported from a wide range of soil types (Lagerwerff et al, op. cit; Motto et al, op. cit; Maclean et al, op. cit), and this is true even if Pb is added in a soluble form (Motto et al, op. cit; Maclean et al, op. cit). Since little Pb is transported down the profile,

roadside soils can be regarded as a repository of Pb, irrespective of whether it has arrived from the air, with precipitation, or the vegetation. Indeed, Goodman and Roberts (1971) suggest that soils may be used as integrators of heavy metal pollution, binding most heavy metals without appreciable loss, over long periods of time.

Plants appear to translocate only small amounts of Pb (Arvik and Zimdahl, 1975; Baumhardt and Welch, 1972; Jones and Clement, 1972; Mitchell and Reith, 1966; Rains, 1971). Maclean et al (op. cit) showed that increasing pH and organic content of the soil reduced uptake of added Pb by oats and alfalfa while phosphates and lime had a similar, if reduced, effect. Arvik et al (op. cit) demonstrated that Pb uptake at the roots is likely to be passive (in Soybean, Bean and Maize), though Pb becomes more available with soil pH less than 7.

Höll and Hampp (1975) give a comprehensive review of all varieties of interaction between plants and Pb, and they note that those parts engaged in 'large-scale gaseous exchange' tend to have higher Pb levels, while the cell wall is now known to be the main site of Pb accumulation. They suggest that photosynthesis may be inhibited by high levels of Pb, although Bazzaz, Carlson and Rolfe (1974) note that Pb is the least effective of several heavy metals which they tested. Development of the phloem of lettuce, cauliflower, carrot and potato may be arrested by high Pb content (Barker, 1972), though this is known to vary from species to species.

Tolerance to Pb was investigated by Jowett (1964) in Agrostis tenuis, using plants growing on spoil heaps in Cardiganshire. The addition of Ca to the soil increased the growth of both tolerant and non-tolerant plants, but he also found that tolerant plants were adapted to low Ca and phosphate

levels. Höll et al (op. cit) note that Pb tolerance in plants invariably implies the ability to precipitate Pb in the roots.

Ziegler (1973) considered the effects of airborne Pb on plants and noted the role of phosphates in precipitating Pb inside the plant. More extensive work has been done on the ability of mosses to bind airborne Pb (Rühling and Tyler, 1969; Goodman et al, op. cit), which have been used to monitor heavy metal pollution. The nature of this binding mechanism has not been fully described, particularly in higher plants.

The seasonal variation in Pb levels in standing vegetation has received little attention. Daines et al (op. cit) have described a winter maximum in airborne Pb, which they attribute to local climatic conditions (New Jersey, USA), particularly wind movement and atmospheric mixing, both of which were low during the winter. They do not relate this to their subsequent work on soils and vegetation (Motto et al, op. cit), though Rains (1971) recorded Pb levels of Avena fatua around San Francisco, which showed a winter maximum. This was preceded by a slow summer rise and a spring low. A similar record for privet is reviewed by Hammond et al (op. cit), also in America, but no comparable work has been reported from Britain.

By proper monitoring of the climatic conditions, seasonal changes in the Pb content of the vegetation could be related to particular factors responsible for enhancing Pb deposition. Measurement of these changes per unit area of the vegetation throughout the year should also give an estimate of the addition of Pb to the soil from the overlying vegetation.

The possible role of precipitation in producing enhanced Pb levels in roadside vegetation has not been properly considered. Spray, created by passing vehicles, could be of major importance in producing the typical 'logarithmic' decline in Pb levels discussed earlier. Wind has been shown to be

important in producing this pattern (Daines et al, op. cit), and the generation of a fine spray, and its passage across the verge may also contribute to this rapid decline. If this is the case, major differences in Pb deposition at sites with differing positions and morphologies may be expected. Invariably, previous work has only described the pattern of decline from a single site, with little comparison with other sites of differing aspect and topography.

Pb levels in the litter overlying the soil has received even less attention. Martin, Coughtrey and Young (1976) measured the Pb content of litter around a Pb smelter, and used various extractants to estimate its availability to detritivores. No comparable work on verges has been reported. Similarly, no previous studies have distinguished between the Pb levels of live and dead vegetation, even though they may have different capacities to bind Pb. This represents a major gap in our knowledge of Pb in terrestrial ecosystems.

It is these questions which are examined in this section, as well as attempting to estimate the general levels of Pb contamination of each site studied. The eventual fate of this Pb has been investigated, particularly that which becomes available to higher trophic levels. In this respect, the soil has been regarded as a repository for Pb, and the work has been directed toward Pb in living systems.

## 2.2 Lead levels in live and dead roadside grass

Any random sample of vegetation from a verge will, at most times of the year, consist of a mixture of both living and dead material. The proportions of these components will vary during the year, and any difference in the capacity of either to accumulate Pb may be correspondingly affect the mean Pb level of the sample. As a result of some anomalies in early attempts to estimate the Pb level of roadside grass, experiments were conducted to measure any such difference.

Grass samples were taken from two sites (1 and 5), at 0.5m from the road, and sorted into 'live' (wholly green and healthy grass) and 'dead' (brown and withered grass) components. Only leaf material was selected from each sample, and this was then sub-sampled. One sub-sample was dried and analysed (cf. Methods V.i), while each of the other three were given a different washing treatment, in various solutions, for 10 minutes. This was to investigate the extractability of the Pb. This treatment was followed by rinsing in deionised water, and the material was finally dried, milled and analysed as before. This experiment was repeated on two subsequent occasions with samples from site 1 alone. The results are given in Table 1.

All the samples show a striking difference between the Pb levels of 'live' and 'dead' grass. Although the ratio of Pb in 'live' to that in 'dead' grass varies, 'dead' material always has substantially higher levels, even after the washing treatments. Such a difference has not been previously described, yet it could be of fundamental importance for understanding the process by which Pb is incorporated into plants, and eventually the soil.

SITE	DATE	TREATMENT	STATUS	REPLICATE 1	REPLICATE 2	Mean % Pb REMAINING
1	14/2/75	Unwashed	Live	306.6	298.3	100
			Dead	414.4	407.1	100
		Deionised water	Live	91.1	97.3	31
			Dead	248.6	240.3	59
		10% HCL	Live	74.5	72.5	24
			Dead	99.46	89.1	19
		10% Decon 90	Live	98.3	97.3	32
			Dead	331.5	346.0	67
11	23/4/75	Unwashed	Live	37.2	29.0	100
			Dead	609.1	625.7	100
		Deionised water	Live	29.0	18.6	60
			Dead	420.6	476.5	70
		10% HCL	Live	8.2	8.2	20
			Dead	45.5	49.7	8
		1N $\text{NH}_4\text{NO}_3$	Live	14.5	16.5	39
			Dead	594.6	569.8	97

Table 1 Pb content of grass samples from two sites, after different washing treatments, following separation into live and dead components. (ppm).

SITE	DATE	TREATMENT	STATE	REPLICATE 1	REPLICATE 2	Mean % Pb REMAINING
5	14/2/75	Unwashed	Live	37.2	37.2	100
			Dead	62.1	62.1	100
		Deionised water	Live	18.6	20.7	54
			Dead	37.2	43.5	82
		10% HCl	Live	18.6	10.3	37
			Dead	37.3	43.5	64
1	7/5/75	10% Decon 90	Live	16.5	22.7	51
			Dead	60.1	60.1	96
		Unwashed	Live	37.2	35.2	100
			Dead	130.2	169.9	100
		Deionised water	Live	22.8	22.8	58
			Dead	155.4	147.1	86
		10% HCl	Live	0.0	0.0	0
			Dead	55.9	51.8	31
		$^{14}\text{N}$ $\text{NH}_4\text{NO}_3$	Live	20.7	20.7	55
			Dead	171.9	167.8	97

Table 1 continued.



By far the largest fraction in both 'live' and 'dead' material is only removed by 10% HCl, suggesting that Pb forms a very strong bond. There is little consistency in the size of this fraction, but it is always the largest. Deionised water and 'Decon 90' (a surface-active detergent) were used to estimate purely surface contamination, though both were again variable in their effectiveness, and there was little difference between the two. This surface component is the smallest in both 'live' and 'dead'.

Normal  $\text{NH}_4\text{NO}_3$  is commonly used as a soil extractant to measure the amounts of exchangeable cations of various metals, and it is likely to be equally effective in removing exchangeable Pb from grass. In this case, the ionic Pb would be exchanged for  $\text{NH}_4^+$  groups. This treatment in fact extracted little Pb from 'dead' material, though it was more effective with 'live' grass. This suggests that exchangeable Pb is a much larger fraction in 'live' grass than 'dead', and that there is a difference between the two in their mode of binding Pb.

Further experiments were carried out on the capacities of 'live' and 'dead' grass to bind Pb. Four 10g replicates of 'live' and 'dead' Arrhenatherum elatius (from a uniform bulk sample taken from an area of low Pb contamination) were placed in 250cc cylindrical separating funnels. The grass was retained in these for the duration of the experiment, as different solutions were added and drawn off. Initially, 100cc of 1mM  $\text{Pb}(\text{NO}_3)_2$  was added to each funnel, and agitated regularly for 110 minutes. At given intervals, a small sample (about 2cc) of each solution was drawn off and analysed on the spectrophotometer, after which it was replaced (so there was little net volume change). The mean results are presented in Fig. 7.

Here the rapid and progressive uptake by 'dead' grass

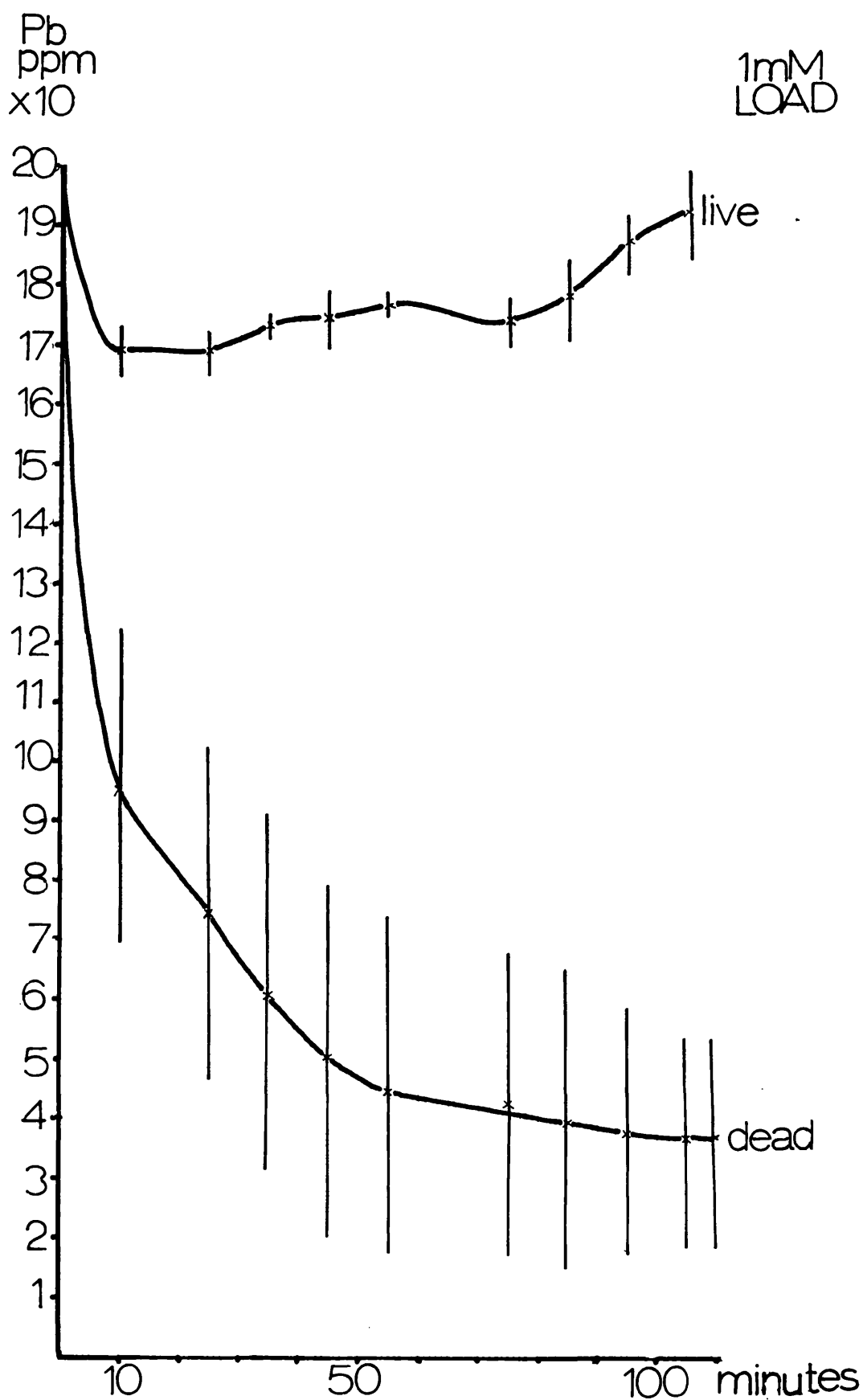
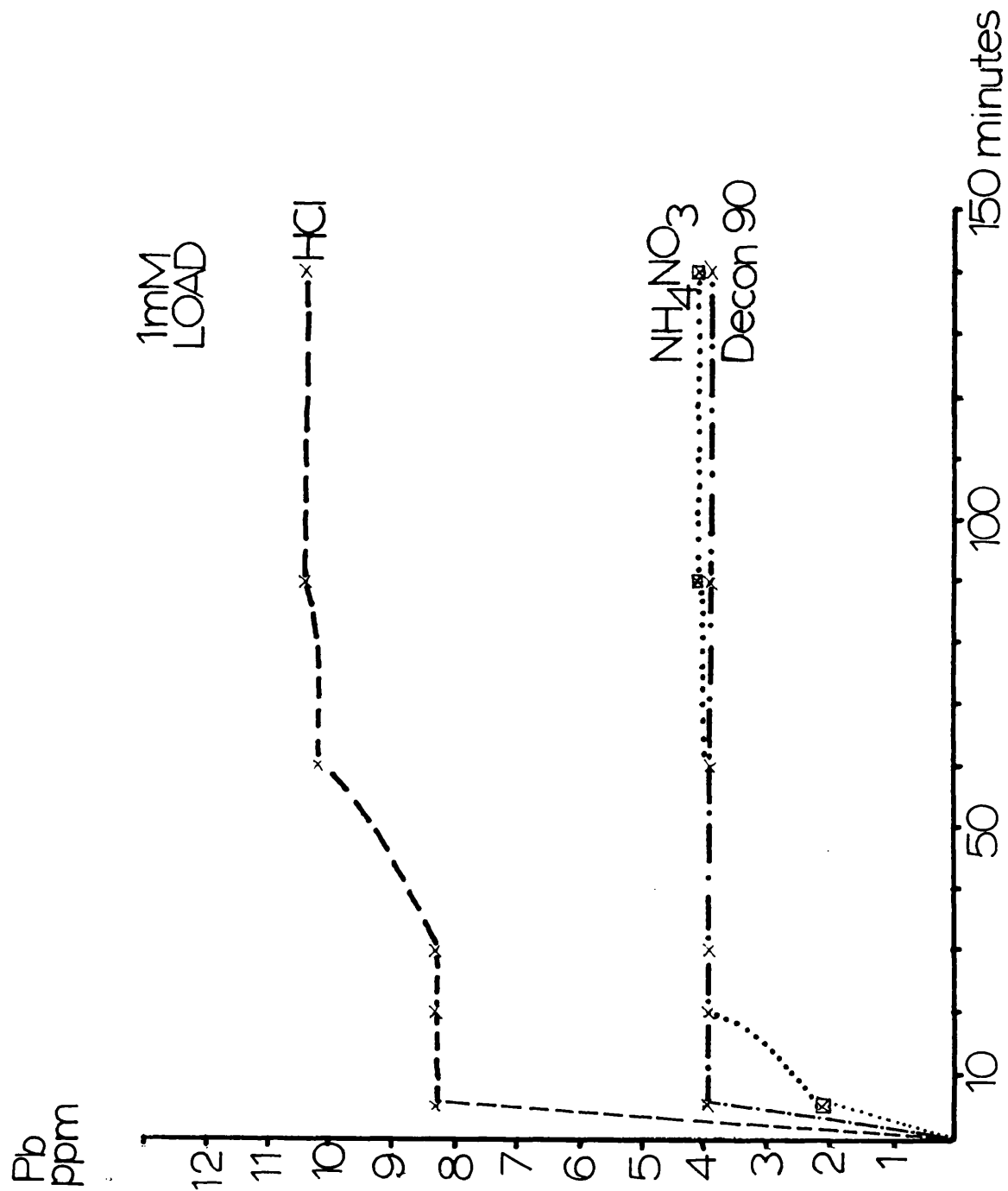


Fig. 7

Levels of Pb remaining in solution which contains 10g of either live or dead grass, with time. This represents reciprocally what has been taken up by the grass. The initial solution was 1mM  $\text{Pb}(\text{NO}_3)_2$ .



**Fig. 8**  
Levels of Pb removed by different extractants from 10g of live grass, with time. This followed loading with 1mM Pb(NO<sub>3</sub>)<sub>2</sub>.

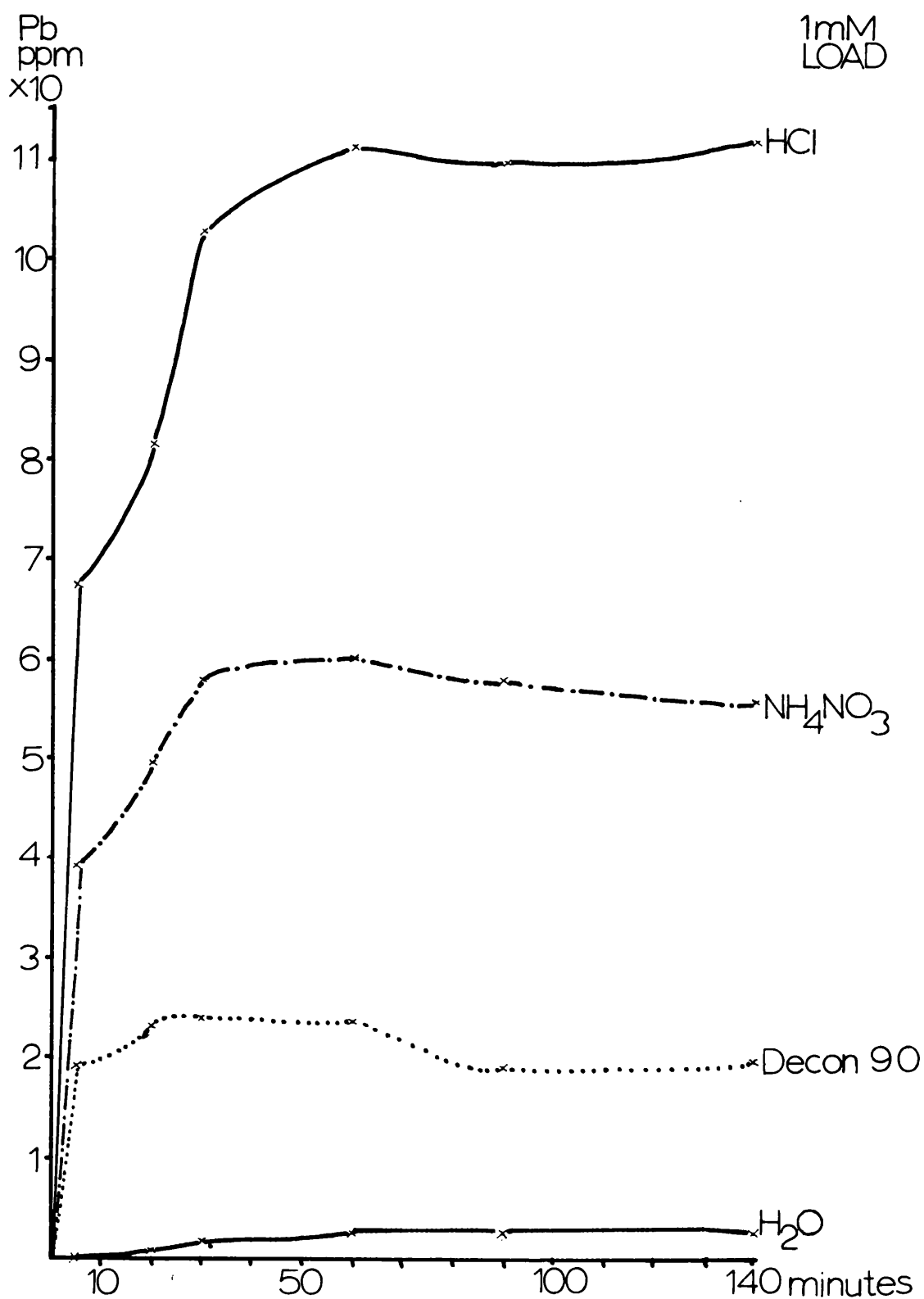


Fig. 9

Levels of Pb removed by different extractants from 10g of dead grass, with time. This followed loading with 1mM  $\text{Pb}(\text{NO}_3)_2$ .

appears to reach an equilibrium with the solution, and this is contrasted with the more variable uptake by 'live' grass. The total uptake by 'dead' grass is a magnitude greater than that of 'live' and this is equivalent to 170 ppm Pb taken up by the 'dead' grass at the end of the experiment, compared to 15ppm in 'live'.

After this 'loading' period, the grass was rinsed in deionised water, and one of four extractants (10% HCl, Decon 90, 1N  $\text{NH}_4\text{NO}_3$  or deionised water) added to each funnel. The concentration of Pb in these extractants was then measured at regular intervals in the same way as given above. The results are shown in Figs. 8 and 9.

As in the material taken from the verge, 10% HCl is the most effective extractant in both 'live' and 'dead' grass. The 'dead' material shows large differences between all extractants, though in 'live' grass the  $\text{NH}_4\text{NO}_3$  and Decon 90 are virtually the same, and the deionised water failed to remove any Pb at all.

This experiment was repeated with a lower 'load' level of 0.1mM  $\text{Pb}(\text{NO}_3)_2$ , and over a shorter period of time. The rapid uptake by 'dead' grass (Fig. 10) is again apparent, with 'live' again removing a small amount (3ppm Pb compared to 20 ppm in 'dead' grass). The removal of the Pb by three extractants (HCl,  $\text{NH}_4\text{NO}_3$ , and deionised water) again show distinct differences (Figs. 11 and 12) in 'live' and 'dead' material, and the order of the efficacy of the extractants is maintained. These curves are flattened toward the end of the experiment, indicating that an equilibrium in the transfer of Pb from the plant to the solution is again being reached. The proportion of the Pb removed by exchange is greater than that found in the verge material, and consistently higher in the 'live' grass than in the 'dead'.

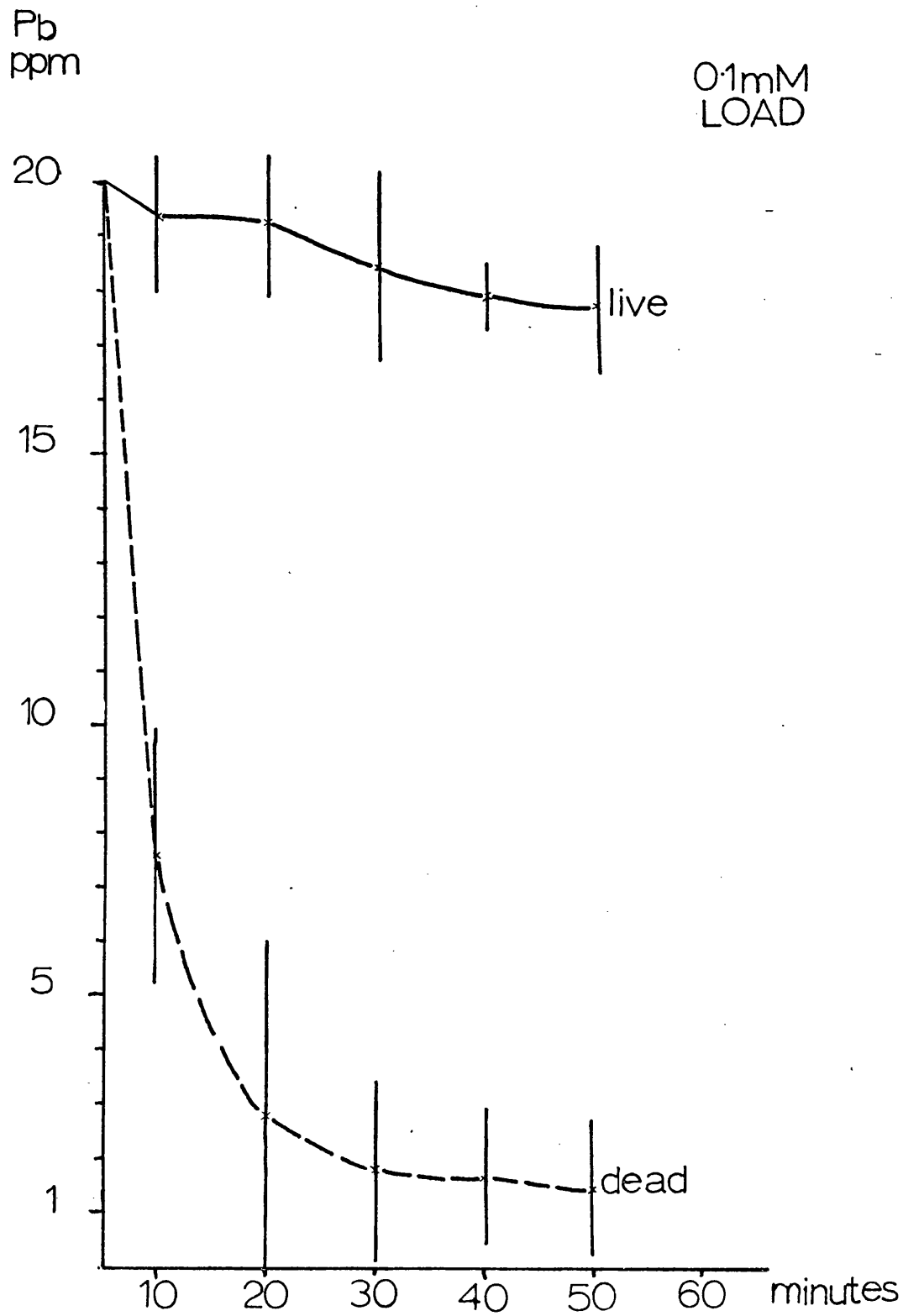


Fig.10

Levels of Pb remaining in solution which contains 10g of either live or dead grass, with time. This represents reciprocally what has been taken up by the grass. The initial solution was  $0.1\text{mM Pb}(\text{NO}_3)_2$ .

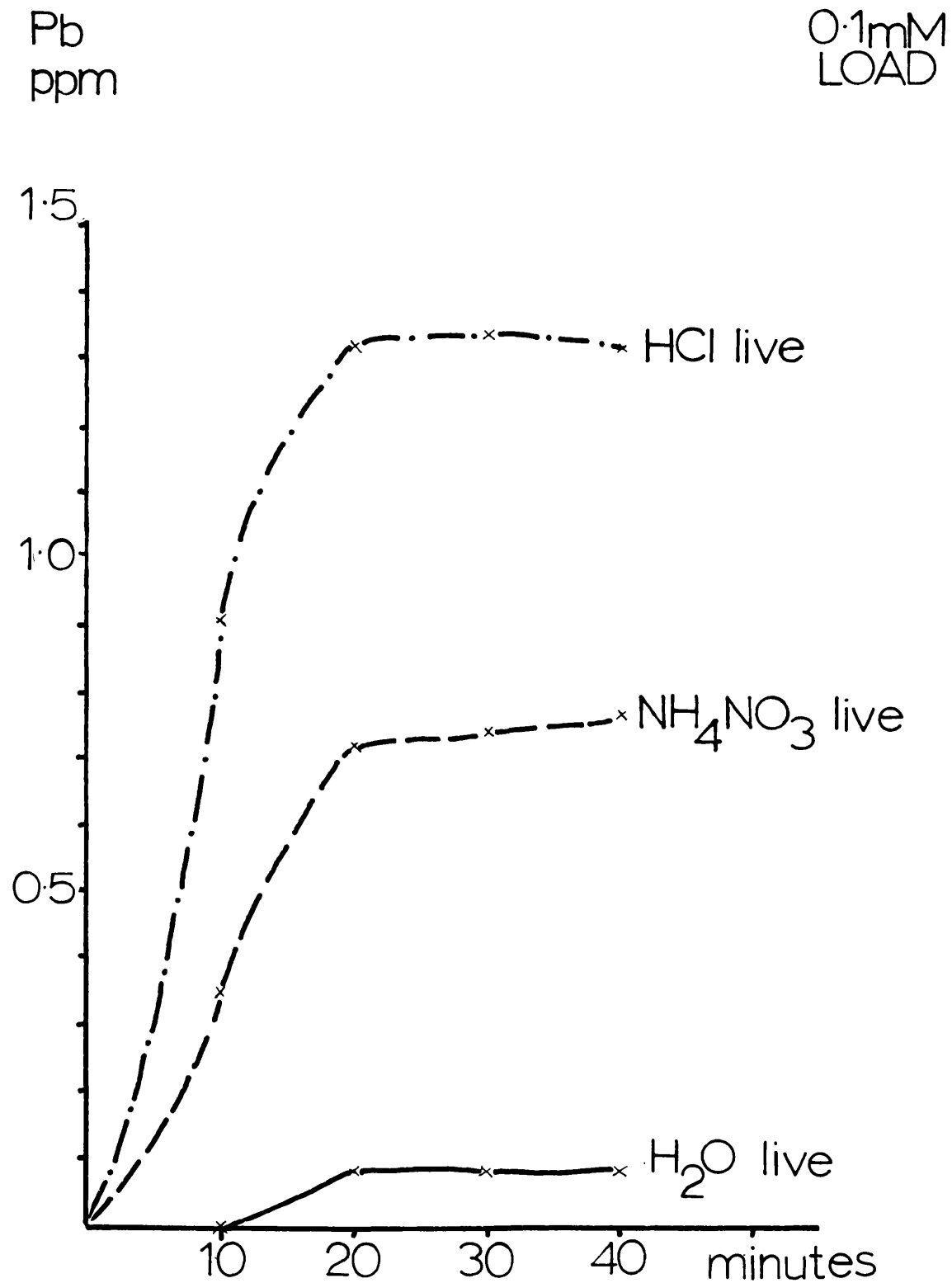


Fig. 11

Levels of Pb removed by different extractants from 10g of live grass, with time. This followed loading with 0.1mM  $\text{Pb}(\text{NO}_3)_2$ .

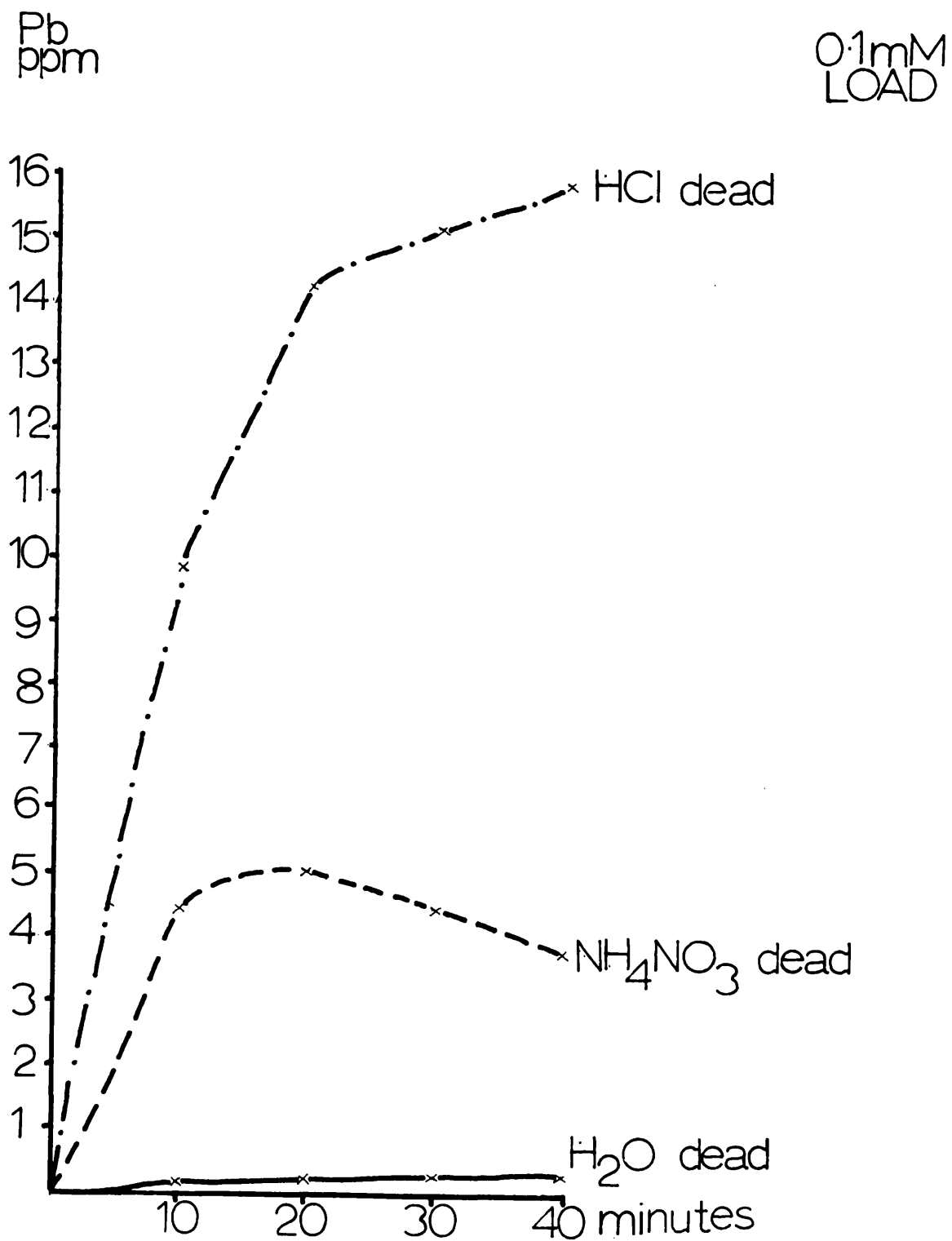


Fig. 12

Levels of Pb removed by different extractants from 10g of dead grass, with time. This followed loading with 0.1mM  $\text{Pb}(\text{NO}_3)_2$ .



The surface-held component in all grass is low (both in the artificially loaded material, and that taken from the verge). Assuming that particulate Pb is bound on the grass surface, then this is only a small fraction of the total Pb content of roadside vegetation.

These experiments are short-term, but are probably comparable to the verge situation since most of the bound Pb must be taken up from solution. If allowed to dry, it is likely that the  $\text{Pb}(\text{NO}_3)_2$  would break down to a more insoluble salt, such as  $\text{PbO}_2$ , in the experimental material, and this may happen on the verge. This would account for the large proportion only removed by 10% HCl.

In the 'live' grass the ionically-held component is seen to represent a much larger fraction of the total Pb, compared to 'dead' grass. One of the major differences between 'live' and 'dead' grass is almost certainly the condition of the cuticle, and the access which solutions have to the intercellular spaces of the plant. A third experiment tested the ability of an intact cuticle in excluding Pb from 'live' grass. Young, healthy shoots of Arrhenatherum elatius were cut and their severed ends dipped in molten beeswax/petroleum jelly, to seal the cut-end; another set were left untreated. This was to estimate the amount of Pb absorbed at the cut-end, compared to that entering over the leaf surface. Three 10g replicates of each set were placed in separating funnels and loaded with 1mM  $\text{Pb}(\text{NO}_3)_2$  over a period of 30 hours. Again, these were agitated occasionally, and samples of solution periodically analysed.

Although the mean Pb level of the unwaxed grass is consistently lower than that of the waxed material (Fig. 13), the standard error terms show the difference to be slight. After 25 hours it was noted that the grass had begun to deteriorate, and it became totally 'wetable'.

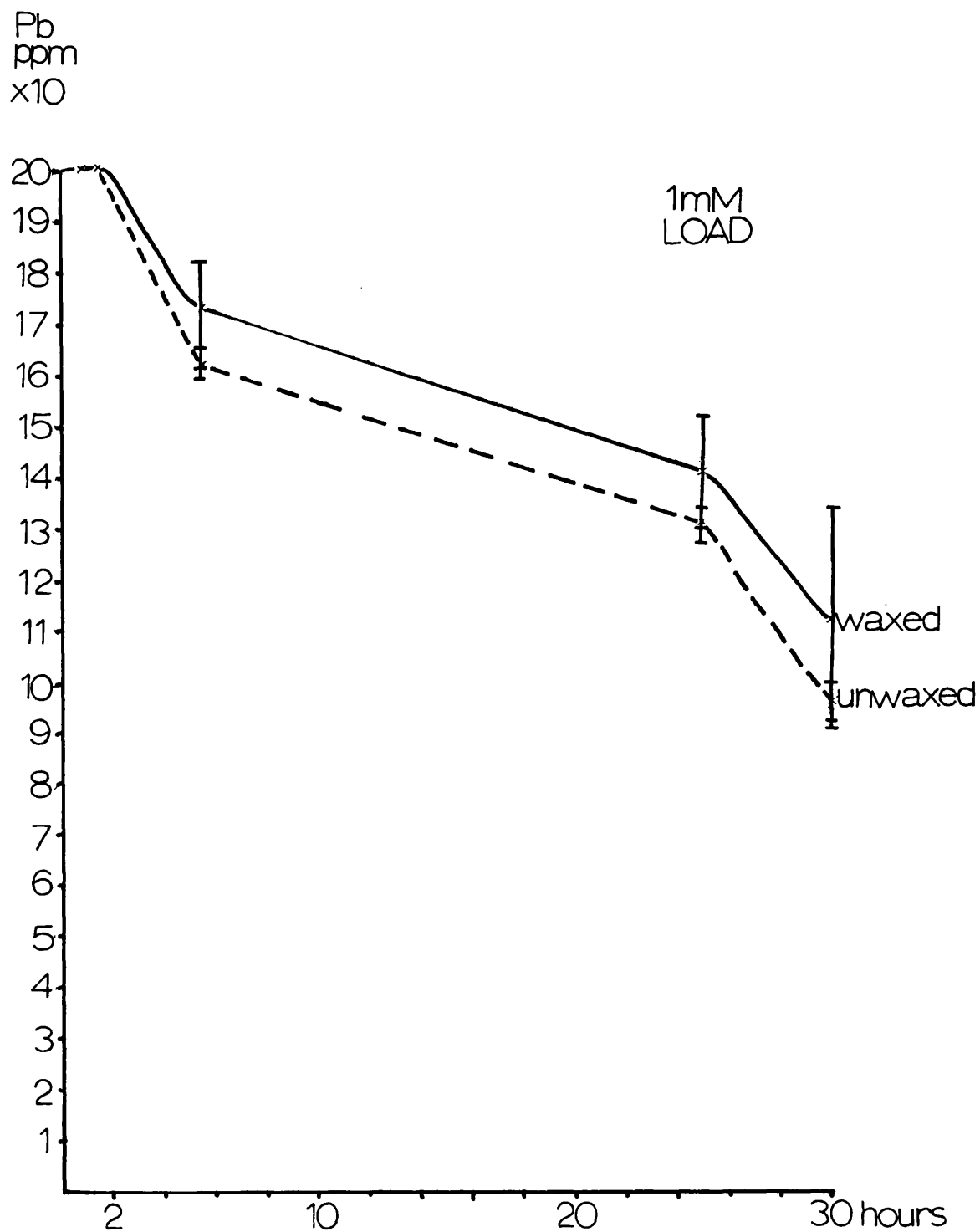


Fig.13

Levels of Pb remaining in solution which contains 10g of live grass, either with the cut-ends waxed or unwaxed, with time. The initial solution was 1mM  $\text{Pb}(\text{NO}_3)_2$ . This represents reciprocally what has been taken up by the grass.

At this time, it was apparent that the intercellular spaces had become injected with the solution, and this was reflected in the increased uptake of Pb (Fig. 13). In terms of its appearance, the grass closely resembled the grass at the road margin following severe weather conditions in the winter. Although these situations are not totally comparable, this does suggest that saturation of the intercellular spaces of the grass is responsible for the extremely high levels of Pb recorded occasionally in 'live' grass during the winter (cf. section 2.4).

Again, following thorough rinsing with deionised water, each replicate was extracted with a different solution (10% HCL, 1N  $\text{NH}_4\text{NO}_3$  and deionised water), and the levels of Pb in the extract measured with time. The relative efficacy of each extractant is similar to that found before (Fig. 14), though obviously there are differences between the waxed and unwaxed material.

These results suggest that the cut-ends are not the major source of entry for Pb in solution (and that there was no significant error in the previous experiments), but that this is across the leaf surface. This may be through the stomata or other discontinuities in the cuticle, though after a sufficient period of soaking, the grass appears to become wettable and the solution penetrates the plant tissue. This results in increased uptake. Whether this increase follows fracturing of the cuticle or not is not known.

Earlier, less controlled experiments, also suggest that the total capacity of either 'live' or 'dead' grass to bind Pb was not reached. This may be in excess of  $1.5 \times 10^3$  ppm Pb for both.

The ability of the cell wall to bind Pb (Höll et al, 1975) has been established, and this may well be the main site of

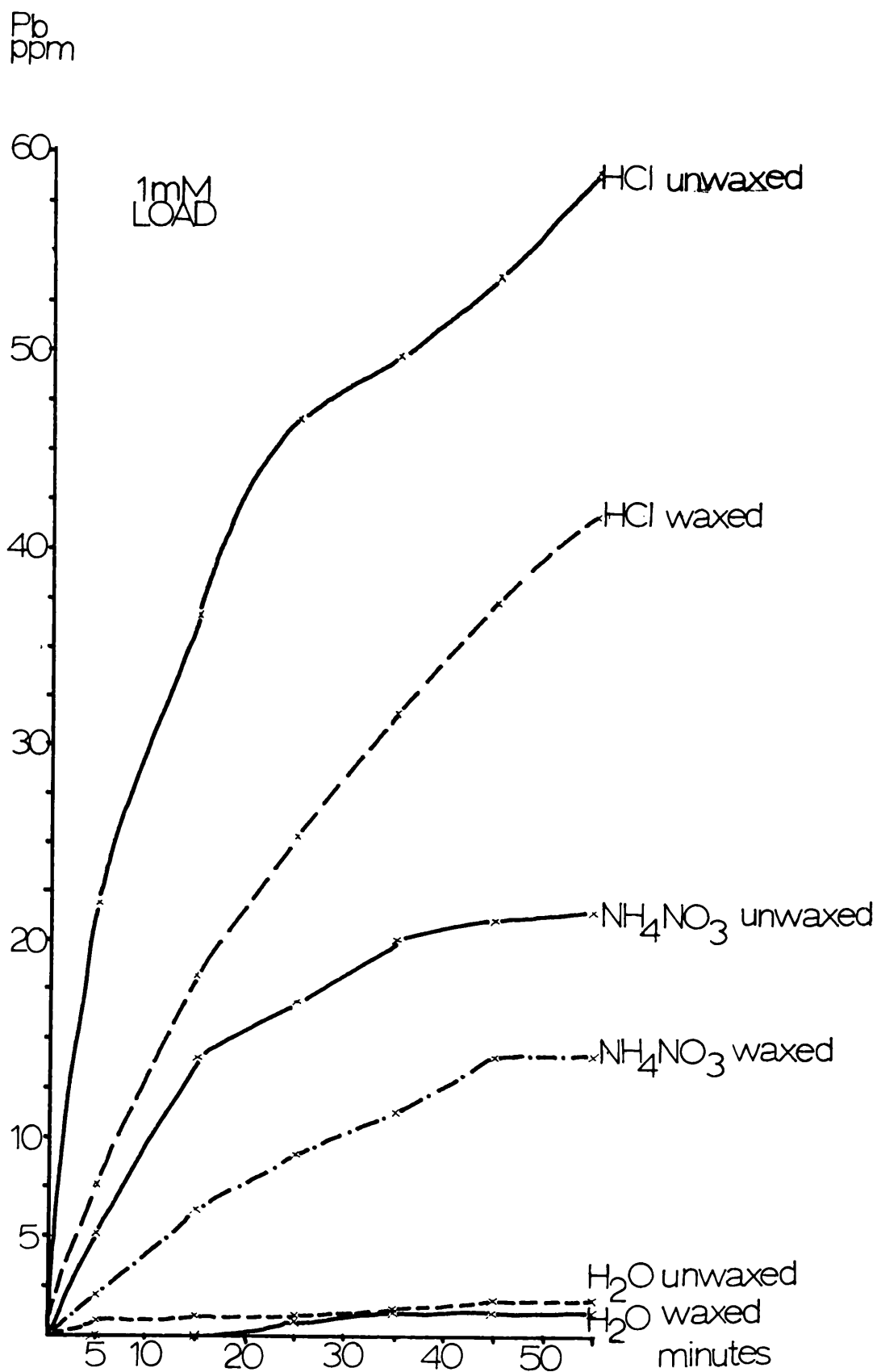


Fig. 14

Levels of Pb removed by different extractants from 10g of live grass, either with the cut-ends waxed or unwaxed. This followed loading with 1mM Pb(NO<sub>3</sub>)<sub>2</sub>.

Pb binding in these experiments. Certainly, these results suggest that the condition of the cuticle is likely to be the major difference between 'live' and 'dead' grass, determining the degree of exposure of the tissues and their consequent ability to bind Pb.

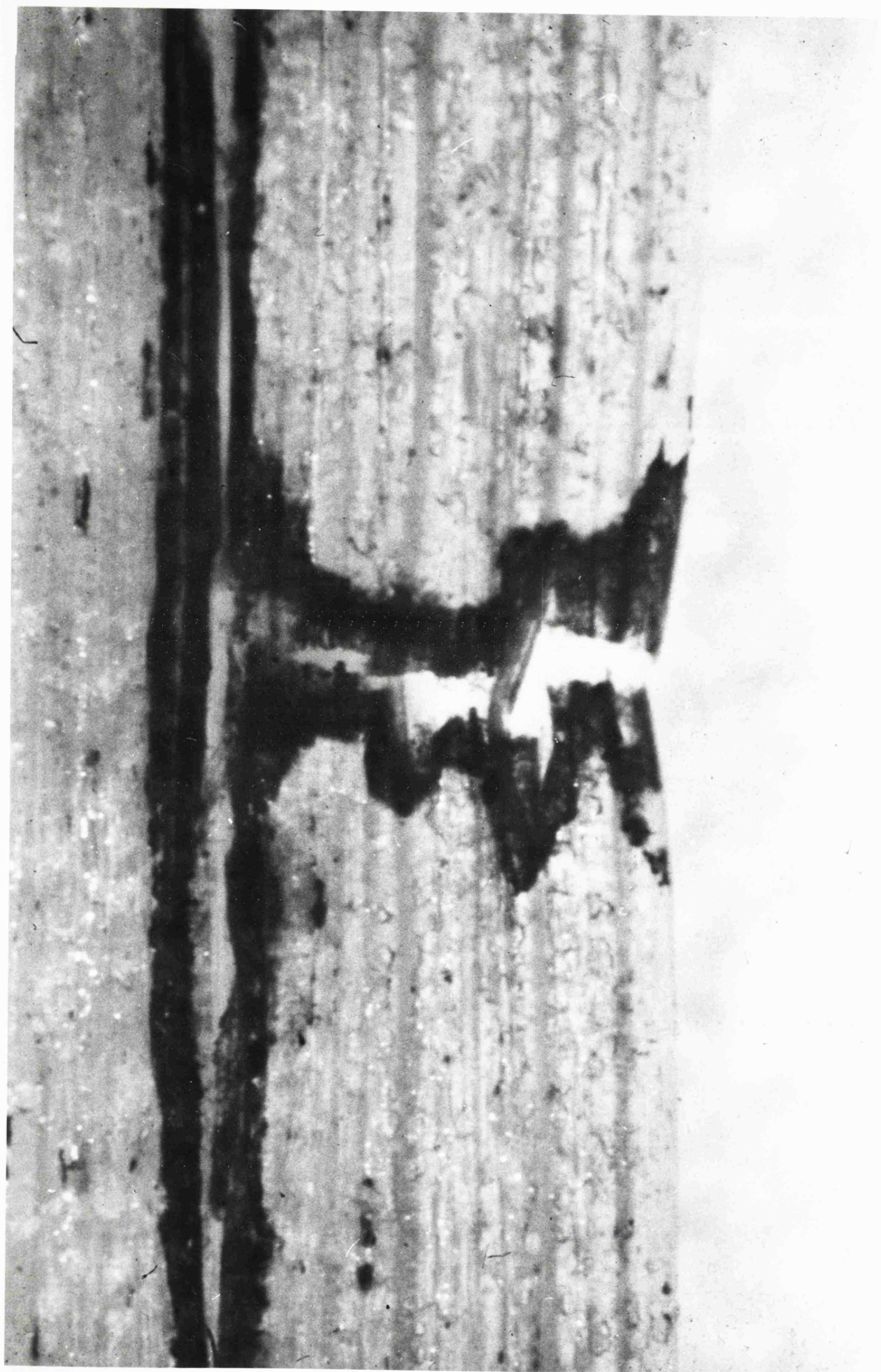
How the Pb is bound in grass is not known. The HCl-extractable component may well be that <sup>which is</sup> chelated or chemically bound by the grass tissue, though this may also be due to the insoluble Pb salts. These experiments suggest that rapid uptake of Pb in solution takes place by the grass, and that some fraction of this is exchangeable (as the component removed by  $\text{NH}_4\text{NO}_3$ ), though this may decompose to an insoluble Pb salt. The results from the various extractions of roadside grass support the view that much of the Pb is bound in solution (by the size of the HCl- and  $\text{NH}_4\text{NO}_3$ -extractable components), and consequently, that the state of the cuticle is the major difference between 'live' and 'dead' material.

Attempts were also made to stain grass sections for Pb using the Sodium Rhodizenate technique discussed by Glater and Hernandez (1972). This was only successful with heavily contaminated grass, that was unsectioned and fully wettable. It was totally ineffective on properly fixed and embedded grass, that had been alcohol dehydrated. Sodium rhodizenate (0.2% aqueous solution) reacts with Pb to leave a scarlet red stain in a buffer of pH 2.8 (1.5g Tartaric acid and 1.9g Sodium bitartrate in 100cc of water), and an example is shown in Fig. 15. Here the Pb stain is shown around a damaged area of the blade of grass, after it had been loaded with 1mM  $\text{Pb}(\text{NO}_3)_2$  and then extracted with  $\text{Ca}(\text{NO}_3)_2$  (which should only extract the exchangeable component). Only the most firmly-held

Fig. 15

Pb around a damaged zone in the surface of a blade of Dactylis glomerata. This is shown by the dark (stained red) area around the horizontal and vertical fractures.

The grass was stained with Sodium Rhodizenate, after loading with 1mM  $\text{Pb}(\text{NO}_3)_2$  and extraction with 10mM  $\text{Ca}(\text{NO}_3)_2$  (see text). About 20x.



Pb has been stained, and it is apparent how the fracture has allowed the Pb to enter, if only a short distance, into the tissues.

### 2.3 Lead deposition on roadside vegetation with distance from the road

All of the early work on levels of Pb deposition in roadside vegetation consisted of samples where no distinction was made between living and dead material, yet the pattern reported was always of a particular form: high levels immediately next to the road which rapidly fall off with distance to a low basal level.

To confirm this was the case in both 'live' and 'dead' grass, the vegetation of site 1 was sampled along a transect from the road margin to the hedgerow, and into the field behind the hedge (February 1975). The samples were returned to the laboratory and 'live' and 'dead' components extracted for each distance. These were then dried and analysed in the normal way (of. Methods V.i). The results are shown in Fig. 16; no 'dead' grass was found in the field and so only the Pb content of the 'live' grass is shown at this distance. The rapid decline in the Pb levels of both components is apparent and is comparable to the typical pattern described by previous workers (Motto et al, 1970; Fidora, 1972). At the road margin 'dead' grass is about five times the level of 'live' grass, but because the pattern of decline is similar, an unsorted sample would probably show a comparable decline with distance from the road. In this case, however, the actual degree of contamination would be somewhere between these two levels in an unsorted sample, but would be a less informative figure. They may also differ if one component shrouds the other, leading to differential rates of accumulation.



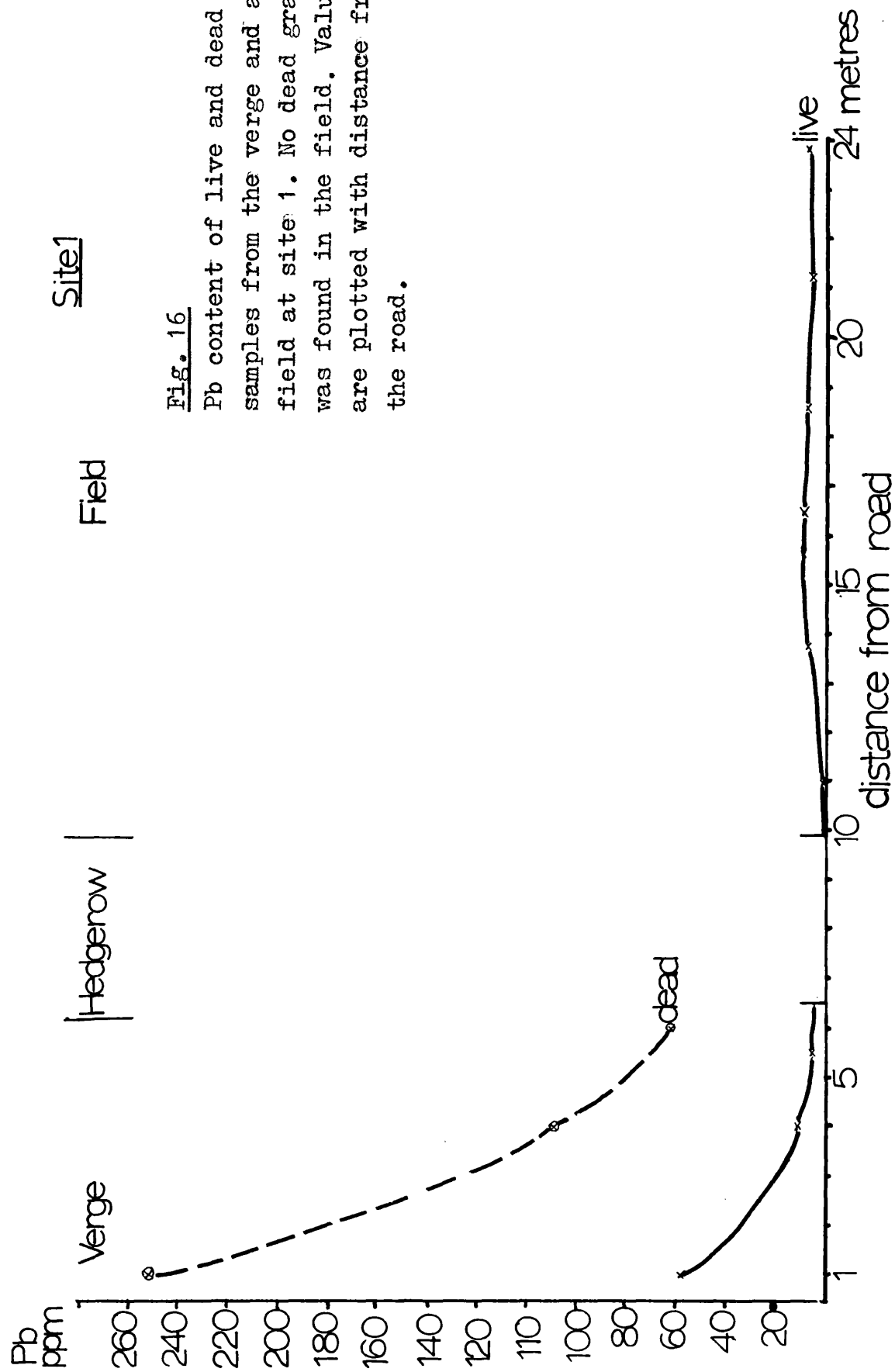


Fig. 16

Pb content of live and dead grass samples from the verge and adjacent field at site 1. No dead grass was found in the field. Values are plotted with distance from the road.

Similar transects were taken at six other sites for comparison, by taking grass samples from three distances from the road (October 1976). These were the minor sites and three of the major sites (1,  $\alpha$ , and 5). No 'dead' grass was found at the A50 site, at this time.

The major sites all face East and have comparable verge morphologies (cf. section 1.1); the Pb levels in their vegetation are shown in Fig. 17 and 18. Elevated levels at the road margin are particularly apparent in the 'dead' grass, though a much flatter profile is seen at site 5. Here the traffic frequency is less, but also the hedgerow is smaller than at sites 1 and  $\alpha$ , and this may be a contributory factor in determining the rate of decline of the Pb levels.

The same pattern is true of the A47, which is of similar form to site 5, except that it faces South. As a result, the aspect of the site with regard to the predominantly westerly winds is probably responsible for the small fall in Pb levels with distance from the road, by dispersal of airborne Pb away from the verge. This may also be true of the flattened profile seen in the 'live' grass of the A46, which faces West. A much steeper and regular decline occurs in the Pb levels of the 'dead' grass (Fig. 18) which formed the more extensive cover at this site. This verge again has a low hedgerow, with an extensive ditch, leaving only about 4m of level surface. Also, there is a  $\frac{1}{2}$ m-wide paved area next to the road. The 'dead' grass shrouded the 'live' grass over most of the site, and this may account for the different patterns from the 'live' and 'dead' components. Again, aspect with regard to the predominant winds is the most likely control on the rate of decline of Pb levels in 'dead' grass.

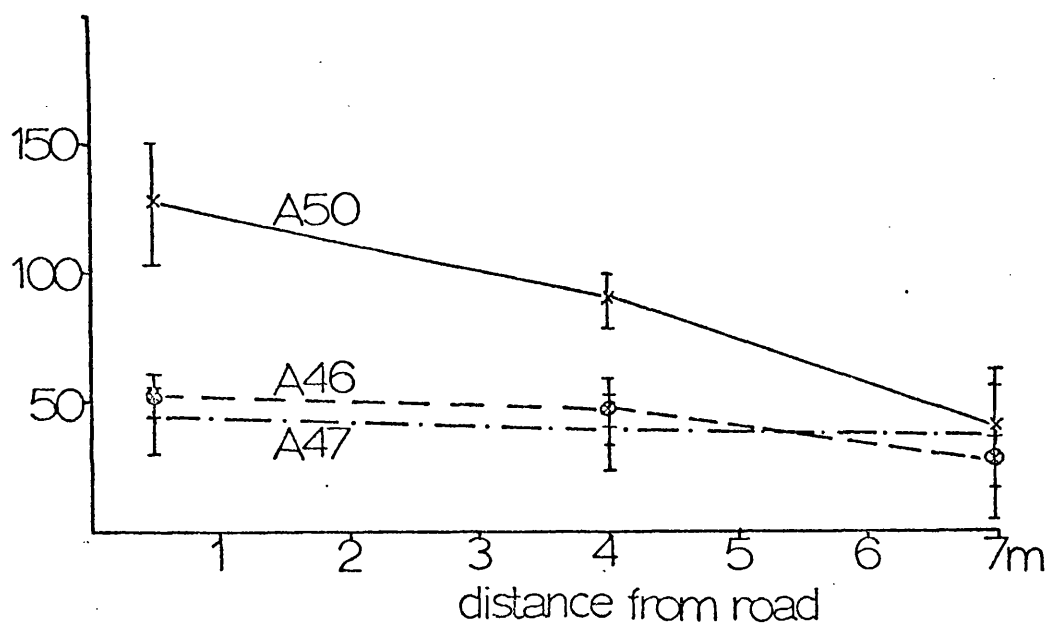
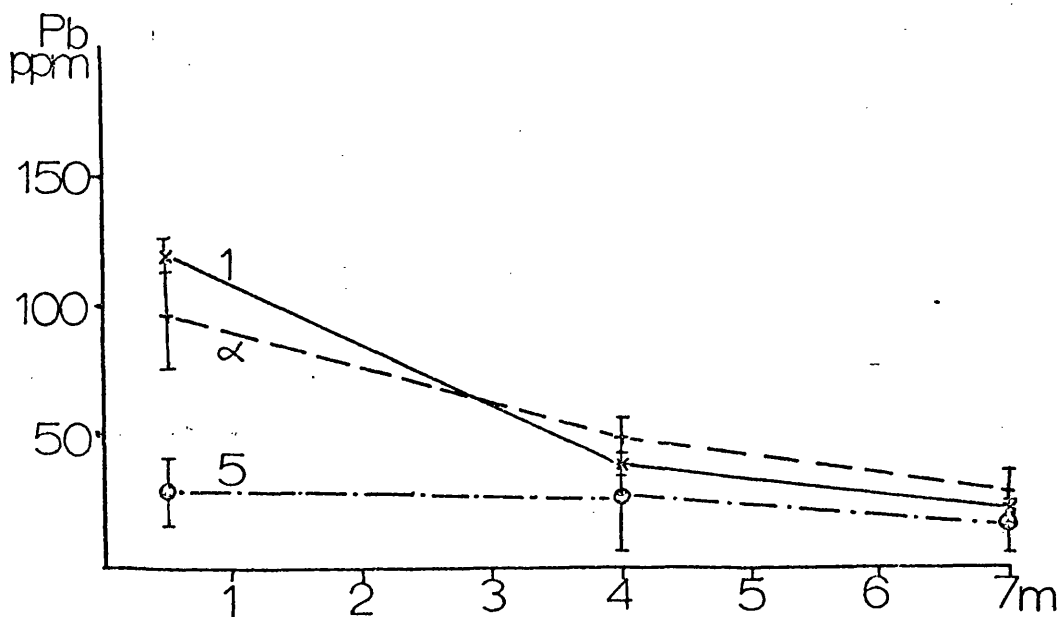


Fig. 17

The decline in the Pb content of live grass with distance from the road, at three of the major sites and the minor sites (15/10/76).

all live  
unwashed

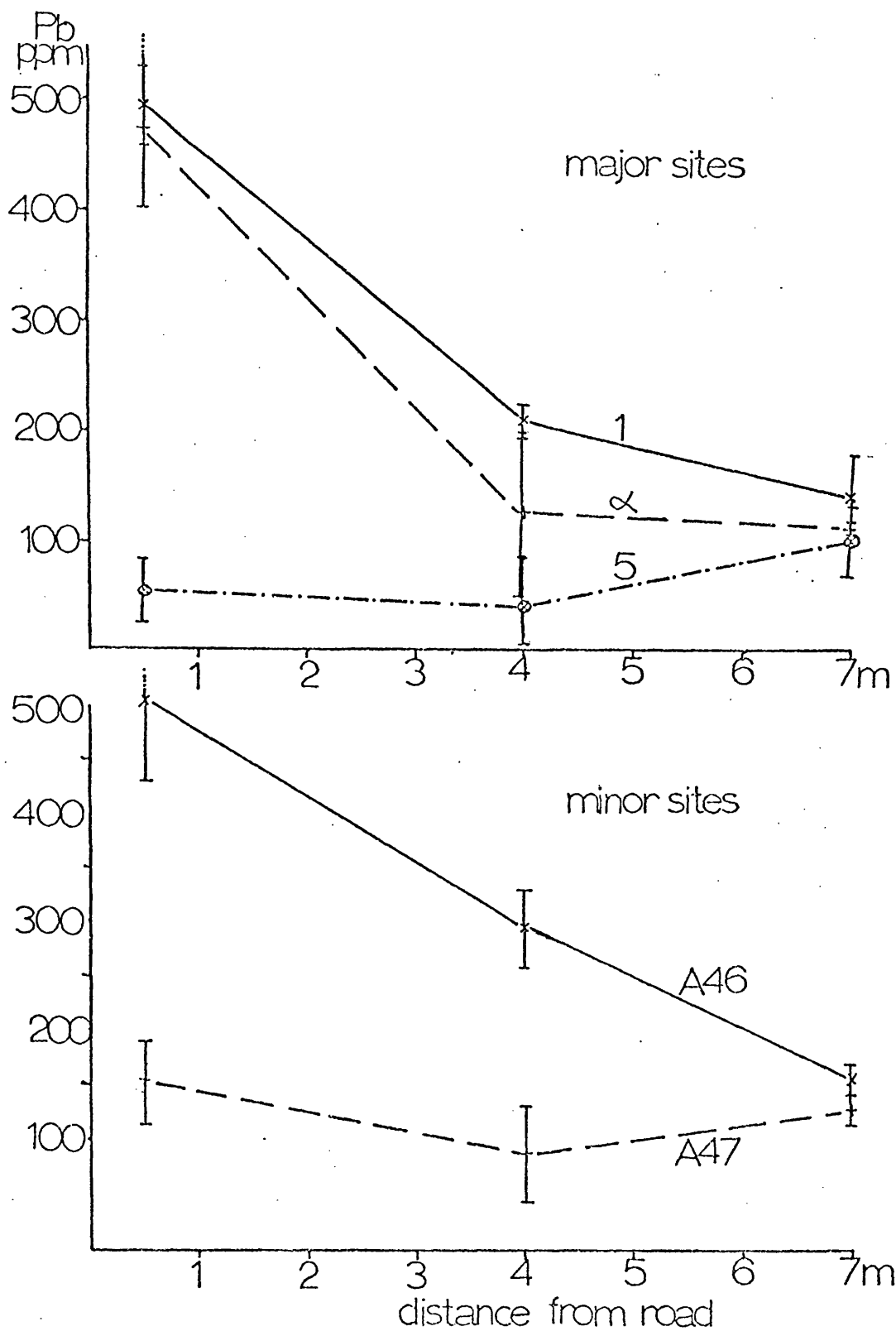


Fig. 18

The decline in the Pb content of dead grass with distance from the road, at three of the major sites and the minor sites (15/10/76).

all dead  
unwashed

The Pb levels of the 'live' grass of the A50 show a regular decline with distance from the road. This site is flat, without a ditch, and with a rock outcrop (about 3m high) in place of the hedgerow. It faces North on an East-West dual carriageway, and aspect may again be the main factor producing the pattern of the decline of Pb levels in the grass.

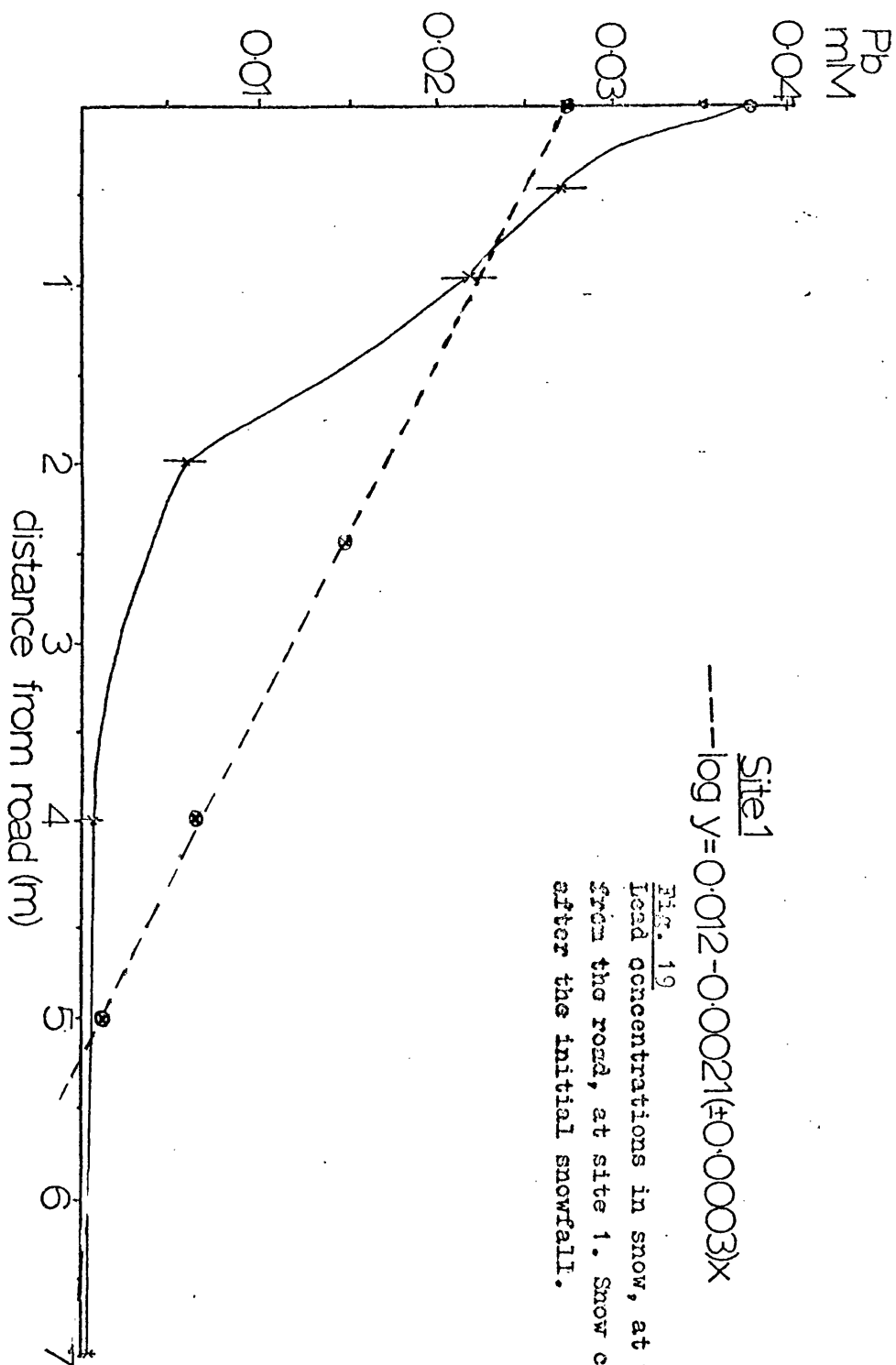
The effects of precipitation on the typical pattern of Pb deposition has recieved little attention. It may be important for several reasons, such as washing particulate Pb from vegetation, making soluble Pb available for uptake by grass, and in producing wet roads, so that passing vehicles generate a fine spray which passes onto the verge. Indeed, the movement of this spray across the verge has been observed on several occasions, and at several sites, and in visual terms at least, would seem to illustrate perfectly the pattern of turbulence described in the wind measurements of section 1.3.

Two experiments were carried out to estimate Pb deposition by spray 'fall-out' during wet conditions. One involved the collection of snow samples, at various distances from the road, 24 hours after the initial snow fall. About 6" of snow fell within three or four hours, and began to melt on the road, immediately after the cessation of the fall. The snow on the verge was very soon heavily discoloured by the consequent spray. Samples were collected by placing clean polypropylene containers of 10cm diameter over the snow to obtain a core, by pressing down to the vegetation surface. The full container was then removed and returned to the laboratory to melt at room temperature. The volume of the liquid was measured and transferred

to a clean glass beaker, and evaporated to dryness in an oven at 70°C. 20cc of 50%  $\text{HNO}_3$  was then added to each beaker and stirred until all deposits were in suspension. This was evaporated to dryness on a hot-plate, and the cooled residue taken up in 10cc of 25%  $\text{HNO}_3$ . This was placed in a Kjeldahl flask and gently boiled for 1 hour, before filtering into a 100cc volumetric flask and making up to volume with deionised water. Spectrophotometric analysis was performed as for vegetable material (cf. Methods V.i), and the results are given in Fig. 19.

A second experiment used 10cm wide strips of paper with one side polythene-coated ('Benchkote'), suspended across site 1. The strips were pinned <sup>horizontally</sup> to wooden stakes inserted into the ground, so that the paper was just above the level of the vegetation. This was carried out on two occasions, immediately following heavy rainfall, collecting the strips after 4 and 24 hours respectively. The strips were rolled and returned to the laboratory (there was little transfer of particles from the absorbant face to the adjacent polythene face) to dry at room temperature. 50cm<sup>2</sup> samples were cut at various distances along the strip, and also three control squares from an unexposed section of paper. Each square was cut into smaller fragments and placed in a glass beaker, to which was added 30cc of concentrated  $\text{HNO}_3$ . This was shaken and allowed to stand for 12 hours in a fume cupboard. The acid was drained off into a clean glass beaker, and the paper rinsed with deionised water. The resulting liquor was then evaporated to dryness and treated as in the snow experiment. The results are presented in Fig. 20.

Before cutting the paper, the coverage of particulate matter on the paper was measured with an EEL reflectance densitometer (calibrated against an unexposed portion of



HP  
(mM)  
x0-0001

site 1

Fig. 20

Lead concentrations in paper strips

after exposure on the verge for 4

hours (24.1.77) and 24 hours (2-3.11.77)

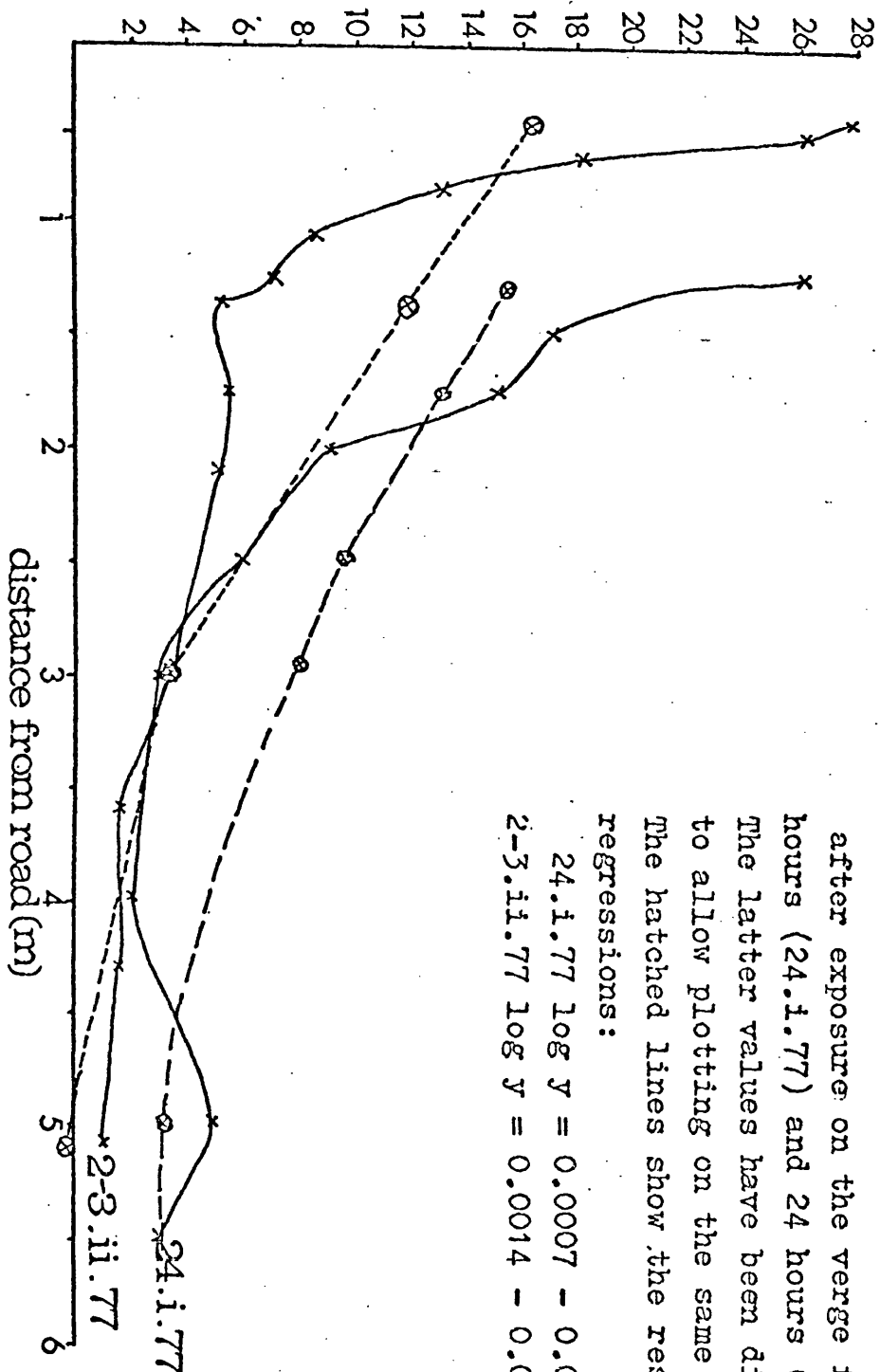
The latter values have been divided by 2  
to allow plotting on the same graph.

The hatched lines show the respective

regressions:

$$24.1.77 \log y = 0.0007 - 0.00017(\pm 0.00005)x$$

$$2-3.11.77 \log y = 0.0014 - 0.00039(\pm 0.00009)x$$





paper). The discolouration of the paper with distance from the road is shown in Fig. 21.

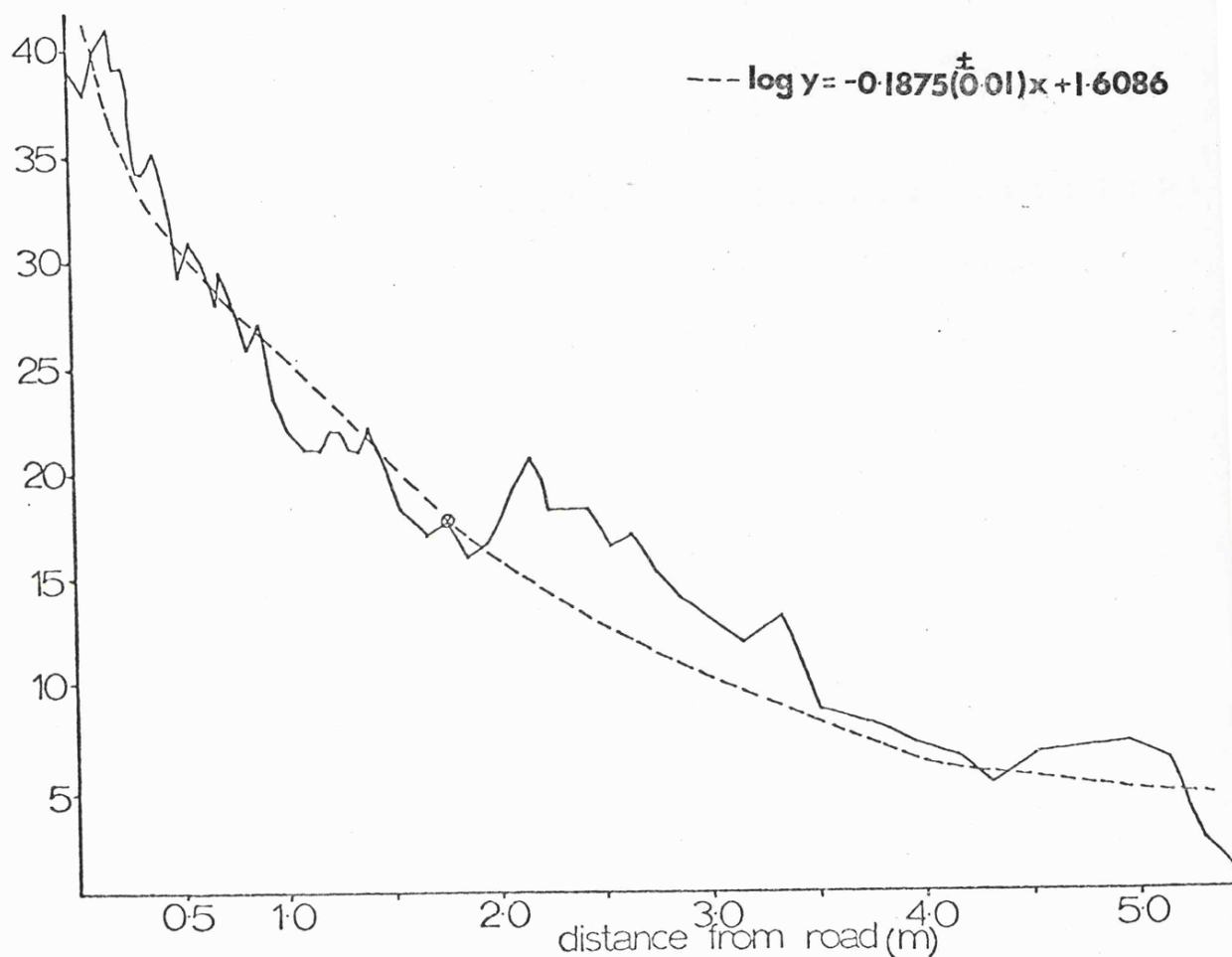
The Pb content of both the snow and paper strips show the enhancement of levels next to the road margin, and the subsequent rapid decline. A regression analysis shows no significant difference between these curves ( $t = 1.0317$  NS). These results demonstrate that this typical pattern is not simply a 'dilution' effect - the decline of Pb levels with distance from the road is a real fall in the rate of deposition, rather than a uniform rate being diluted by different thicknesses of vegetation (which increase toward the back of the verge).

In the snow samples, the elevated levels of Pb next to the road represent a very large input of Pb over the 24 hour period, - about  $173 \text{ mg/m}^2$ . This is compared to 15 mg for the 24 hour paper sample; the difference is most probably due to the larger amounts of water on the road during the snow experiment.

The results from the reflectance densitometer readings of the paper (24 hour sample) show a similar decline in discolouration as observed with the Pb levels. In fact, these two curves are not significantly different ( $t = 1.8302$  NS), as shown by regression analysis of distance against the log. of either Pb level ( $r = -0.7617$ ,  $P < 0.01$ ) or discolouration ( $r = -0.9306$ ,  $P < 0.001$ ). This shows a correlation of the deposition of particulate matter on the paper strip with Pb level: circumstantially, this may mean that most of the Pb is deposited in particulate form, though further experimentation would be needed to confirm this.

If spray was of importance in enhancing Pb levels in roadside vegetation, it might be expected for values to be elevated after a period of heavy rainfall. Conditions under

%  
discolouration



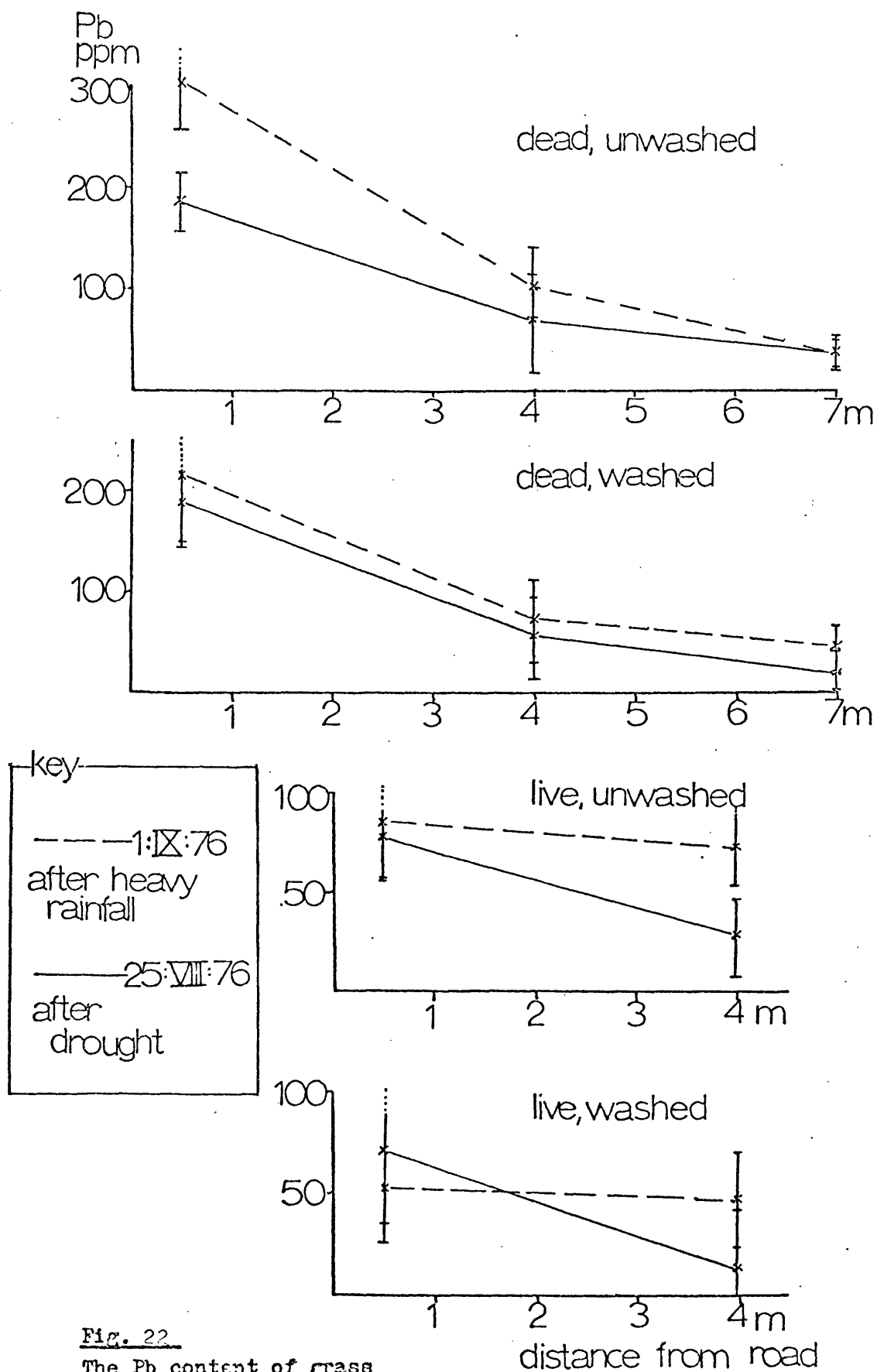
**Fig. 21**

The discolouration of a strip of paper after being exposed at site 1 for 24 hours (2-3/11/77), used to measure particle deposition across the width of the verge. The regression of discolouration against distance is also shown ( $r = -0.9306$ ,  $P > 0.001$ ). This was measured with a reflectance densitometer, and the results are expressed as percentage discolouration compared to an unexposed strip of paper (see text for details).

which to test this arose at the end of the drought of 1976, when the weather was about to change, and rainfall was expected. A transect of grass samples across site 1 was taken a week before the drought ended, and <sup>a second transect</sup> after a period of heavy rainfall. Both 'live' and 'dead' components were extracted, and further sub-divided into two parts, one of which was washed in deionised water (to estimate surface contamination) and the other left untreated. No 'live' grass was found at the 7m distance, since the vegetation here consisted of dead Arrhenatherum elatius alone. The results are given in Fig. 22.

Elevated levels are apparent over the whole width of the verge in the 'dead' samples, after the rainfall. The 'live' material shows no clear-cut enhancement, probably because little 'live' material existed over the verge, and that sampled was shrouded in dead grass. The 'dead' unwashed grass Pb level is most enhanced (by about 50%) next to the road, though this declines with distance from the road. This would appear to be mostly surface contamination as the elevation in the Pb levels of the 'dead' washed sample is more regular over the whole width of the verge.

Spray from vehicles does appear to increase Pb deposition on the verge, and the extremely wet conditions produced by snow enhanced deposition by a magnitude greater than that of more normal wet conditions. Turbulence from passing vehicles renews the supply of contaminated air at the road margin, and in both wet and dry conditions this <sup>alone</sup> would induce the high levels on the verge next to the road. The typical pattern is probably a reflection of the reactions of airborne Pb with time and particulate fall-out immediately after emission, roughly corresponding to the pattern described in the air above the verge studied by Daines et al (1972). That this pattern is not repeated at the minor sites is probably a result of the



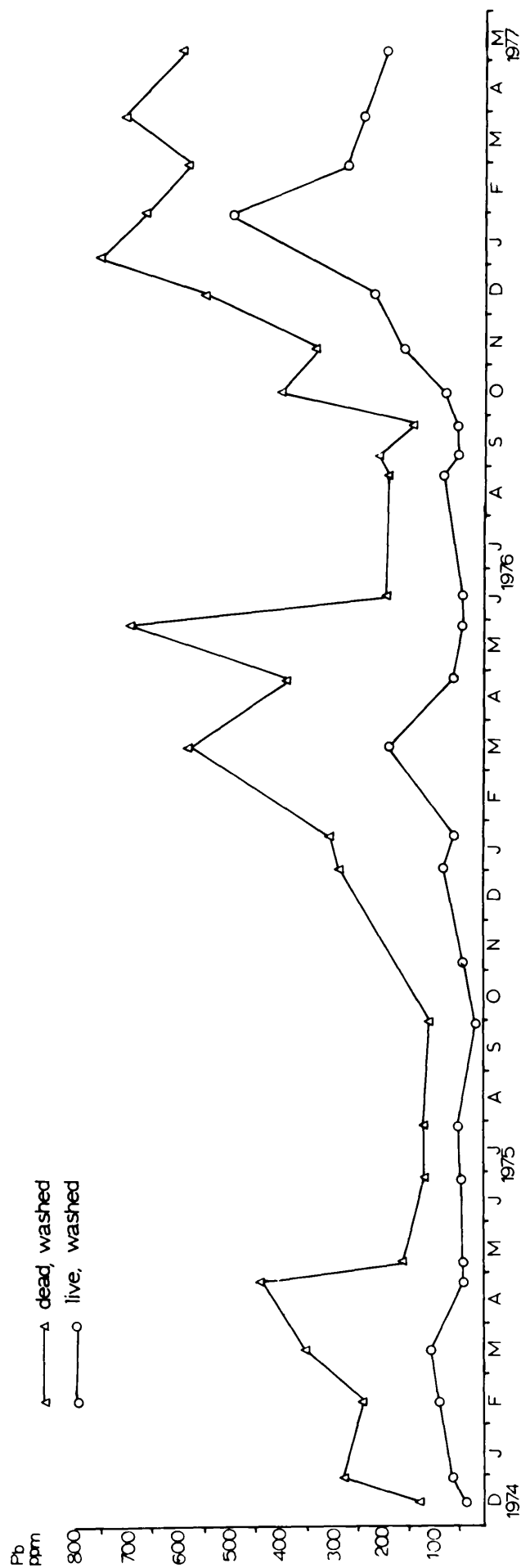
effect of aspect of the verge in relation to the predominant winds altering the pattern of Pb deposition in both wet and dry conditions. Dry deposition and wet deposition probably only differ in degree, and their relative importance will depend on climatic conditions. What effect the diffusion of fine particles of Pb (the Pb aerosol) have on this pattern is difficult to estimate, though in any case, this will probably comprise the fraction blown away from the roadside (Hammond et al, 1972).

#### 2.4 Seasonal Changes in the Pb content of roadside vegetation

The ability of rainfall to increase Pb deposition on roadside vegetation is likely to result in seasonal changes in the Pb level found in the grass. Such changes have been considered only by a few authors, notably Mitchell and Reith (1966) and Rains (1970), though Daines et al (1972) describe a seasonal fluctuation in airborne Pb (New Jersey, USA). All these studies show a winter maximum, which, in the case of the airborne Pb is attributed to low atmospheric mixing in the winter. No comparable, long term studies have been reported from Britain.

In the present study, a sampling programme was run from December 1974 until May 1977. Monthly grass samples were taken at 0.5m from the road margin at site 1, and 'live' and 'dead' fractions extracted. Each fraction was further sub-divided into two parts, one of which was washed in deionised water for 10 minutes (to estimate superficial deposits) and the other was analysed unwashed (cf. Methods V.i). Rainfall was also measured at this site during the period of sampling. The results are shown in Fig . 23 and 23a. Sampling





**Fig. 23a** The Pb content of live and dead grass samples (after being rinsed in deionised water) from site 1, 1/2m from the road, over a two year period.

was slightly irregular, partly because of the absence of 'dead' grass at various times, and partly because of transport difficulties. The adjacent hawthorn hedgerow was also sampled during the period of its foliage cover in 1976. Samples were taken at about 1.7 m high, as twigs, which were taken to the laboratory and stripped of the leaves. The leaves were left unwashed, prior to analysis in the normal way (Fig. 24).

The condition of the grass on the verge underwent large changes from season to season, and there were also differences from year to year. Typically, in the summer there was mostly live grass, with a rapid turnover in both live and dead, so that little dead material was left standing for a long period of time. With the fall in temperatures of the autumn, this rate of turnover slowed, so that both live and dead grass were standing for increasingly longer periods, until, by the winter, there was little change. At this time, dead grass dominated much of the verge, but both live and dead were replaced at a very slow rate. Next to the road margin, the dead<sup>grass</sup> was often flattened by precipitation and formed a thick, sodden mat at the ground surface, with live shoots forming most of the standing crop. The higher temperatures of the spring increased both the rate of growth and decay, and the dead grass rapidly disappeared. The standing live grass of the winter died and was replaced by new shoots, returning to the rapid turnover conditions of the summer. In terms of the productivity of a unit area, these changes can be seen in the results from a later experiment,<sup>which</sup> (Figs. 25 - 27) shows the winter maximum in the weight of the standing crop (due to the low turnover rate), that falls to the summer low (high turnover).



Parallel with these changes in the composition of the roadside grass are changes in the Pb level (Fig. 23 and 23a). Under summer conditions, neither 'live' nor 'dead' grass accumulate high levels of Pb because of the short standing time (and low rainfall), but as this increases in the autumn, so do the Pb levels. A winter maximum of Pb content is reached when little growth and decay is possible in the low temperatures, and the standing time is increased. The Pb levels of the grass fall rapidly after the commencement of growth in the spring and the concomitant increase in the rates of decomposition. A natural result of this system is that a high level of Pb in 'live' grass will subsequently become a high level in 'dead' grass. The rise in the Pb content of the 'live' grass of the autumn will become part of the Pb level of the 'dead' material in winter. Thus, 'dead' grass maxima tend to follow 'live' grass maxima, and this is most apparent in Fig. 23a.

There is also a correlation between rainfall per month and Pb levels in 'live' unwashed samples ( $r = 0.6163$ ,  $P < 0.01$ ), and in 'dead' unwashed grass ( $r = 0.4393$ ,  $P < 0.01$ ). These coefficients are low because of the longer term factors involved in Pb accumulation (such as standing time), and this is likely to be the cause of the lack of correlation in the washed samples. There is little consistency in the amount of Pb removed by the deionised water wash from either 'live' or 'dead' grass, though notably the mean percentage removed from 'live' grass (43%) is higher than that of 'dead' (21%). This is compatible with the experiments of section 2.2 (p. 29), where the solution loading of 'live' and 'dead' grass suggest a similar difference. As expected, the Pb level is always greater in the 'dead' grass than the 'live' ('dead' unwashed mean level 515 ppm, 'live' unwashed mean level 191 ppm),

though on one occasion in the winter of 1977 the situation is reversed, This is almost certainly due to the poor condition of the 'live' grass at this time, when it closely resembled the wetted grass in the experiment described in section 2.2 (cf. Fig. 13), with its intercellular spaces similarly injected with solution. At the time of sampling the 'dead' grass formed a mat of prostrate leaves, and this may be responsible for its lower Pb level.

The difference between consecutive winters is less easily explained; for example, the winter of 1975/76 shows a higher Pb level than the previous winter, even though the rainfall was less abundant. On the other hand, the highest winter peak was in 1976/77 when the highest rainfall was recorded, yet there was no apparent increase in traffic over the period of the study. These differences may well be due to variations in the standing time of the grass in markedly different weather conditions. The density of the sward also varied from winter to winter, and this also might have affected the capacity of the grass to accumulate Pb.

The results from the hawthorn (Fig. 24) show a regular rise in its Pb level during the extremely dry summer of 1976. This increase probably represents the effect of standing time on Pb deposition and accumulation under dry conditions, with little effective growth during the summer. Its regularity would suggest a uniform input of Pb over the period sampled, entirely due to dry deposition.

A second experiment measured changes in the Pb levels of the standing crop of unit areas of the verge. Successive  $\frac{1}{2}\text{m}^2$  quadrats were cropped of vegetation to ground level at sites 1 and  $\propto$  (cf. Methods I.ii). This second site was used to compare Pb deposition at an unmown site ( $\propto$ ) with that

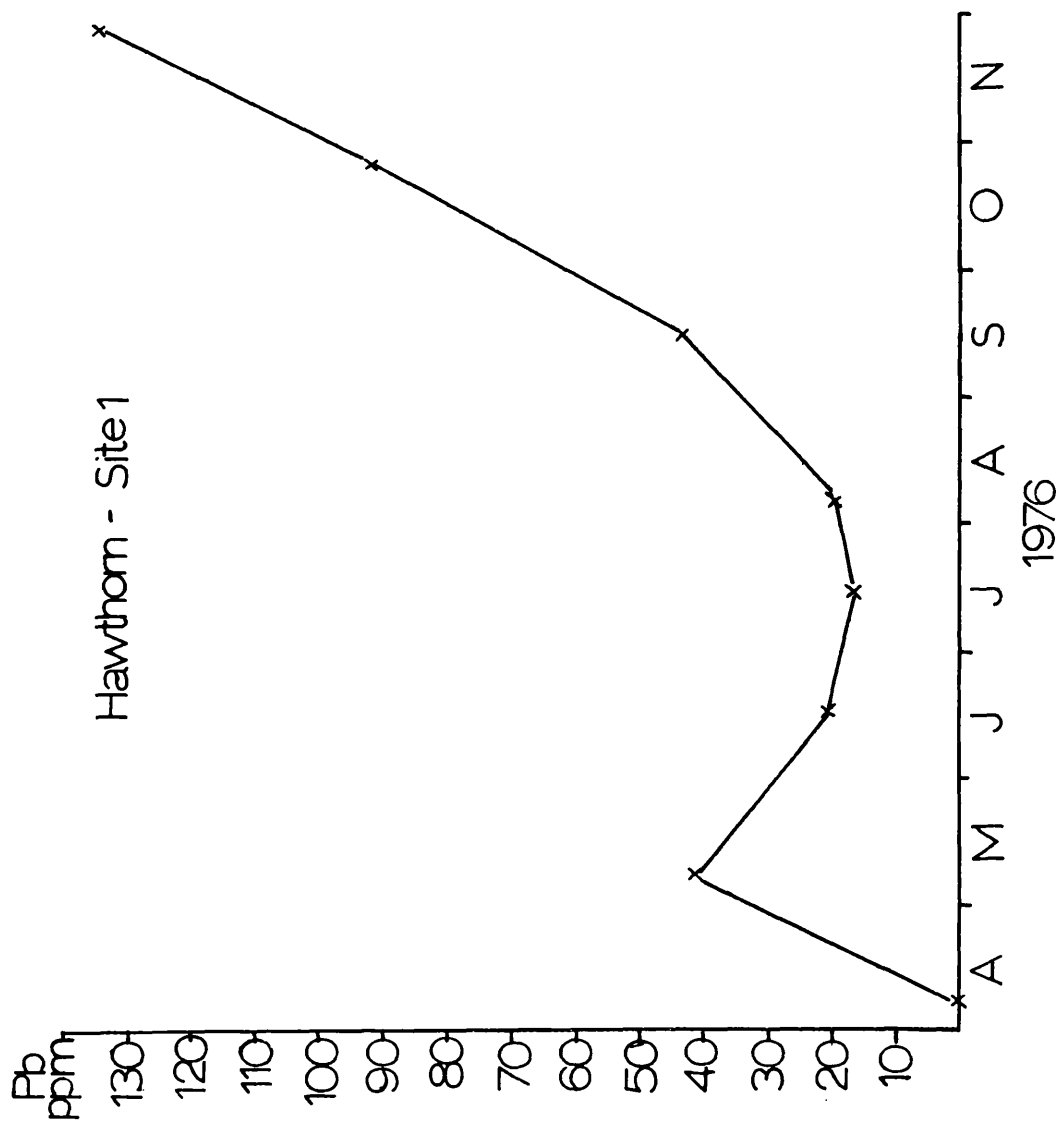


Fig. 24  
Pb content of the  
foliage of the site 1  
hawthorn hedgerow  
during 1976. All  
samples were collected  
at head height.

of a mown site (1). A third sample was taken at 7m from the road at site  $\alpha$ , in the zone which was never mown at either site. Assuming no filtering effect of the vegetation cover of the front of the verge, this would represent the same conditions at this distance of both site 1 and  $\alpha$ . The samples were taken at monthly intervals and left unsorted (due to their large size); they were analysed in the normal way (cf. Methods V.i). The dry weight of the grass cropped (this was referred to as the standing crop, comprising both live and dead grass) was recorded and the amount of Pb (in ppm and  $\text{g/m}^2$ ) was calculated. The results are given in Fig. 25 -27.

Generally, two maxima in the amount of Pb per  $\text{m}^2$  can be seen in each figure: a winter peak followed ( in both years) by a spring peak. From these results, it is possible to estimate the total input of Pb into the soil from the vegetation by assuming that whenever the  $\text{Pb/m}^2$  is lower than on the previous sampling occasion, the difference has been added to the soil. This will be a minimum estimate for several reasons, most particularly because Pb is likely to be added to the soil throughout the year, and not simply when the apparent level in the grass falls. The total for each site is given below:

Site 1,  $\frac{1}{2}\text{m}$  ....  $1.387 \text{ g/m}^2/\text{year}$

Site  $\alpha$ ,  $\frac{1}{2}\text{m}$  ....  $1.073$  "

$\alpha$ , 7m ....  $0.347$  "

There is no statistical difference in the  $\text{Pb/m}^2$  between site 1 ( $\frac{1}{2}\text{m}$ ) and site  $\alpha$  ( $\frac{1}{2}\text{m}$ ) ( $t = 1.0500$  NS), and so a rough estimate for the  $\frac{1}{2}\text{m}$  distance is about  $1.2 \text{ g Pb/m}^2/\text{year}$ .

How low this estimate is cannot be easily assessed, though it may be compared to an estimate of the yearly addition of Pb to the grass, which was next calculated. The period of the

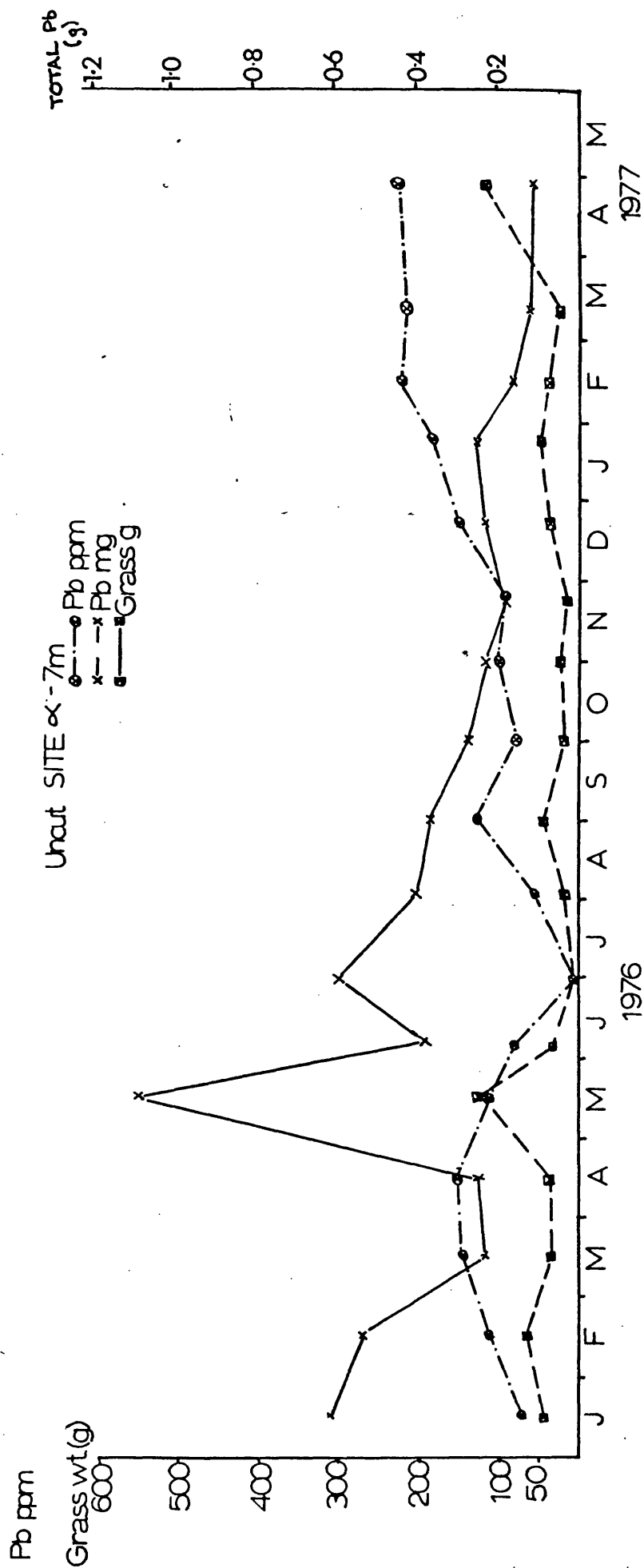
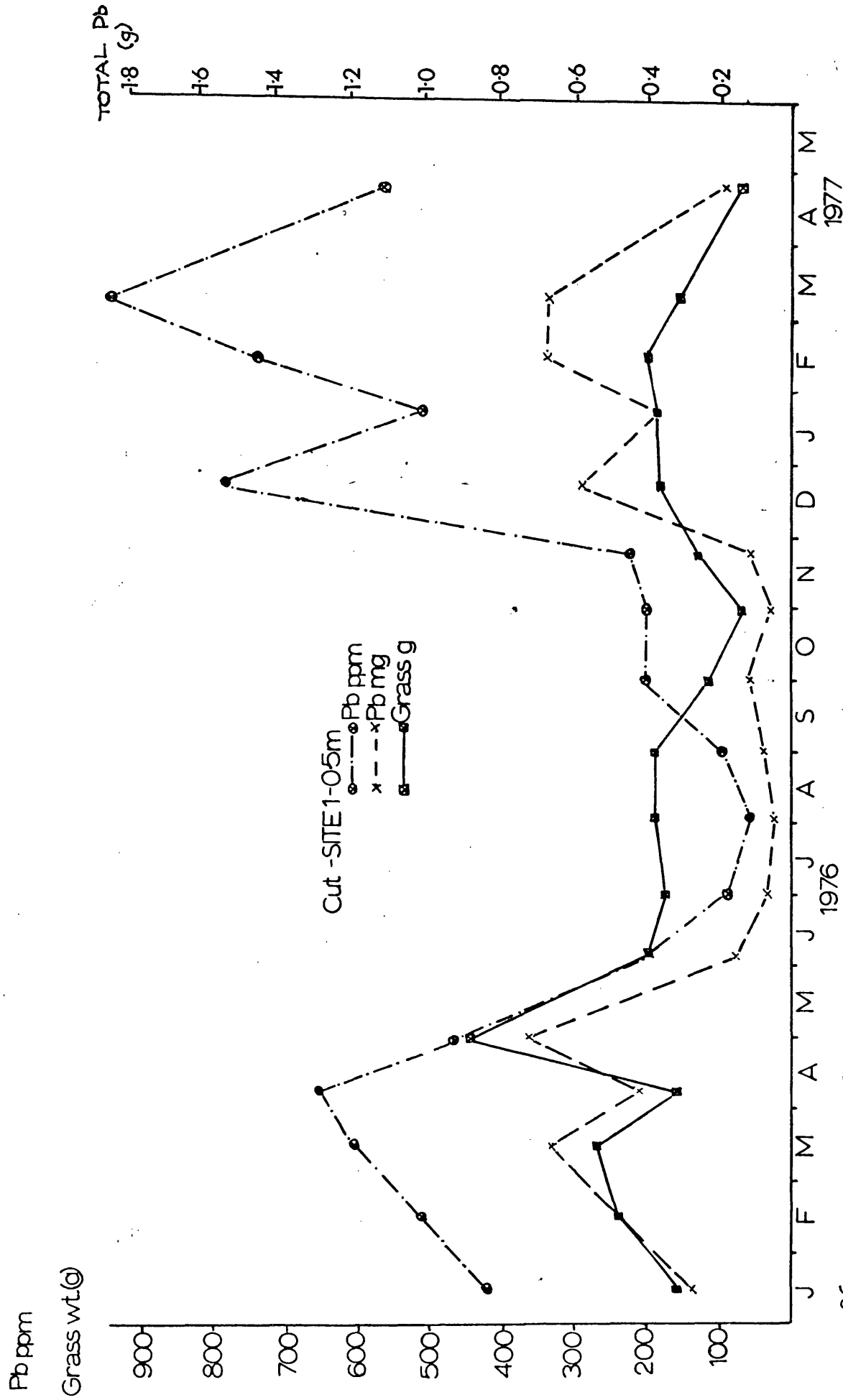


Fig. 25 The concentration of Pb and weight of grass per  $m^2$ , for site  $\alpha$ , 7m from the road, 1976 to May 1977. The third plot, total Pb per  $m^2$ , is derived by calculation from the other two.



**Fig. 26** As for Fig.25, except for site 1, 1/2m from the road.

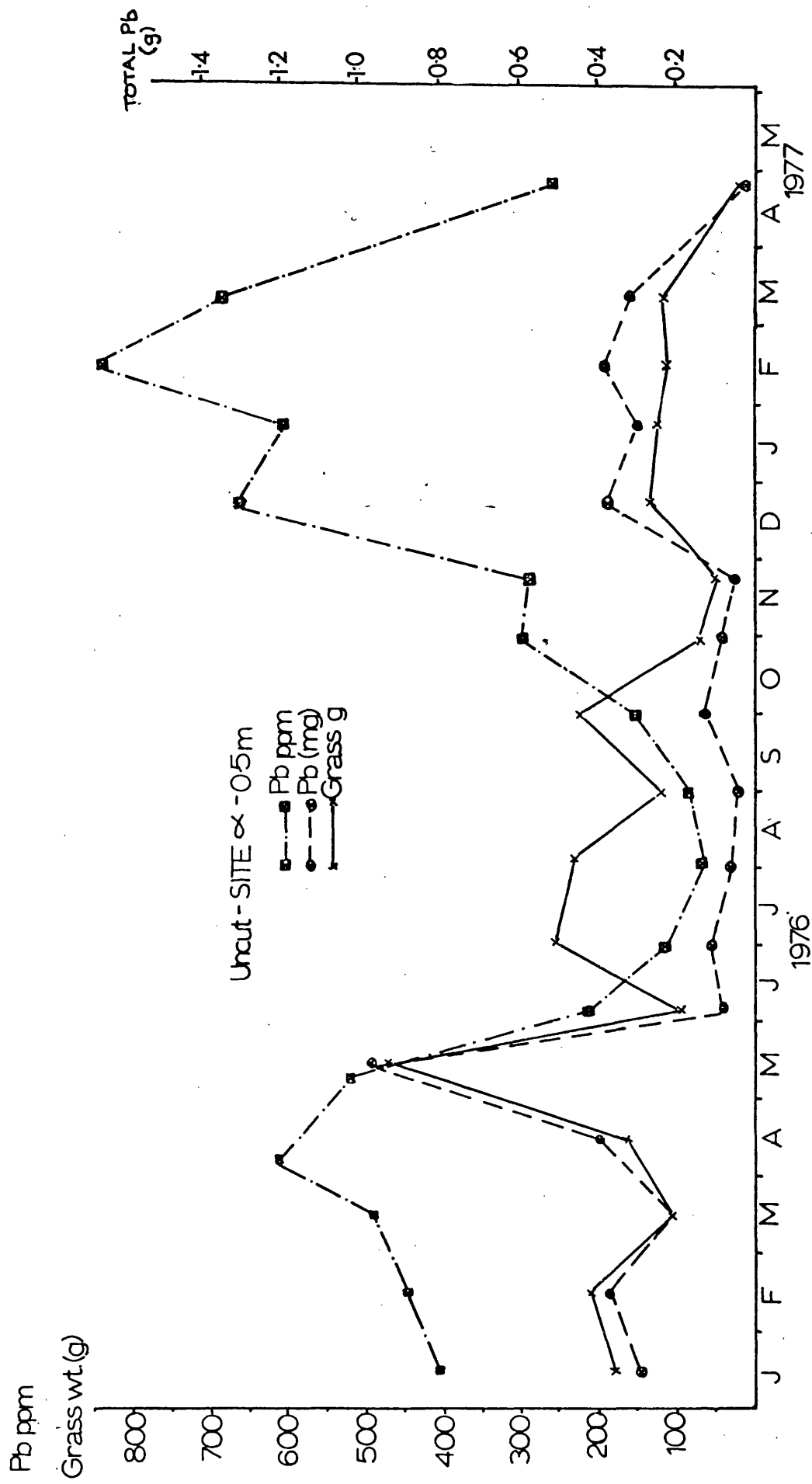


Fig. 27 As for Fig. 25, except for site  $\alpha$ ,  $\frac{1}{2}$  m from the road.

greatest increase in the Pb content of the grass will occur when there is the least change in the standing crop: loss to the soil will be at a minimum, and there will be little grass growth to 'dilute' the Pb. At this time there will be the greatest increase in the Pb content of the grass between two sampling occasions, which, in the present case was April/May 1976. This may be assumed to be the total input of Pb in one month: if the input is taken to be uniform throughout the year, then the following values would represent the yearly addition of Pb to the vegetation:

Site 1,  $\frac{1}{2}$ m ....5.4g/m<sup>2</sup>/year (1976)

$\alpha$ ,  $\frac{1}{2}$ m ....4.7 "

$\alpha$ , 7m ....2.1 "

This is likely to be an overestimate since the two standing crops used for the measurement were subject to a higher rainfall than the mean value for the rest of the year. It does at least place a maximum value on the Pb input onto the vegetation.

These results may be compared to those of the snow and paper strip experiments. Assuming that Pb deposition in these cases is comparable to that on the vegetation, then the integrated Pb input at the  $\frac{1}{2}$ m distance was 176 and 15mg Pb/m<sup>2</sup>/day respectively. The snow sample was obviously exceptional and even the contamination of the paper strip cannot be considered characteristic for the whole year. However, this latter method would estimate the input as 5.6g Pb/m<sup>2</sup>/year for  $\frac{1}{2}$ m from the road, which is obviously comparable with the figures given for the yearly addition to the vegetation.

One possible way to test these figures would be to estimate the excess Pb in the soil of the verge at this distance and divide by the number of years since it was last



disturbed. This follows from previous work which has shown that no Pb is lost from the soil profile (Hildebrand and Blum 1975), and this is known to produce a rapid decline in Pb levels with depth of the soil, due to the binding of Pb by the organic fraction of the upper layers.

Three soil profiles were cut at site 1 ( $\frac{1}{2}$ m), exposing a 'core' of about  $\frac{1}{4}$ m<sup>2</sup>, to a depth of about 30cm. A fourth core was cut at a newly made-up verge near site 5 (site $\beta$ ) for comparison, and to estimate background Pb. Each core was cropped of vegetation and litter, and then divided into six layers, placing each into a clean polythene bag. In the laboratory, the layers were dried and passed through a Christy-Norris laboratory mill, before being sub-sampled and analysed for Pb (cf. Methods V.i). The Pb content of the soil layers are shown in Fig. 28.

The rapid decline in Pb levels with depth is apparent except at site $\beta$ , where there is no vertical change. Throughout its profile, this site has a level of about 50ppm, and the other cores reach this level around 25 cm deep. This was taken to be the background level of Pb, and the total amount of Pb above this concentration was considered as excess. The mean total excess for all three cores was 38.319 g Pb/m<sup>2</sup> in 1976. The County Engineer and Surveyors Department last made-up this verge 8 years before the cores were taken, giving an annual input of 4.82 g Pb/m<sup>2</sup>/year.

This is not greatly different from the previous two estimates. This does mean that the <sup>minimum</sup> estimate of Pb added to the soil from the vegetation is only a quarter of this level, but the latter is nearly the same total as for the yearly addition to the vegetation. Certainly, part of the difference will be due to direct fall-out of Pb since the quadrats sampled at the  $\frac{1}{2}$ m distance included some bare soil at various times of the year.

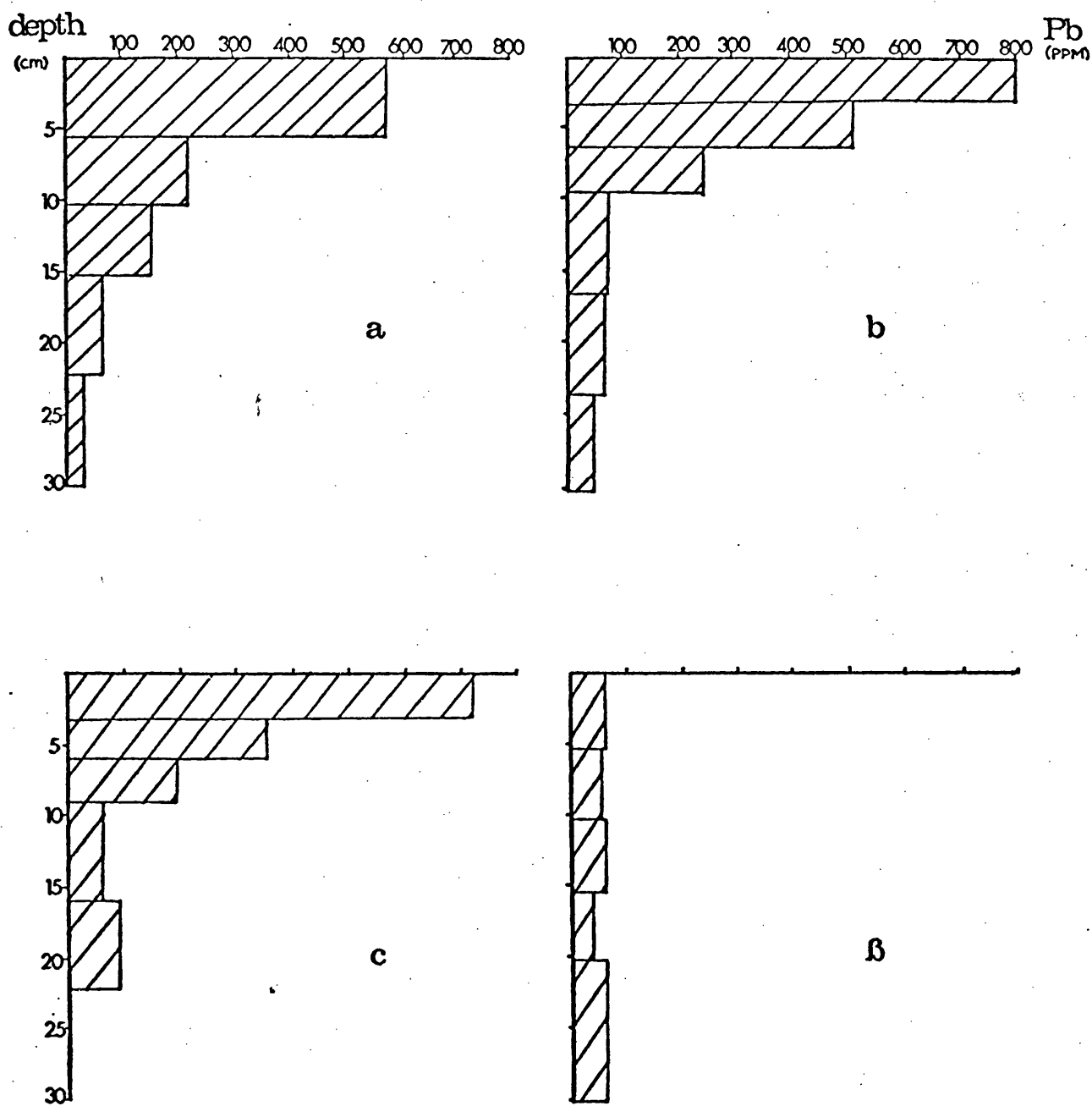


Fig. 28

The Pb content of successive layers of soil (taken 0.5m from the road at site 1) from three soil cores. A fourth core was also taken from the same distance at a newly made-up verge, site β. (21/4/76)

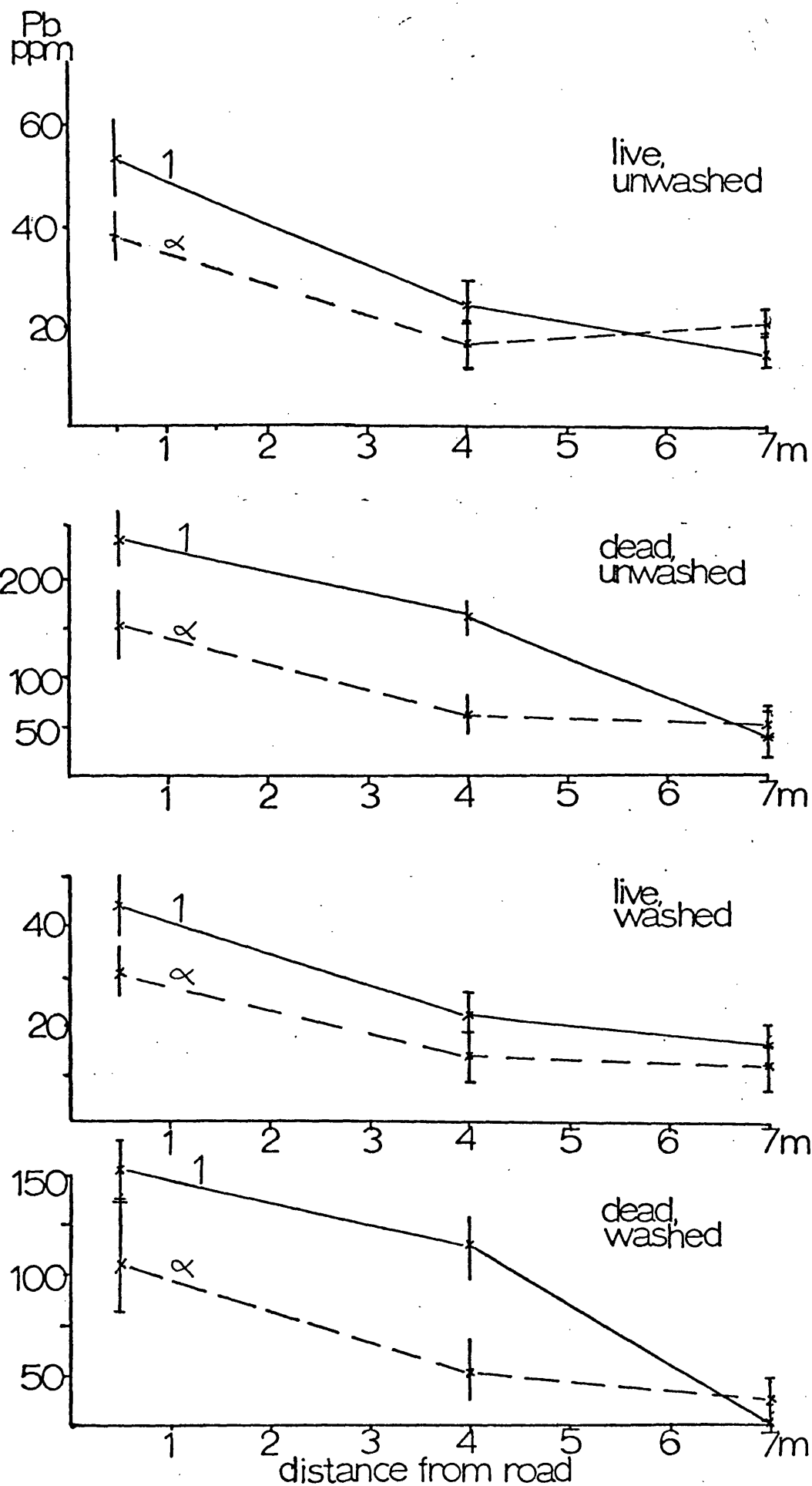
Generally, the three methods used to estimate the total addition of Pb to the soil at this distance show comparable levels, a mean estimate of which would be around  $5.3 \text{ g Pb/m}^2$  /year.

Finally, an estimate can also be made of the total Pb input across a metre wide strip of the width of the verge, (site 1), in one year, from the paper strip and snow experiments. Here, the total Pb deposited in 24 hours for each experiment was calculated by integrating the amount of Pb beneath the regression curves of each set of results (Fig. 19 and 20). Again, these can only be treated tentatively, since they are probably not typical occasions. For the snow, 362 mg Pb were deposited across the metre wide strip of the verge, which is equivalent to 132 g/year. The corresponding figures for the paper strip experiment are 38 mg/24 h and 14g Pb/year. Obviously, the figures from the paper strip experiment are more likely to be indicative of the real situation.

## 2.5 Comparison between sites

To compare the decline in Pb levels in the vegetation with distance from the road of the mown and unmown sites, transects of grass samples were taken from sites 1 and  $\alpha$ . These were sorted and analysed in the normal way. The results are given in Fig. 29.

Generally, the grass of site  $\alpha$  has a lower Pb level than that of site 1, both in 'dead' and 'live' material. This is in contrast to independent experiments carried out in the lead dosing chamber which suggest that denser swards collect more Pb (Woodings, Undergraduate project 1977). These results are not directly comparable because the swards used in the chamber were not as dense as those on the verge, and the latter would effectively dilute the Pb.



**Fig. 29**

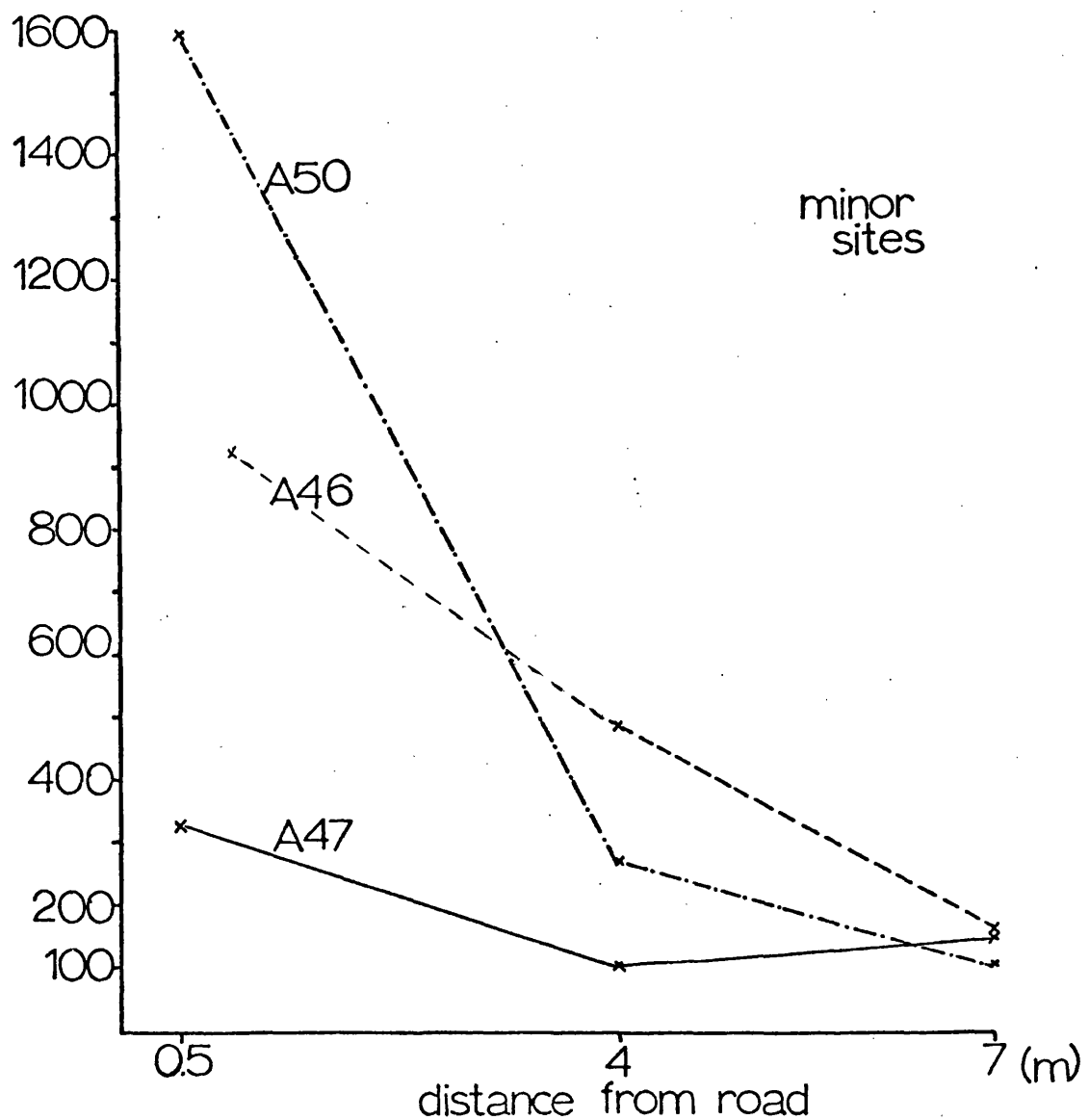
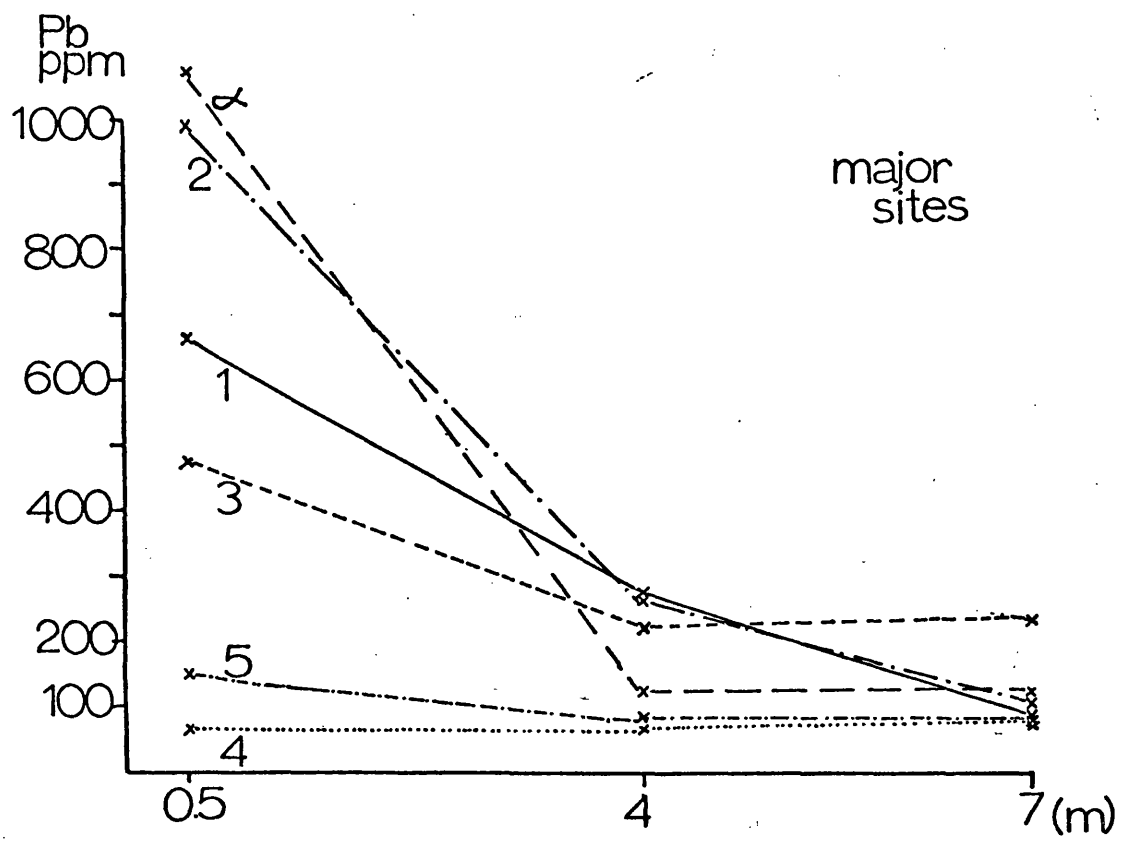
Comparison of the Pb content of the grass at site 1 (mown) and site alpha (unmown), with distance from the road. 5/11/75

The Pb contamination of the other sites studied was estimated by sampling the soil of each site at three distances from the road. 5cm deep soil cores were cut with an auger of 25cm diameter, and standing vegetation and litter were removed. The core was dried in an oven at 60°C and then milled and analysed as for vegetation (cf. Methods V.i). Loss on ignition measurements were also made (to estimate organic content) by heating two 1g samples of each core in a muffle furnace at 550°C for 2 hours, before cooling and reweighing. The milled soil was also measured for pH. The results are given in Table 2; the Pb levels are shown graphically in Fig. 30.

The soil levels of sites 1 and  $\alpha$  are comparable and the apparent difference at  $\frac{1}{2}$ m distance from the road is probably sampling error alone, rather than a real difference. Sites 1, 2, 3 and  $\alpha$  all show roughly the same fall-off of Pb levels with distance from the road, while the other two major sites (4 and 5) on the less-trafficked roads, have less elevated levels at the  $\frac{1}{2}$ m distance.

The A47 has a similar pattern while the Pb levels in the soil of the A46 show a more linear decline with distance. Only the A50 conforms to the pattern most typical of the major sites. Generally, the overall Pb levels in the soil of the minor sites reflect their relative traffic frequencies: immediately next to the road at the A50 1600 ppm is recorded (with about 13,000 vehicles per 16 hour day), compared to the A47 with 400 ppm (7,000 vehicles per 16 hour day). The exceptional site is the A46 with 14,000 vehicles per 16 hour day, but with only 900 ppm Pb next to the road. This is probably due to the paved area next to the road, so that no estimate of the Pb in the soil from the  $\frac{1}{2}$ m distance is possible.

These soil Pb levels almost certainly reflect differences



**Fig. 30**

The Pb content of the soil at three distances from the road, at all the sites studied (16/3/77)  
Each value is the mean of three replicates.

between the morphology and wind characteristics of these sites (cf. section 2.3 p. 36), but may also be a result of the time since the last disturbance of the verge by roadworks.

## 2.6 Availability of lead to consumers

The results given in Table 2 show that the soil characteristics of all sites are comparable, at least in terms of the factors examined. The pH of the soil and its organic content decides, to some degree, how much Pb is available to plants and animals (Hildebrand et al, op. cit). Most of the sites are decidedly alkaline next to the road, and this is clearly due to road salting (Ranwell et al, 1973). This may account for the nature of the plant community next to the road (Ranwell et al, op. cit; Davison, 1971; Westing, 1969).

The relatively high pH of the soil at most distances from the road, at all sites, means that little Pb would be readily available to plants and animals (Hildebrand et al, op. cit)

Litter has necessarily a high level of Pb and attempts were made to estimate what proportion of this was available to detritivores at each site where woodlice were collected (1, 4, 5, A47, A46, A50). Samples were collected by hand at  $\frac{1}{2}$ m from the road, and sub-divided into two parts: one was extracted in the normal way (25%  $\text{HNO}_3$ ) to estimate total Pb, and the second was soaked in 10% acetic acid for 10 minutes, rinsed in deionised water, and then extracted in 25%  $\text{HNO}_3$ . The difference in the Pb levels between the two sub-samples was taken as an estimate of what might be available to detritivores. Martin, Coughtrey and Young (1976) used 1% acetic acid to measure the proportion available to Oniscus asellus, but this is likely to underestimate the value. The 10% acetic acid may well overestimate this, but it does, at least, place a ceiling

SITE	Distance	pH	Mean % Loss on Ignition	Mean Pb ppm
1	0.5 4.0 7.0	7.65 6.70 5.65	14.0 21.7 23.1	668.247 276.62 89.10
2	0.5 4.0 7.0	7.65 7.50 6.80	13.3 11.9 18.0	1099.24 128.47 113.96
3	0.5 4.0 7.0	8.00 7.75 7.45	12.5 12.2 13.3	488.97 224.82 237.25
4	0.5 4.0 6.0	7.30 7.30 7.10	13.6 21.6 24.2	67.33 65.26 80.81
5	0.5 4.0 7.0	7.50 6.40 4.95	18.2 31.4 34.1	151.26 82.88 73.55
A47	0.5 4.0 7.0	7.70 7.60 7.70	12.9 12.7 21.8	327.39 102.56 150.22

Table 2  
Pb content, pH and organic  
content of soil from three  
distances from the road at  
all sites - 16. iii. 77.



SITE	Distance	pH	Mean % Loss on Ignition	Mean Pb ppm
A46	0.5	7.8	12.8	912.76
	4.0	7.7	15.1	436.17
	7.0	7.1	28.7	211.35
A50	0.5	7.8	9.0	1593.44
	4.0	6.55	20.0	269.37
	7.0	5.55	14.9	108.78

m

Table 2 - continued.

Site	Distance	Litter mean Pb ppm	SE Pb ppm	Litter - Pb ppm Acetic Acid extract.	SE Pb ppm	% of Total Pb
1	0.5	857.84	$\pm 19.14$	119.66	$\pm 3.31$	10.14
2	0.5	964.21	$\pm 16.75$	128.98	$\pm 3.91$	10.39
5	0.5	198.92	$\pm 3.58$	15.02	$\pm 0.99$	7.55
A47	0.5	645.80	$\pm 8.655$	75.11	$\pm 3.20$	11.63
A46	0.5	744.57	$\pm 23.39$	92.20	$\pm 8.43$	12.38

m

Table 3

Litter data for various  
verges where woodlice were  
sampled. Total Pb was extracted  
with 25% HNO<sub>3</sub>; Acetic acid was  
10%.

over what might be taken up. The results are given in Table 3. On average, only about 10% of the Pb in the litter is removed by the acetic acid, substantially lowering the effective amount of Pb available to litter consumers.

Since Pb is known to be bound by the organic fraction of the soil, the same is likely to be true of the Pb available to earthworms. Pb levels in roadside earthworms are described by Gish and Christensen (1973), though the assimilation of Pb by one particular species (Dendrobaena rubida) is considered in much more detail by Ireland (1975). Only small numbers of earthworms were found at any of the sites studied (probably due to the heavy clay-loam soil), and they were therefore not considered in any detail.

## 2.7 Discussion

The analysis of the Pb contamination of roadside vegetation has shown that there are major differences between live and dead grass, probably as a result of the condition of the cuticle. The degree of contamination of both components vary with distance from the road, generally with the factors described by Daines et al (1972) but also with precipitation. Seasonal changes are also apparent, with the highest levels occurring in winter. This is most likely a product of the increased standing time of the grass with the lower temperatures of the winter allowing the vegetation longer to accumulate more Pb.

Any threat to roadside animals from Pb thus depends on the state of the food consumed, the season, the road, and the distance from the road. Thus detritivores are seen to be the group most likely to be feeding on a high Pb diet, though in real terms, the amount of Pb available is much less than the total amount present.

### 3. Community and Population studies of the roadside invertebrate fauna

#### 3.1 Introduction

No previous work has made any attempt to describe and analyse the whole range of the roadside faunal communities. Williamson and Evans (1972) examined a number of invertebrate types in their survey of Pb levels in the verge fauna, but did not consider the species composition of the community. Other workers have investigated single species, for example, earthworms (Gish and Christensen, 1973) and deer mice (Mierau and Favara, 1975). Price, Ratchke and Gentry (1974) and Giles, Middleton and Grau (1973) examined insect communities on the verge, and both groups of workers attempted to show the movement of Pb through a series of trophic levels. Although both report higher Pb levels in predatory insects, the small number of samples involved means that the evidence is not conclusive. Generally, there is a lack of information on the possible concentration of Pb in higher trophic levels, as there is on other effects of Pb on animal communities.

Quarles, Hanawalt and Odum (1974) looked at the small mammal population on verges in the United States, and concluded that Pb was not limiting the numbers of individuals. Other workers have been concerned simply with the amounts of Pb in roadside vertebrates (Jeffries and French, 1972; Welch and Dick, 1975).

With proper sampling and statistical techniques it may be possible to distinguish a response in the community as a whole to a zone of high Pb contamination. The initial idea in the present study therefore, was to see if enhanced Pb levels were associated with a lowering of diversity in the invertebrate community toward the road, where the highest Pb

levels (in soil, grass and air) have been shown to exist.

In simple terms, the diversity of a community of animals would be expected to decrease if certain species were lost due to some ecological stress, such as pollution. The ability to detect such an effect depends on being able to measure quantitatively 'diversity'. A numerical index of diversity should be capable of reflecting changes both in the number of species and number of individuals (Williams, 1964): it may be that only one of these components will change as a response to changing environmental conditions. Williams (op. cit) has considered the various theoretical approaches to the problem, while Bullock (1971) has compared several indices for their ability to describe a sample quantitatively.

In the present study, the index chosen was the Shannon-Weiner Information Statistic, that is given by the formula:

$$H' = \log.N - \frac{1}{N} \sum_{i=1}^S n_i \log.n_i$$

where N = total number of individuals  
 $n_i$  = number of individuals in the  $i^{th}$  species (1,2,3....S)  
 S = number of species

This is a transformation of an original equation that measured the information content or entropy of a message. In this form the index is measuring the 'disarray' or entropy of a sample resulting both from the number of taxa and their relative abundance.

The maximum value of the index is set by the total number of individuals ( $\log.N$ ), from which is subtracted a calculated value of species composition of the sample ( $\frac{1}{N} \sum n_i \log.n_i$ ). If all the individuals were of a different species, then  $\sum n_i \log.n_i$  is zero and the second term of the equation is zero. Thus, the  $\log.N$  does not change and the index is simply the logarithm

of the number of species ( = number of individuals).

Conversely, if all the individuals are of the same species, the diversity and the  $H'$  value are zero.

One possible objection to this index is that the values obtained do not follow a statistical distribution, and more particularly, the log. series distribution that would be expected under diverse conditions (Williams, op. cit; Bullock, op. cit). As a consequence, Bullock (op. cit) points out that  $H'$  is insensitive to changes in species with a small number of individuals. In addition,  $H'$  will remain constant if the relative proportions of the species remain the same, even though the total number of individuals may have altered. Thus,  $H'$ , when measuring diversity, relies totally on the number of species and the proportions between them. Nevertheless, the ease and simplicity with which this index can be applied and its sound basic principle were the main considerations for its selection in the present work.

All this presumes that a standard <sup>sampling</sup> technique is used, and two methods were employed in the present study - pitfall traps and Tullgren soil extractions. The problems associated with pitfall traps are discussed by Southwood (1966), who cites the work of Greenslade, showing that pitfalls selectively catch active ground-living invertebrates. Although various modifications to the basic pitfall trapping technique were outlined by Southwood (op. cit), a simple form was used (as shown below), enabling a large number of samples to be taken.



Simple Pitfall Trap

EACH PITFALL OCCUPIED AN AREA OF 23 cm<sup>2</sup>

### 3.2 Sampling and identification programme

Ten pitfall traps were laid in a line at right angles to the road on the verges of all the major sites. The position of each pitfall on each site is shown in Fig 3 (cf. section 1.1). When collected, the contents of the trap were emptied into a plastic tube, and the plastic cup refilled with ethylene glycol and replaced in the glass jar. The traps were sampled every 2 weeks from December 1974 until December 1975, making 26 sampling occasions. Each occasion is denoted by a letter of the alphabet (Table 4). Only samples collected on selected dates were examined, and the animals identified; these samples are shown graphically in Table 4. Interference, either by vehicles transgressing on to the sites, or by other factors, was a constant problem accounting for most of the missing plots.

Two identification programmes were carried out. Initially, animals trapped were identified to the levels shown below:

Acari - sub-order	Hymenoptera - family
Annelida - class	Hymenoptera larvae - sub-order
Aranea - species	Hemiptera - family
Chilopoda - sub-order	Hemiptera larvae - sub-order
Coleoptera - species	Isopoda - species
Coleoptera larvae - family	Lepidoptera larvae - family
Collembola - sub-order	Mollusca - sub-order
Dermaptera - species	Psocoptera - family
Diplopoda - sub-order	Siphonaptera - species
Diptera - family	Thysanoptera - family
Diptera larvae - sub-order	

These were termed the 'first series' of pitfall samples, on which the most intensive analysis was carried out. This series included the first three sampling occasions (A, B, and C - during the winter maxima in Pb levels in the vegetation), and a single spring set, I (during the spring low in Pb levels). Sampling at these times was confined to sites 1 - 5.



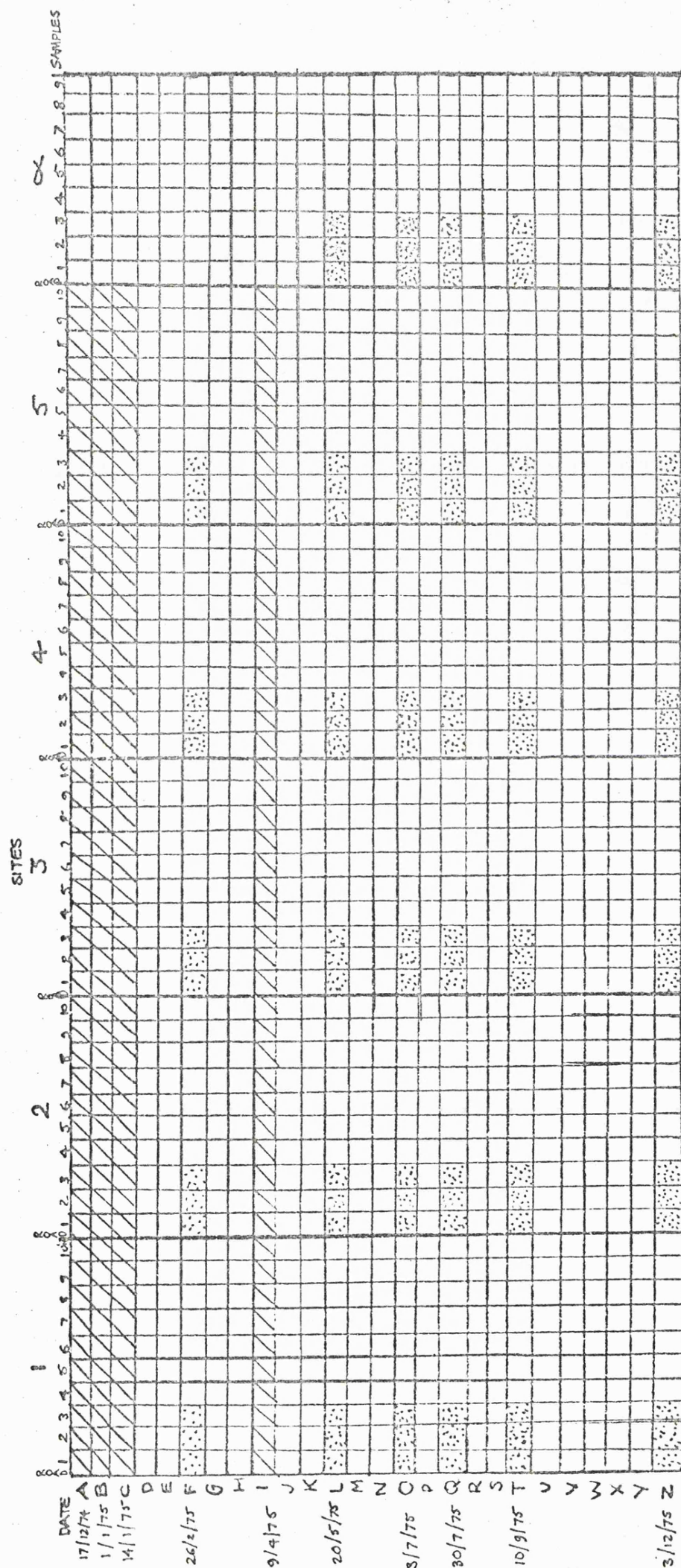




Table 4  
Pitfall Sampling Programme  
1974/1975

First Series.....

Second Series.....



The second series of pitfall samples had a more restricted programme of identification. Here, only the samples from the three pitfalls nearest to the road (nos. 1 -3, in the zone of highest Pb contamination) were examined and identified to the levels given above. The series of samples comprised sets F, L, O, Q, T, and Z (sampling all seasons - Table 4) and included samples from site  $\alpha$ .

The presence or absence of a particular taxon in all the pitfalls are given as a series of lists in Appendix III. The families of the Diptera have been omitted.

### 3.3 Results from the pitfall traps

The matrix of values of  $H'$  calculated from the data of the first series of samples is given in Table 5, which also shows the overall mean  $H'$  of all sites, for each sampling occasion. Table 6 shows the mean  $H'$  for each pitfall trap, with means for each distance from the road. The results from the second series of samples is shown in Table 7. With the former series, an analysis of variance (with calculated values for the missing plots) was performed, using the second order interaction for the error term (due to the lack of replication). The results are shown in Table 8.

Significant differences in  $H'$  values are found only between sites and sampling dates. Samples from various distances from the road (position) show no significant difference, and neither do any of the first order interactions.

The possible effects of Pb on the diversity of the samples were tested by two methods - firstly by comparing sites with different degrees of Pb contamination, and secondly, by comparing samples from areas of the verge with different Pb levels. Only the former is significant, and then a multiple range test shows that only site 2 and 3 ( $P < 0.01$ ) and 2 and 4 ( $P < 0.05$ ) are significantly different from each

Samples											
Date	Site	1	2	3	4	5	6	7	8	9	10
A 11/12/74	1	0.74	0.93	1.15	0.76	0.97	0.66	0.85	0.81	0.93	0.63
	2	---	0.66	1.10	1.12	1.14	0.55	1.04	1.08	0.95	0.89
	3	0.88	1.02	0.90	0.64	---	0.62	0.68	0.38	0.56	0.48
	4	0.75	0.89	1.04	1.25	0.79	1.09	1.05	0.66	0.36	0.79
	5	0.77	1.04	0.90	0.80	0.96	1.04	1.00	0.41	1.05	0.48
B 1/1/75	1	0.80	0.79	1.04	0.91	0.99	0.93	0.81	1.02	0.92	1.12
	2	0.95	1.24	1.24	0.84	1.06	1.07	0.90	1.06	0.97	1.36
	3	0.78	0.93	0.73	0.55	0.89	0.86	0.69	0.72	0.59	0.63
	4	0.44	0.63	0.86	0.64	0.51	1.07	0.85	0.72	1.07	0.61
	5	---	0.85	1.24	0.87	1.02	0.87	1.35	0.67	0.45	0.54
C 11/1/75	1	1.01	0.77	1.00	1.24	0.94	0.76	1.30	0.74	1.13	0.53
	2	0.96	0.82	0.93	0.60	1.06	0.98	0.82	0.96	0.61	1.05
	3	0.65	---	0.55	0.80	0.86	0.67	0.71	0.85	0.65	0.20
	4	0.78	0.34	0.56	0.85	0.76	0.47	0.78	0.90	0.43	0.73
	5	0.95	1.48	0.72	1.04	1.04	0.97	0.60	0.00	0.49	0.51
I 9/4/75	1	0.25	0.10	0.41	0.41	0.59	0.11	0.43	0.45	0.14	0.30
	2	0.24	0.64	0.46	0.27	0.30	0.45	0.58	0.69	0.85	0.00
	3	0.75	---	0.36	0.53	0.88	0.59	0.19	0.46	0.45	0.00
	4	---	---	0.21	0.47	0.43	0.17	0.31	0.59	0.50	0.34
	5	0.00	0.00	0.95	0.41	0.45	0.47	0.27	0.00	0.57	0.28

SE of mean:  
n = 50; ± 0.03  
n = 48; ± 0.03  
n = 47; ± 0.03

ple 5

Mean (occasion)

0.84

0.88

0.79

0.42

SE of mean:

n = 50;  $\pm 0.036$

n = 48;  $\pm 0.036$

n = 47;  $\pm 0.037$

Table 5

H' for the first series of pitfall samples. Each sample number corresponds to the trap's position on the verge (see Fig. 3) - the larger the sample number, the greater the distance from the road. The mean for each sampling occasion is also given.

Site	Samples										Mean (site)	SE of mean:	sites (3,4)
	1	2	3	4	5	6	7	8	9	10			
1	0.70	0.64	0.90	0.83	0.87	0.61	0.84	0.76	0.79	0.64	0.76	n = 38; $\pm 0.0325$	(3,4)
2	1.03	0.84	0.93	0.71	0.89	0.76	0.84	0.98	0.85	0.83	0.84	n = 39; $\pm 0.0321$	(2,5)
3	0.77	0.98	0.64	0.63	0.82	0.69	0.57	0.60	0.56	0.33	0.62	n = 40; $\pm 0.0317$	(1).
4	0.66	0.62	0.67	0.80	0.62	0.70	0.75	0.72	0.59	0.62	0.69		
5	0.57	0.84	0.95	0.78	0.87	0.84	0.81	0.27	0.64	0.45	0.74		
mean	0.69	0.76	0.80	0.75	0.82	0.72	0.76	0.65	0.67	0.58			

SE of mean:

Samples

n = 17;  $\pm 0.0618$  (1,2)

n = 19;  $\pm 0.0585$  (5)

n = 20;  $\pm 0.0570$  (3,4,6,7,8,9,10)

Table 6

Mean values of H' for the first series of pitfall samples (n = 4). The mean for each site, and for each distance from the road are given with associated standard errors (derived from the analysis of variance).

Date	Site	Samples			Mean	Mean for the sampling occasion
		1	2	3		
F 26/2/75	1	1.44	1.01	0.87	1.11	0.74
	2	---	---	---	---	
	3	1.20	0.47	0.91	0.86	
	4	0.97	0.53	---	0.75	
	5	0.47	0.62	0.53	0.54	
L 20/5/75	1	0.00	0.47	0.80	0.42	0.85
	2	1.02	0.81	0.76	0.86	
	3	0.96	0.90	1.02	0.96	
	4	1.06	---	1.08	1.07	
	5	---	---	---	---	
O 3/7/75	1	0.61	0.53	0.77	0.64	1.26
	2	---	0.71	---	0.71	
	3	1.27	1.43	1.33	1.34	
	4	1.42	1.57	1.31	1.43	
	5	1.37	1.17	1.30	1.28	
Q 30/7/75	1	1.18	1.39	1.17	1.25	1.05
	2	0.89	1.29	---	1.09	
	3	1.17	1.24	1.09	1.17	
	4	---	1.16	1.26	1.21	
	5	1.30	1.31	1.21	1.27	
T 10/9/75	1	---	1.05	1.29	1.17	0.61
	2	1.24	0.83	0.93	1.00	
	3	---	---	0.89	0.89	
	4	1.18	1.08	0.00	0.75	
	5	0.41	0.79	0.59	0.60	
Z 3/12/75	1	1.23	---	0.81	1.02	0.67
	2	0.53	0.39	0.13	0.35	
	3	0.54	0.22	0.34	0.37	
	4	0.67	0.63	0.33	0.54	
	5	1.10	0.67	0.64	0.80	
Mean		0.92	0.85	0.80		

Table 7

H' for the second series of pitfall samples. Traps 1 -3 are equivalent to the same number traps as in the first series, covering the 0.5 -1.5m distance from the road. The values for each sampling date have been added and a mean derived for that occasion.

Items	df	MS	VR
Sites	4	1.0108	15.5467***
Position	9	0.1005	1.5462 NS
Time	3	3.2869	50.5532***
Time x Site	12	0.1603	2.4655 NS
Time x Position	27	0.0554	0.8520 NS
Site x Position	36	0.0001	0.0158 NS
Error	107	0.0650	

Table 8

Analysis of variance for the first  
series of pitfall samples.

other. Thus site 2, one of the most highly contaminated of the major sites (cf. section 2.5), has the most consistently high diversity. Notably, there is no parallel decline in diversity with the pattern of Pb contamination at each site and this is further evidence that no decline in diversity of the pitfall samples can be attributed to the Pb levels at these sites.

The highest mean diversity at site 2 may, in part, be due to the very shallow ditch at this verge: the mean  $H'$  for the pitfall traps nearest to the ditch are higher than of any of the other sites. This suggests that the verge invertebrate fauna may be added to from the hedgerow population, possibly enhancing the diversity of the site as a whole.

$H'$  on sampling occasion I is significantly lower than the other first series dates. Although the diversity would have been expected to increase in the spring, the decline in  $H'$  at this date is probably a consequence of the broad taxonomic divisions used: the number of taxa did not increase greatly, but the number of individuals had, and the net effect was a reduction in  $H'$ .

Sampling date B is also significantly higher than the following occasion, C. This may be due to the weather differences during the two sampling periods, though no relevant weather data are available.

In the second series of pitfall samples greater consistency is achieved by the identification levels being more uniform (generally to family level). Most noticeable is the increase in  $H'$  toward the summer (Table 7) and its decline again in the autumn and winter. Here comparison is possible between site 1 and the adjacent site OC. These sites differ only in their

mowing regimes, but the  $H'$  value of site  $\alpha$  is, on average, significantly higher than that of site 1 ( $t = 2.235$ ,  $P < 0.05$ ). It has to be assumed that this is due to the difference in the height of the vegetation cover over the part of the verge sampled.

Clearly, neither the first nor the second series of pitfall samples show any variations in diversity that could be attributable to differing Pb levels. The only significant differences are between sites and between sampling dates, and do not reflect variations in Pb levels of the vegetation. Consequently, an alternative method of considering the pitfall data was adopted, by looking at individual taxonomic groups. Only those taxa with sufficiently large numbers to be meaningful are examined here.

For this purpose, the first series pitfall data has been amalgamated for all occasions, and the numbers of a taxon in adjacent pitfall traps combined at each site. Thus the total number of individuals found in two pitfalls were counted (eg. 1 and 2 = A, 3 and 4 = B etc.), giving five plots per site. The results are given in Fig. 31.

The only group which shows any decline in numbers toward the road is that of the snails. This is not so at site 4 and 5, where the pattern is ambiguous, but <sup>here</sup> the number of snails at the edge of the road is greater than at the same position at the main road sites; this cannot be simply attributed to any affinity for tall vegetation (at the back of the verge) since they occur in appreciable numbers in the short sward at sites 4 and 5. This suggests that at the road margin, the snail population may well be reacting to the enhanced Pb levels at sites 1, 2 and 3, particularly since the soil pH does not differ (Table 2) between all the major sites. Salt levels could be a contributory factor, though road salting

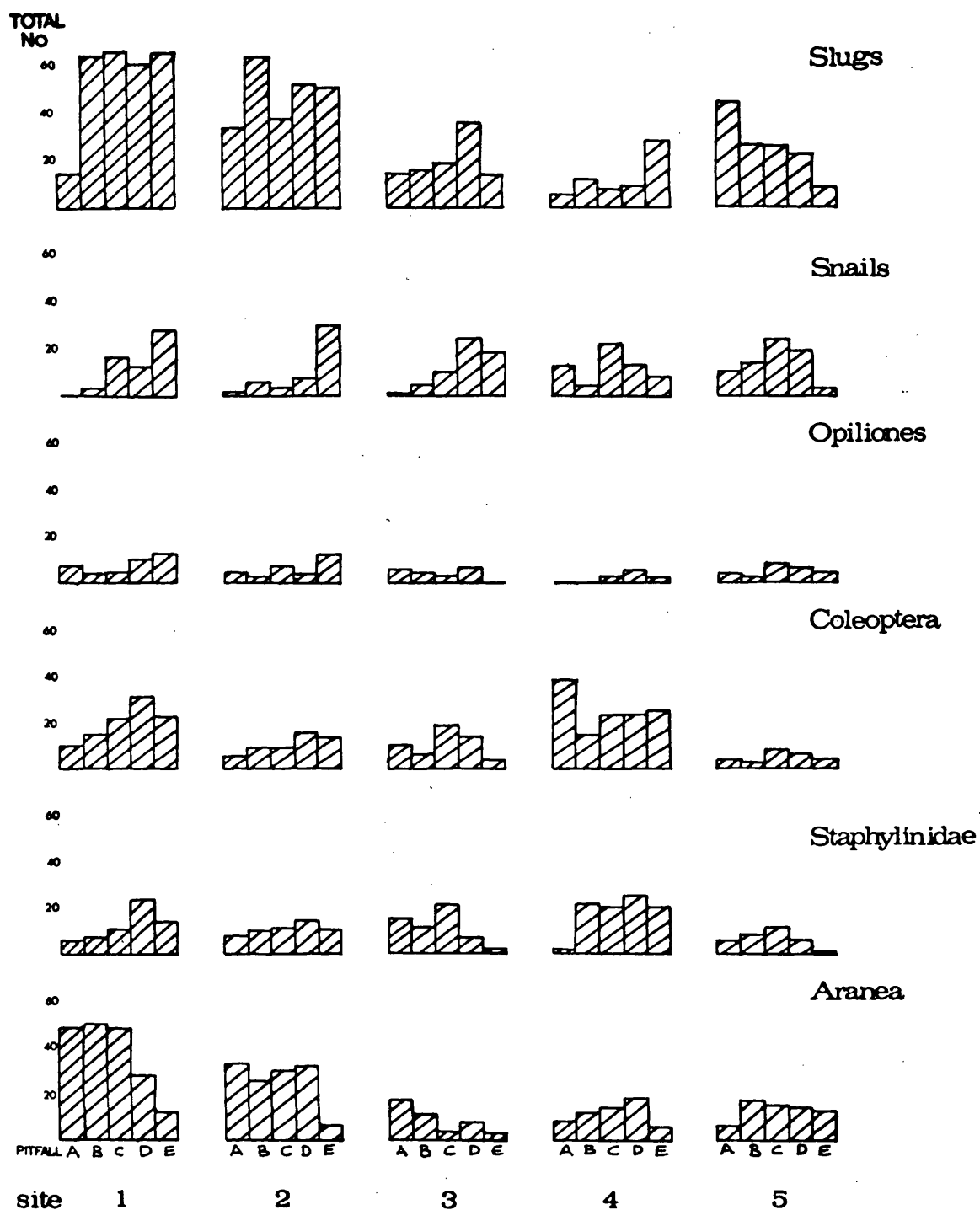


Fig. 31

The total numbers of six taxonomic groups caught in the pitfall traps at the major sites. The numbers from two adjacent traps have been amalgamated, providing the five plots A - E at each site.



was rarely carried out during the sampling period.

The Coleoptera (minus the staphylinidae), and the Staphylinidae themselves, both show a peak of numbers in a central position at the major sites. Generally, the pattern of these two groups at each site is very similar, though the numbers are relatively even over the width of the verge. The slight tendency towards a peak of numbers in the middle of the verge is probably a reflection of the environmental stability (particularly temperature) of the middle region of the verge (c.f. section 1.3).

The most uniform distribution of numbers across the sites is shown by the Opiliones, though small numbers are involved.

Spiders are the only group whose numbers tend to decline with distance from the road (with the possible exception of site 4). This group is most abundant at sites 1 and 2, the least densely vegetated of the sites. This would also account for the larger numbers at the roadside, and may reflect the habits of the largest family of spiders found, the Linyphiidae. The most abundant species found of this essentially ground-living group (Locket and Millidge, 1953) were Centromerita bicolor, C. concinna and Lepthyphantes zimmermani.

The pattern of the distribution of slugs varies from site to site, though large numbers are involved. Like the spiders, the greatest numbers are recorded from sites 1 and 2, but this is not easily accounted for and there is no obvious explanation.

A third way of examining the pitfall data is to calculate, for each site, the mean number of individuals per trap over all sampling occasions (first and second series). The results are shown in Fig. 32. The six taxa shown are those with an apparent preference for particular sites. Two taxa

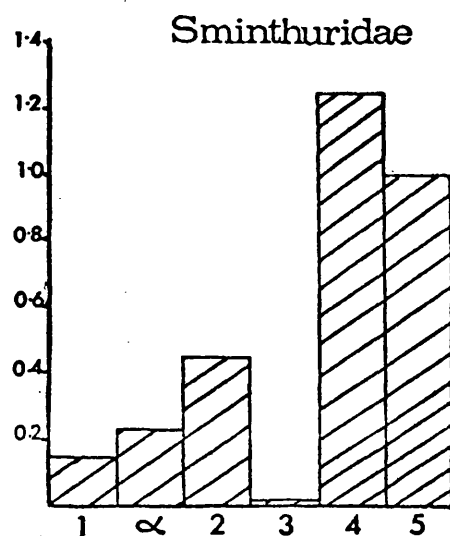
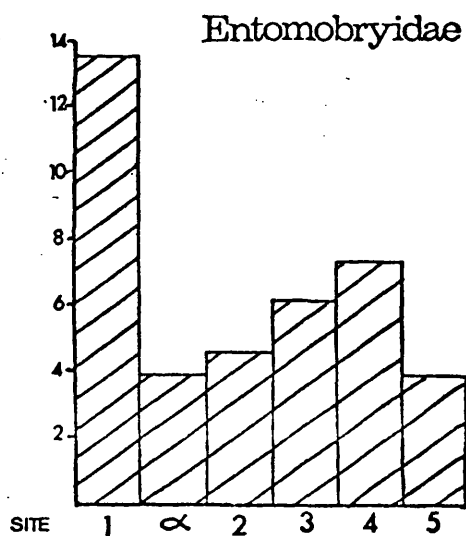
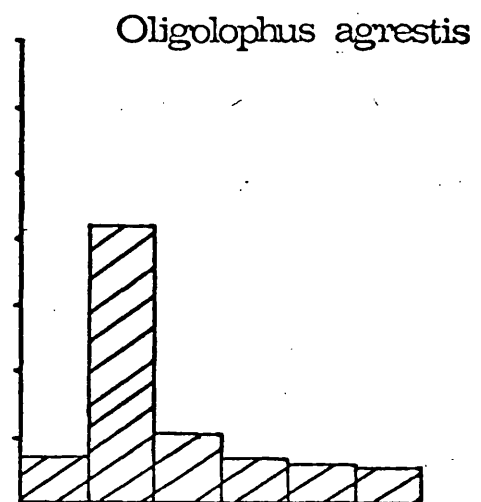
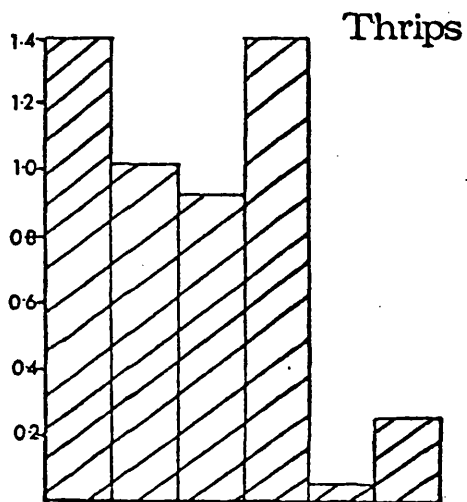
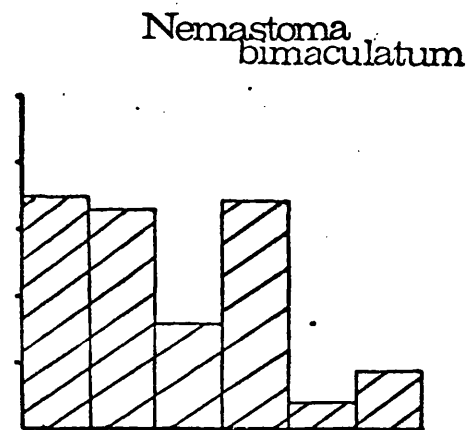
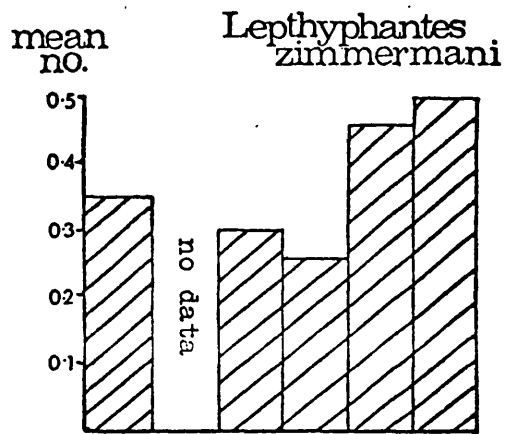


Fig. 32

The mean number of individuals per pitfall trap from the first and second series samples, at all of the major sites.

have low numbers at the sites of high Pb contamination: the sminthurid collembolans and the spider Lepthyphantes zimmermani, both of which have high numbers at sites 4 and 5. This is particularly clear in the sminthuridae, which are principally herbivorous and fungivorous. Why this should also apply to the carnivorous L. zimmermani, as distinct from other linyphiid spiders, is not obvious. However, Bristowe (1971) notes that they feed on collembola, and this may account for this similarity of distribution.

The harvest-man, Nemastoma bimaculatum seems to prefer the main road sites, though this can hardly be a reflection of the level of pollution and no simple explanation is obvious. Finally, another phalangid, Oligolophus agrestis, shows a preference for site  $\alpha$ , possibly because of the denser vegetation (Sankey and Savoury, 1974).

### 3.4 Results from the Tullgren extractions

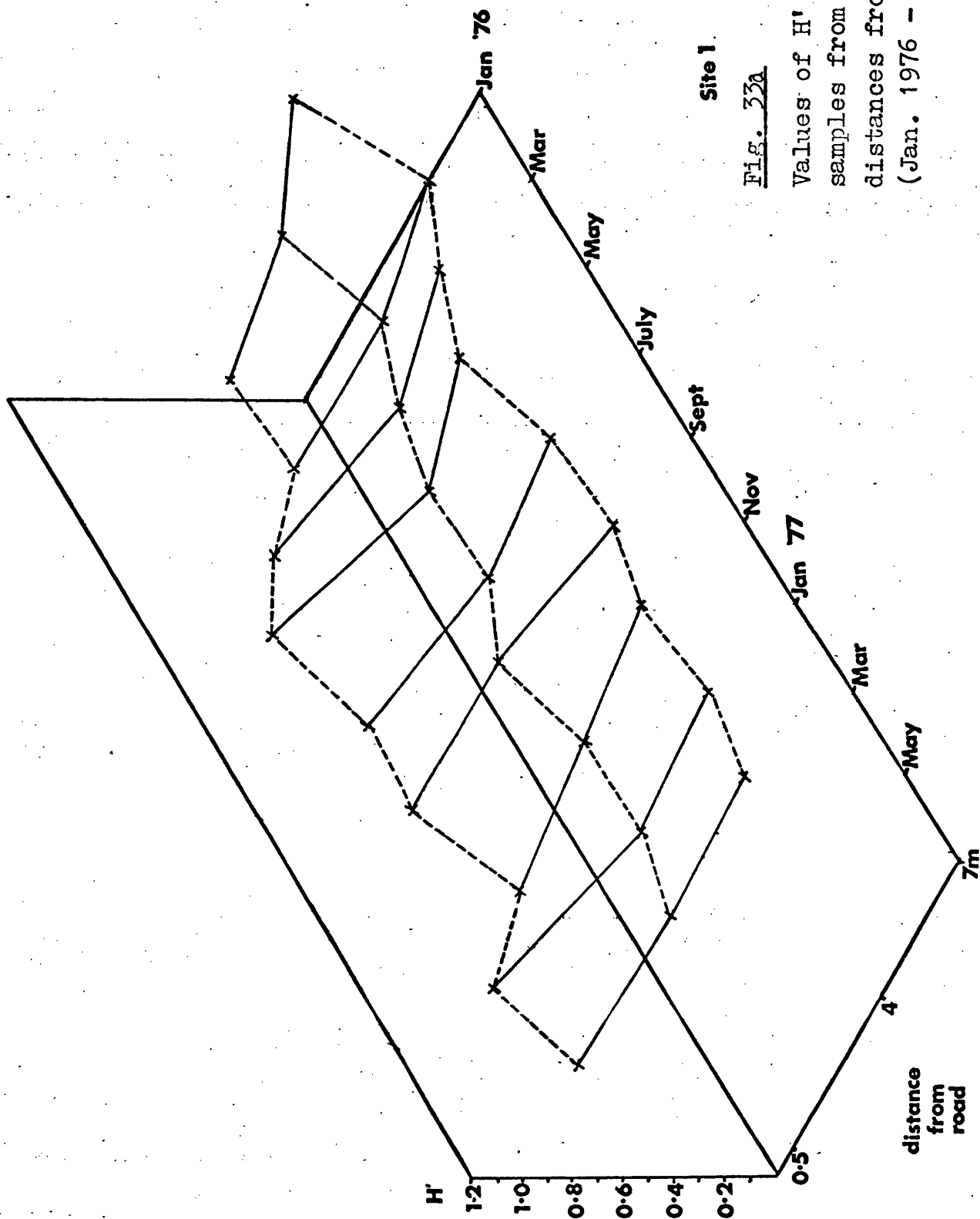
The Tullgren samples were taken to supplement the pitfall data, and to estimate the diversity of the soil fauna of roadside verges. Southwood (1966) discusses the use of Tullgren funnel extraction techniques, but the essential point here is that the extraction process tends to favour the removal of smaller invertebrates. Without elaborate alternatives, this has to be accepted. As the three sites sampled (1,  $\alpha$ , and 5) were all subject to the same technique, this is of little consequence when calculating and comparing diversities.

The samples were taken at three distances from the road (0.5, 4 and 7m), every two months from January 1976 until May 1977. The soil core was cut to a depth of 5cm with an auger of 25cm diameter, and extracted for one week with a bulb of 60w. The animals in the resulting sample were identified to family level only, or, if 'higher', to the level

given for the pitfall samples. Isopods and molluscs were again identified to species. Again, the information statistic,  $H'$ , was used, and the results are given as a series of three-dimensional graphs in Fig. 33a- 33c. An analysis of variance was performed on these results, and along with the means for each location, are given in Tables 9 and 10. The error term for the analysis of variance was again derived from the second-order interaction.

The most significant effect is due to the different sites, and most particularly the large difference between site 5 and the other two sites. Site 5 has a consistently higher diversity at each distance from the road, compared to the more variable site 1 and  $\alpha$  (Table 9). This may be a result of traffic pollution, though the location (with distance from the road) of each sample does not show any significant effect, as might be expected with the increasing Pb levels toward the road margin. There is a slight rise in  $H'$  toward the back of the verge at all sites, but the variability of the samples makes this non-significant. This is most obvious at site 5, where the level of contamination is lowest. Notably, the values of  $H'$  change least with distance from the road at site  $\alpha$ , and this may suggest that the diversity of the Tullgren samples is closely associated with the thickness of the litter layer.

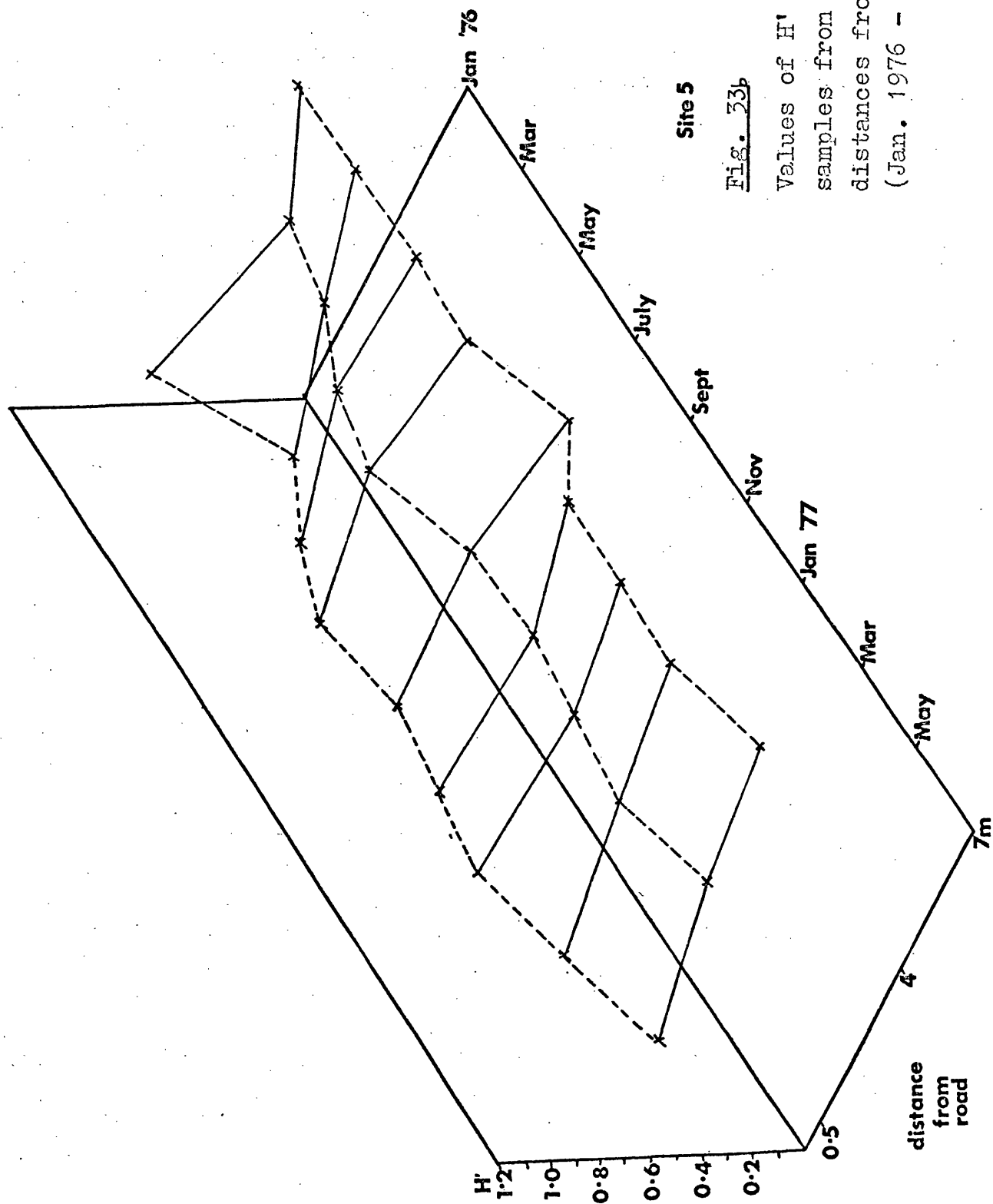
Site and location interact to produce a significant effect, indicative of a different pattern across each site. Time and site also has a significant interaction, which is apparent from Fig. 33a- 33c. Site  $\alpha$  has relatively large seasonal changes, but again, with only slight differences between the different locations on the verge. More erratic changes are seen at the mown sites (1 and 5), both in regard to location and seasonal changes. The 0.5m sample of site 1

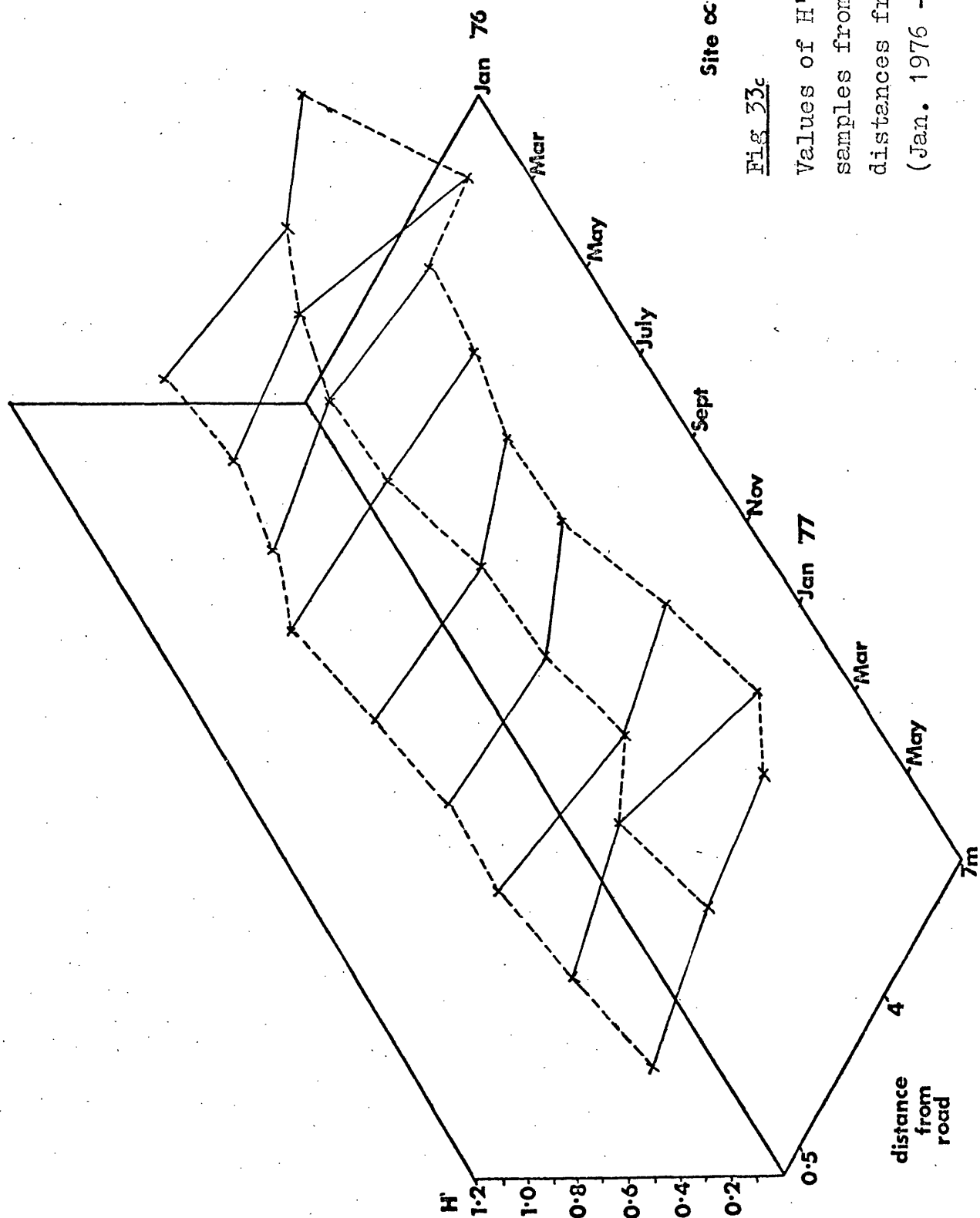


Site 1

Fig. 33a

Values of  $H'$  for the Tullgren samples from site 1, at three distances from the road. (Jan. 1976 - May 1977).





Site  $\alpha$

Fig 33c

Values of  $H'$  for the Tullgren samples from site  $\alpha$ , at three distances from the road. (Jan. 1976 - May 1977).

Site	<u>Distance</u>						<u>Mean</u>	<u>SE site</u> = 0.0203
	<u>0.5m</u>	$S^2$	<u>4m</u>	$S^2$	<u>7m</u>	$S^2$		
1	0.594	0.032	0.519	0.012	0.604	0.011	0.573	
$\alpha$	0.566	0.011	0.573	0.014	0.583	0.027	0.574	
5	0.569	0.017	0.602	0.009	0.666	0.006	0.613	
$\bar{x}$	0.576		0.565		0.618			

SE distance = 0.0203

Table 9

Mean  $H'$  for each location of nine Tullgren soil extractions, with variance. The overall mean for each site and for each distance from the road are also given.

Analysis of Variance

<u>Items</u>	<u>df</u>	<u>MS</u>	<u>VR</u>
Sites	2	0.0531	4.7779*
Time	8	0.0072	0.6462 NS
Location	2	0.0276	2.4889 NS
Time x Location	16	0.0240	2.1621*
Site x Location	4	0.0431	3.8772*
Time x Site	16	0.0207	1.86544 NS
Error	<u>31</u>	0.0111	
	79		

Table 10

Analysis of variance for  $H'$  values from the Tullgren samples. The error term is derived from the second order interaction.



shows the greatest variability (Table 9 ), while the least changes occur at the 7m distance at site 5, amidst a thick bramble and grass cover.

The summer maxima observed at all sites is not unexpected, though each site reacts differently to the seasons. For example, the lowest diversity of site 1 and 5 is in March 1976, whereas it occurs a month earlier at site  $\alpha$ .

There are real differences in the diversity of the soil fauna of these sites, but even though the highest diversity occurs at the least polluted site, this cannot be directly attributable to Pb contamination of the soil. The effect of the overlying vegetation and the thickness of the litter layer may be a factor contributing to the observed differences between sites, though the higher diversity of site 5, compared to the unmown site  $\alpha$  would suggest that some form of pollution may be reducing the diversity of the soil community. In this respect, it is worth noting the work of Williamson and Evans (1973), who added Pb to the soil, but could show no effect on the invertebrate community.

### 3.5 Estimates of the verge woodlice population

The tussocked grass of the verge is likely to provide good conditions for sustaining a woodlouse population (Al Dabbagh, 1976), yet few were found in either the pitfall traps or the Tullgren extractions. The detritivorous woodlice would be feeding on the Pb-rich dead vegetation of the verge, and the possibility arises that their numbers may be limited by the Pb contamination of the roadside litter.

Estimates were made of the woodlice populations on three of the major sites (1,  $\alpha$ , and 5) and the minor sites, using unbaited tiles. Approximately equal areas of tiles were

laid at three distances from the road (1, 4 and 7m). These were inspected every two weeks (April 1976 until May 1977) and any woodlice collected were starved, killed and analysed for Pb and Ca in the normal way (cf. Methods V.ii). The number of animals caught at each location in the first ten months of sampling are shown in Table 11; the species found at each site, for each month, are shown in Fig. 34. The only species to occur in large enough numbers for proper estimation of its Pb and Ca levels was Philoscia muscorum, at two sites (A47 and  $\alpha$ ), shown in Fig. 35.

The total number of woodlice collected at various distances from the road were too small for reliable interpretation, (Table 11), particularly in relation to Pb contamination across the verge.

The low numbers also make comparison between sites rather tentative. Notably, site  $\alpha$  has relatively high numbers of Porcellio scaber and Philoscia muscorum, which, when compared to site 1, is almost certainly due to the increased vegetation cover.

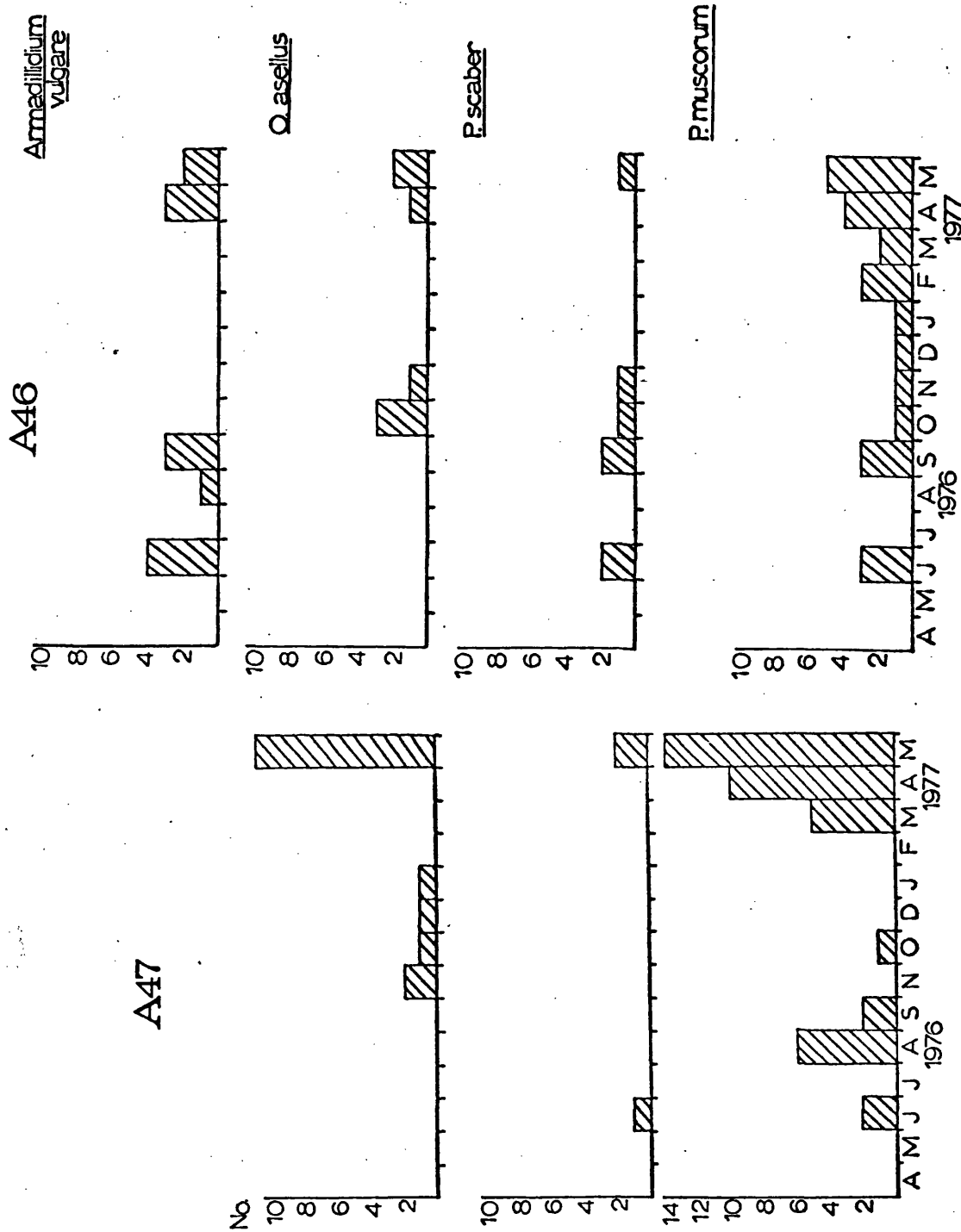
Armadillidium vulgare only occurs at the A46 site, though there are no obvious differences in its soil characteristics (cf. section 2.5<sup>p.49</sup>) or vegetational cover, to account for this. Most notable, perhaps, is the total lack of woodlice from the A50. This site has a well developed tussocked sward, with soil characteristics not greatly different from the other sites. The higher levels of soil Pb here, and the greater pollution of the vegetation (cf. section 2.5) suggest that Pb may at least be a contributory factor in the exclusion of woodlice from this site.

Perhaps the main cause of the low numbers of woodlice during the sampling period was the weather. The extreme dryness and high temperatures of the summer of 1976 were likely to deplete the woodlice populations of the verges. Al Dabbagh (1976)

Distance	Site	1	∞	5	A46	A47	A50
1m		4	10	3	2	11	0
4m		1	8	1	27	1	0
7m		2	18	4	0	4	0

Table 11

The total number of woodlice (all species) collected from six roadside verges in a 10-month period (April 1976 - February 1977), at three distances from the road.



**Fig. 34**

The numbers of four species of woodlice, per month, at two of the minor sites, 1976-77. No woodlice were found at the A50.

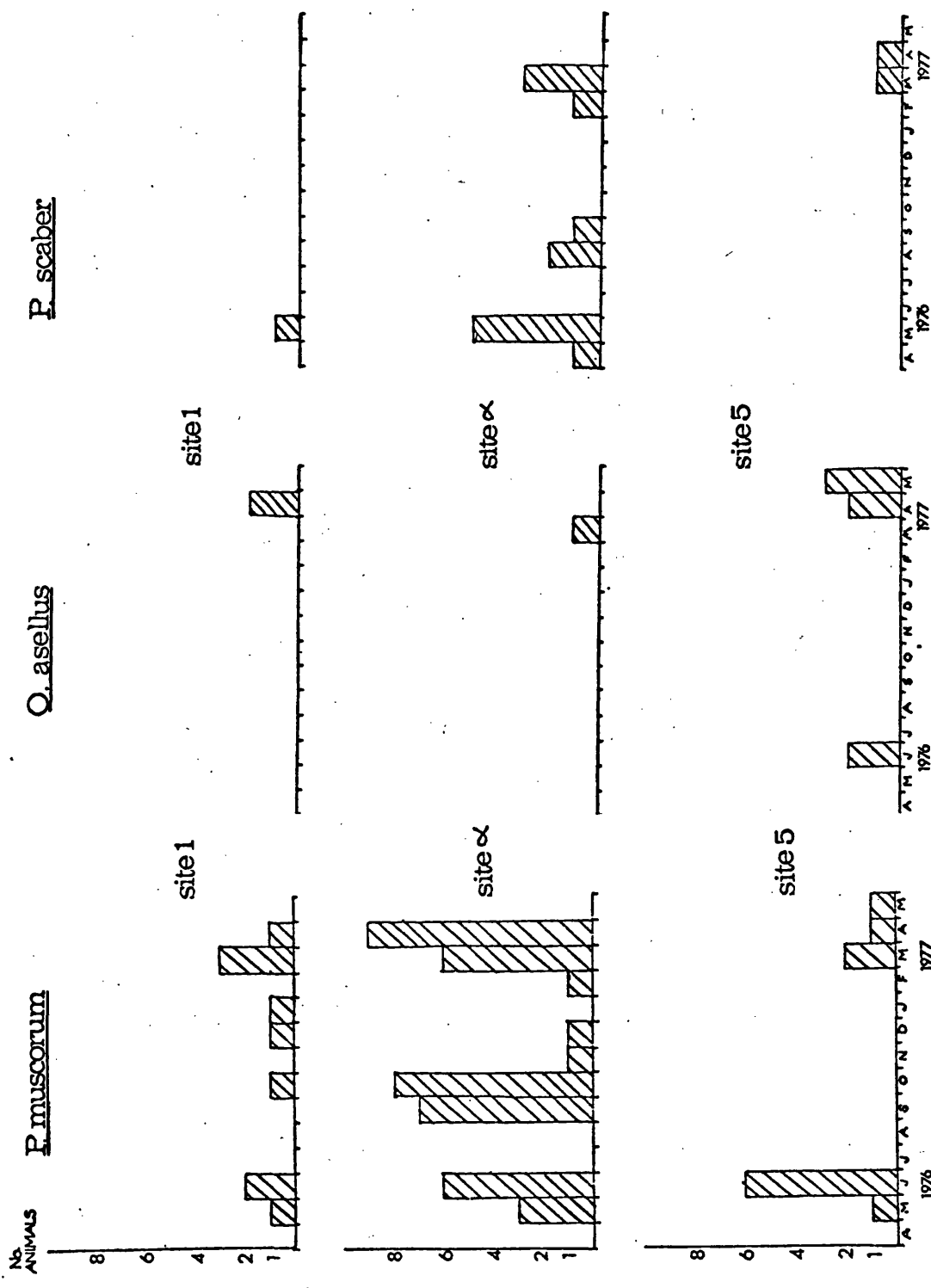


Fig. 34 continued.  
Woodlice numbers  
per month on three  
of the major sites.

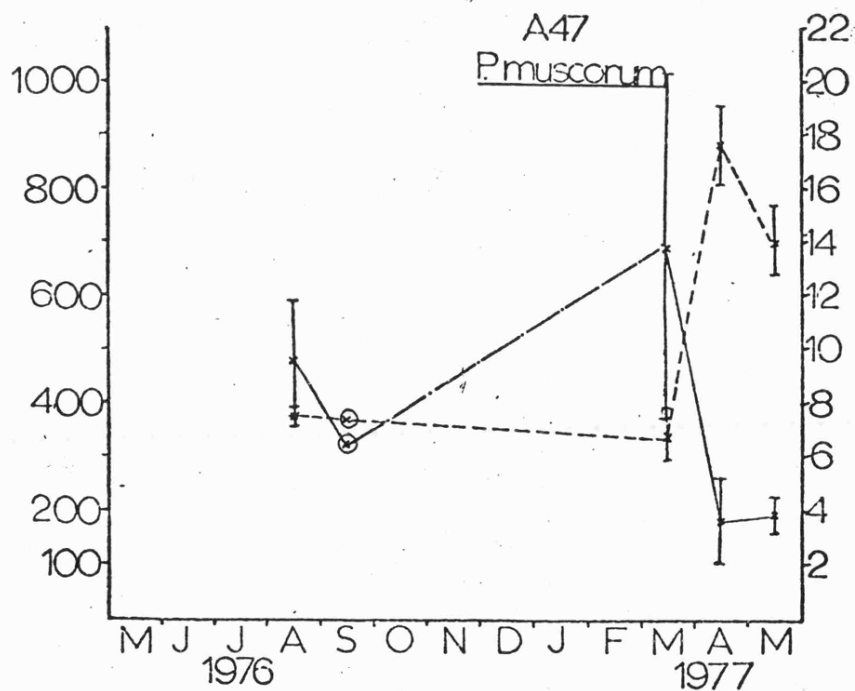
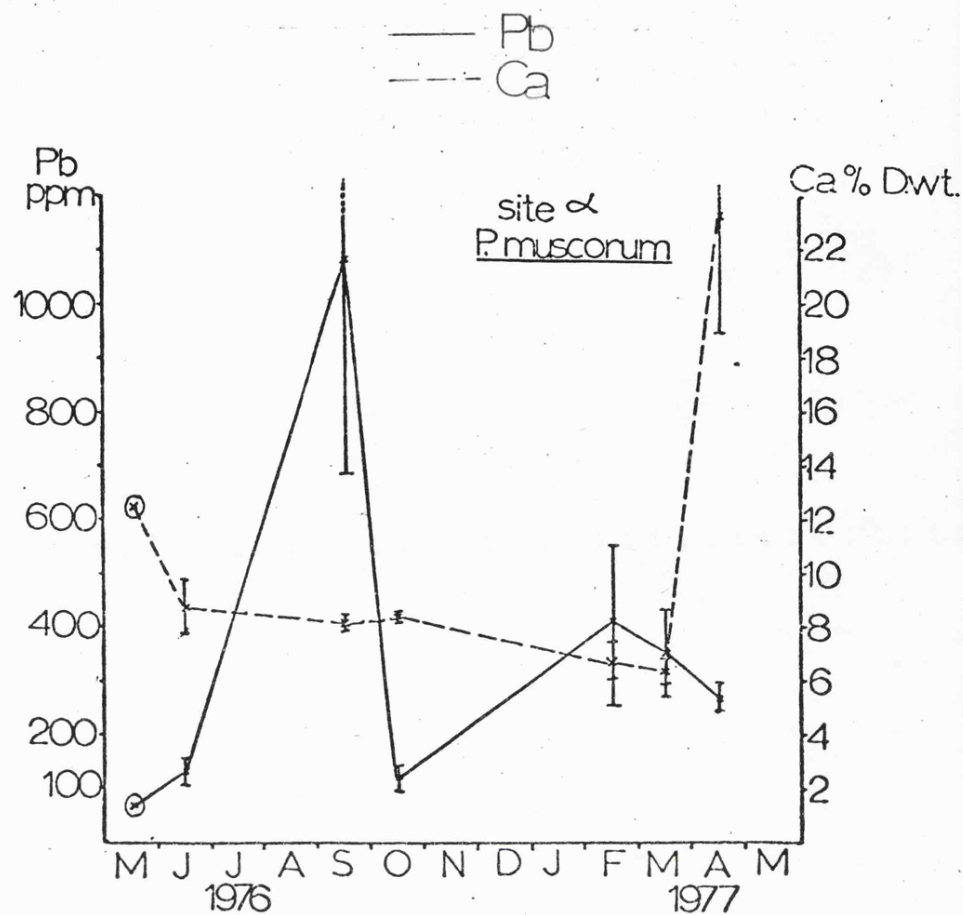


Fig. 35

Levels of Pb and Ca in *Philoscia muscorum* collected at various times of the year from the A47 and site  $\alpha$ . Plots ringed by a circle show the mean alone and no standard error (due to small numbers).

demonstrated that climate is the most important control of woodlice population size in an unpolluted site, and presumably this is true of the verge as well.

The seasonal changes in the Pb and Ca levels of P. muscorum are shown in Fig. 35. Again, only small numbers are involved and this, combined with the inherent variability of the analytical technique (cf. Methods V.ii) accounts for the large standard error term attached to these means. Where fewer than three readings were available no standard error is given.

A distinct spring rise in the Ca level of both sets of P. muscorum is apparent. This may be in some way related to a period of increased moulting with the onset of reproductive activity in the spring: Ca is known to be mobilised within the animal during moulting (Sutton, 1972).

The changes in the Pb levels with season are less clear, though there is a suggestion of winter maxima. This may simply reflect the corresponding rise in the Pb levels of the grass at this time. The results from the other woodlice would appear to support this, but no conclusions can be reached without further data.

To compare the Pb levels in a roadside herbivore with those of the detritivorous woodlouse, Lepidoptera larvae were collected from both the grass and hedgerow of site 1. Satyridae larvae were sweep-netted from the grass and Lasiocampidae larvae collected from the hedgerow by beating. At the same time (June 1977), woodlice were collected from beneath the tiles of this site. The caterpillars and woodlice were both starved before extraction and analysis for Pb and Ca in the normal way (cf. Methods V.ii). The results are given in Table 12.

Pb and Ca levels in both families of Lepidoptera are

Animal	Mean Pb ppm $\pm$ SE	Mean Ca % Dry wt. $\pm$ SE
Philoscia muscorum	122.60 $\pm$ 25.78	8.57 $\pm$ 0.52
Lasciocampidae larvae	4.12 $\pm$ 1.69	0.14 $\pm$ 0.02
Satyridae larvae	5.44 $\pm$ 2.29	0.002 $\pm$ 0.000

Table 12

Comparison of Pb levels in caterpillars and woodlice from site 1, June 1977. The Lasciocampidae larvae were from the hedgerow, the Satyrids from the verge itself.



negligible compared to those found in the woodlouse, P. muscorum. This would be expected from the caterpillars' diet of live grass, with its low summer Pb content. These results follow closely those of Williamson and Evans (1972), who also found higher Pb levels in woodlice. This suggests that woodlice are amongst the roadside invertebrates most likely to be affected by Pb.

### 3.6 Discussion

No clear effect of Pb on the diversity of roadside invertebrates has been demonstrated. This may, in part, be due to the broad taxonomic groups adopted; any more detailed study would have to be planned over a longer time scale, not only to replicate sampling occasions, but also to allow identification to species.

The possible effects of Pb on diversity have been confounded with variations in the vegetation and litter cover with distance from the road. On this point, however, the high diversity of the mown site 5, compared to site  $\alpha$ , suggests that this is not the only factor, and that this may indeed be response to Pb pollution.

The higher diversity of site 2 may be due to the encroachment of the bottom fauna of the hedgerow onto the verge, across the very shallow ditch at this site.

Snails and sminthurid collembolans appear to be the only groups avoiding zones of high Pb contamination. In both groups, this does not appear to be a response to different thicknesses of vegetation, though it may be a reaction to high salt levels. However, little road salting was carried out during the sampling period of the pitfall traps.

Generally, the difficulty in demonstrating a response in the invertebrate community to high Pb levels is quantifying and isolating the effect. The total lack of woodlice at the A50, (and their low numbers on all of the verges studied), and the apparent preference of the snails for areas of low Pb contamination, might indicate that both groups may be reacting to high Pb levels in their diet. Indeed, these results may have been expected from the work of Williamson and Evans (1972), which showed that both woodlice and snails had higher Pb levels than other invertebrates found on the verge.

#### 4. The toxicology of lead in *Forcellio scaber*. Latreille.

##### 4.1 Introduction

Woodlice are the only truly terrestrial crustaceans, though how fully they are adapted to this environment is still a point of contention (Sutton, 1972). Certainly, their salt balance is of great interest, since their ability to selectively remove and concentrate metals from their food is an indication of their adaptation to land. If woodlice are on the edge of their physiological range, the possible relationship between Pb and Ca may make them less able to withstand the toxic effects of Pb, as compared to some more well-adapted group, such as the insects. Pb levels of up to 700 ppm have been reported from roadside *Oniscus asellus* (Williamson and Evans, 1972), though from the present work, a more usual level is around 100 ppm.

The high Ca requirements of woodlice are well established (Heeley, 1941) and these are met by their choice of habitat (generally favouring calcareous soils (Sutton, op. cit)), and detritivore habits, - calcium becomes concentrated in dead vegetation (cf. Appendix IV). This is required for the exoskeleton, which is a chitinous material hardened by the addition of  $\text{CaCO}_3$  (Richards, 1951). The exoskeleton represents a large proportion of the animal's dry weight, suggesting that a calcium/dry weight relationship could be derived, although no such correlation has been reported.

The moulting cycle of the woodlice has to be considered in this respect: it is known to be a time of increased Ca mobility (Sutton, 1972; Wieser, 1968). At this time, Ca is removed from the cuticle to be returned when the old exoskeleton has been shed (Sutton, op. cit). Because of this, all dosing periods

(except in the fecundity experiment) were for two weeks, following two weeks on Pb-free carrot. This was as long as the experiment could be run without the chance of moulting. All experiments were kept at a constant temperature to reduce this possibility further. Any animals which did moult would not feed for several days, and thereby increase the variability of the results. In fact, the woodlice only moulted in the respiration experiment.

The effects of Pb on P. scaber were measured in terms of four main factors. These were :-

- i. the Ca status of the animal
- ii. fecundity
- iii. sexual differences in Pb uptake
- iv. respiration.

Some indications of the effects of the moult cycle on Pb uptake were noted as incidental to the last experiment.

The lack of previous work meant that dosing techniques had to be developed which approximated to the field situation, while ensuring Pb uptake from the food. Generally, the techniques used were successful, and similar levels were attained in the dosed animals compared to those found on the verges.

#### 4.2 Lead and calcium levels in the laboratory culture of P. scaber

A culture of P. scaber was derived from an abundant population of woodlice found in a suburban compost heap. The woodlice were put into a perspex aquarium with soil and litter from the compost, and here the culture thrived and bred freely. All animals for the experiments were derived from this culture.

It was necessary to determine the background Pb and Ca

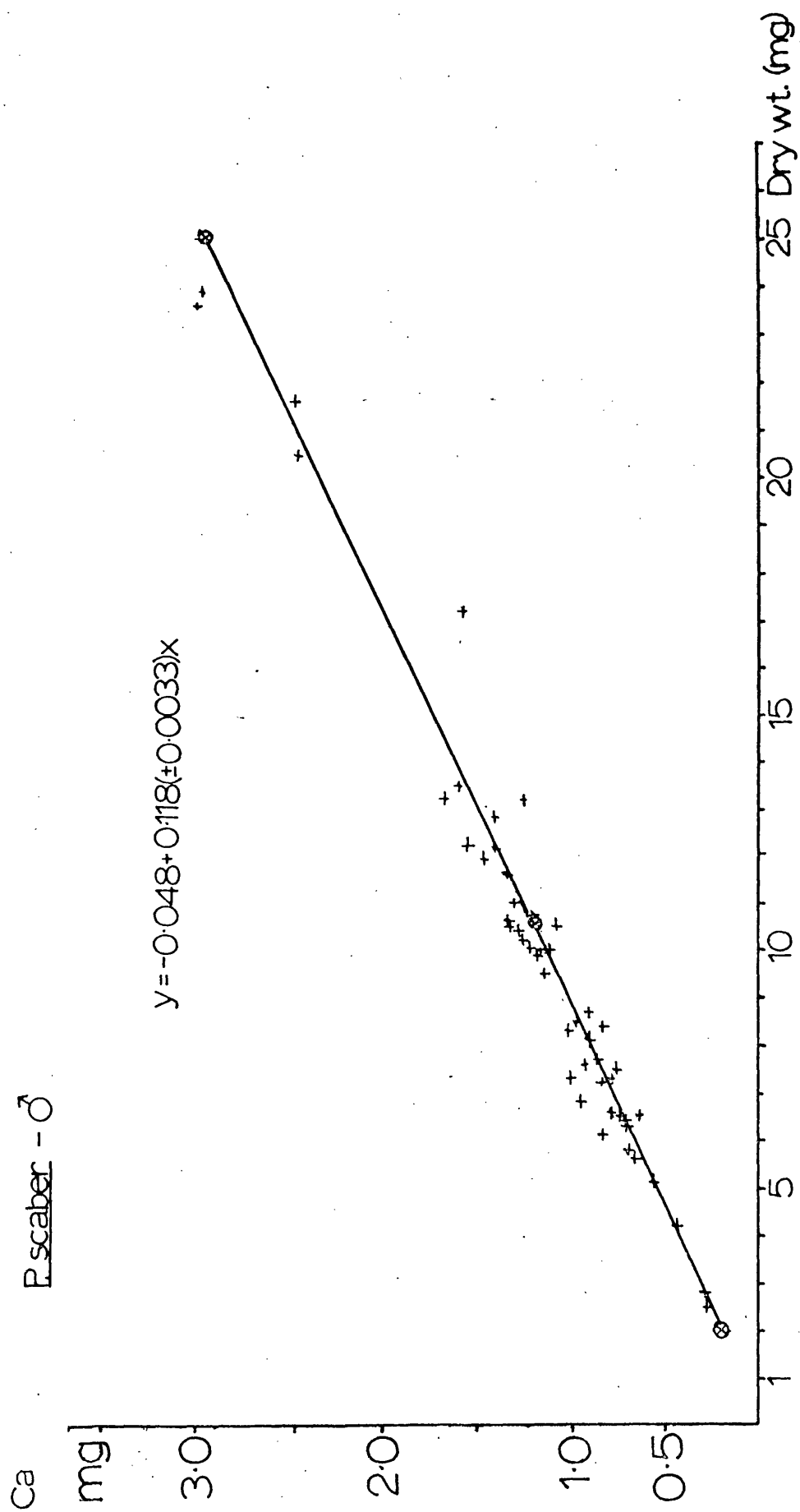


Fig. 36

The regression of Ca content against dry weight in male P. scaber.

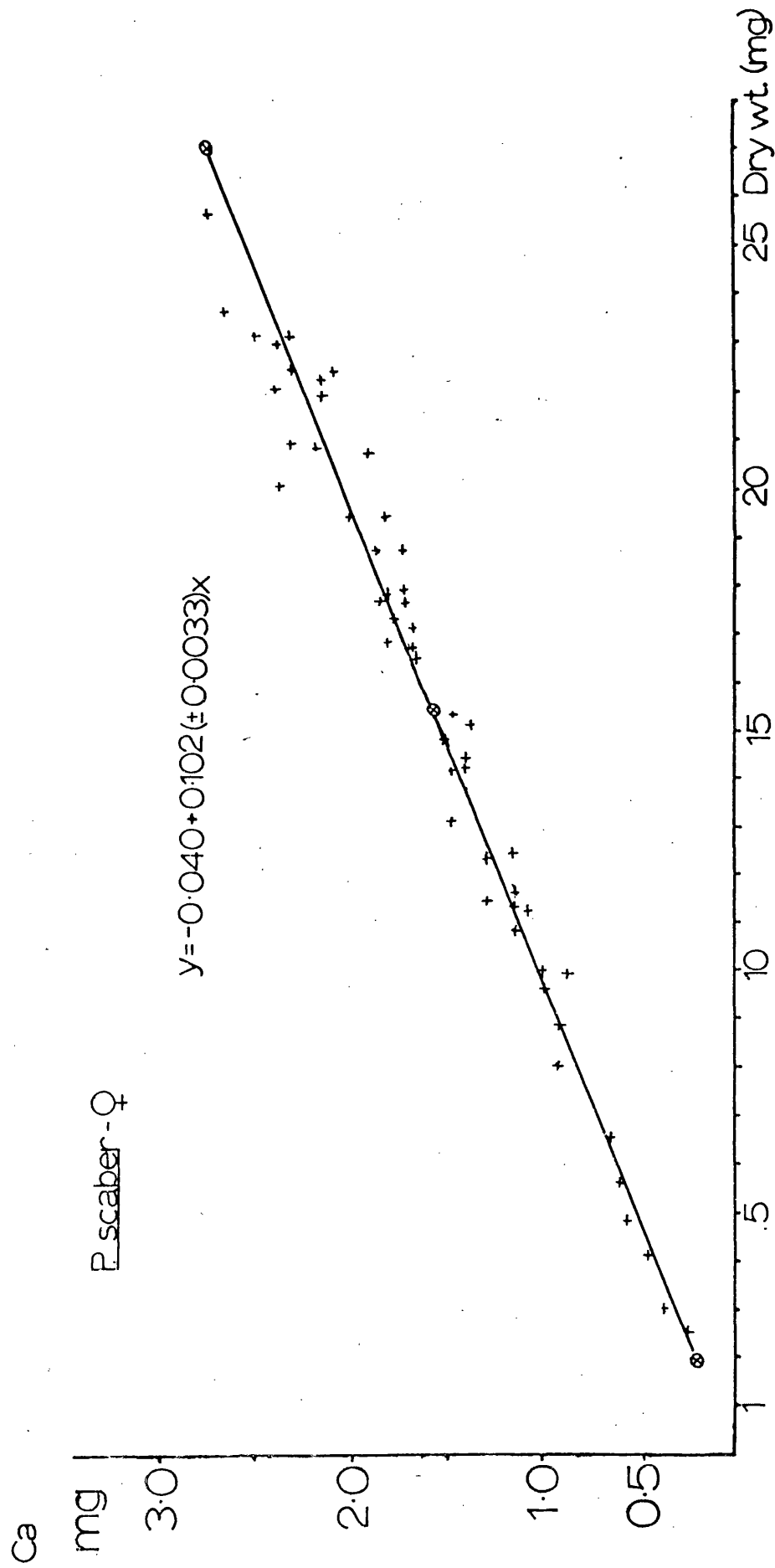


Fig. 37

The regression of Ca content against  
dry weight in females of P. scaber.

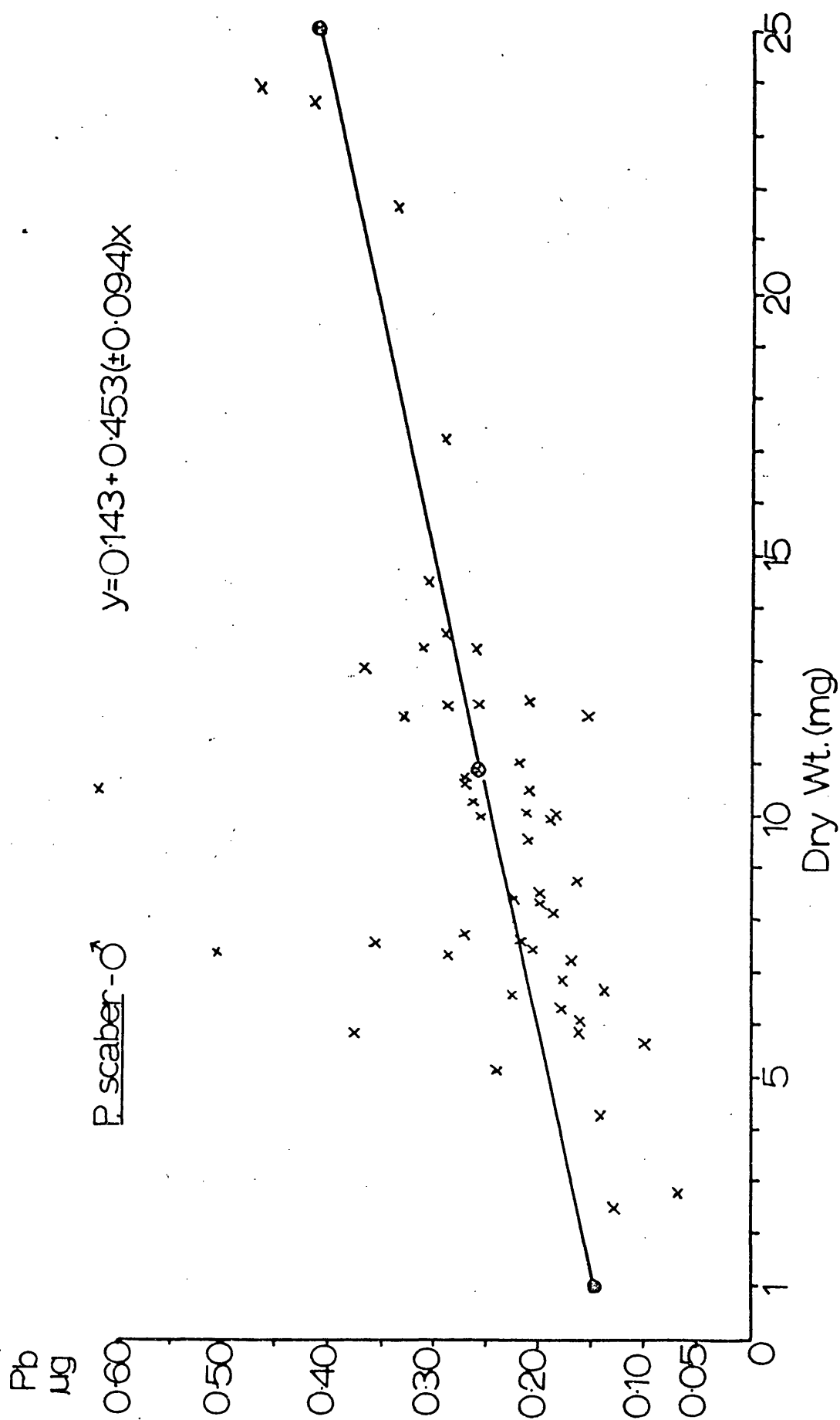


Fig. 38 The regression of Pb content against dry weight in male P. scaber

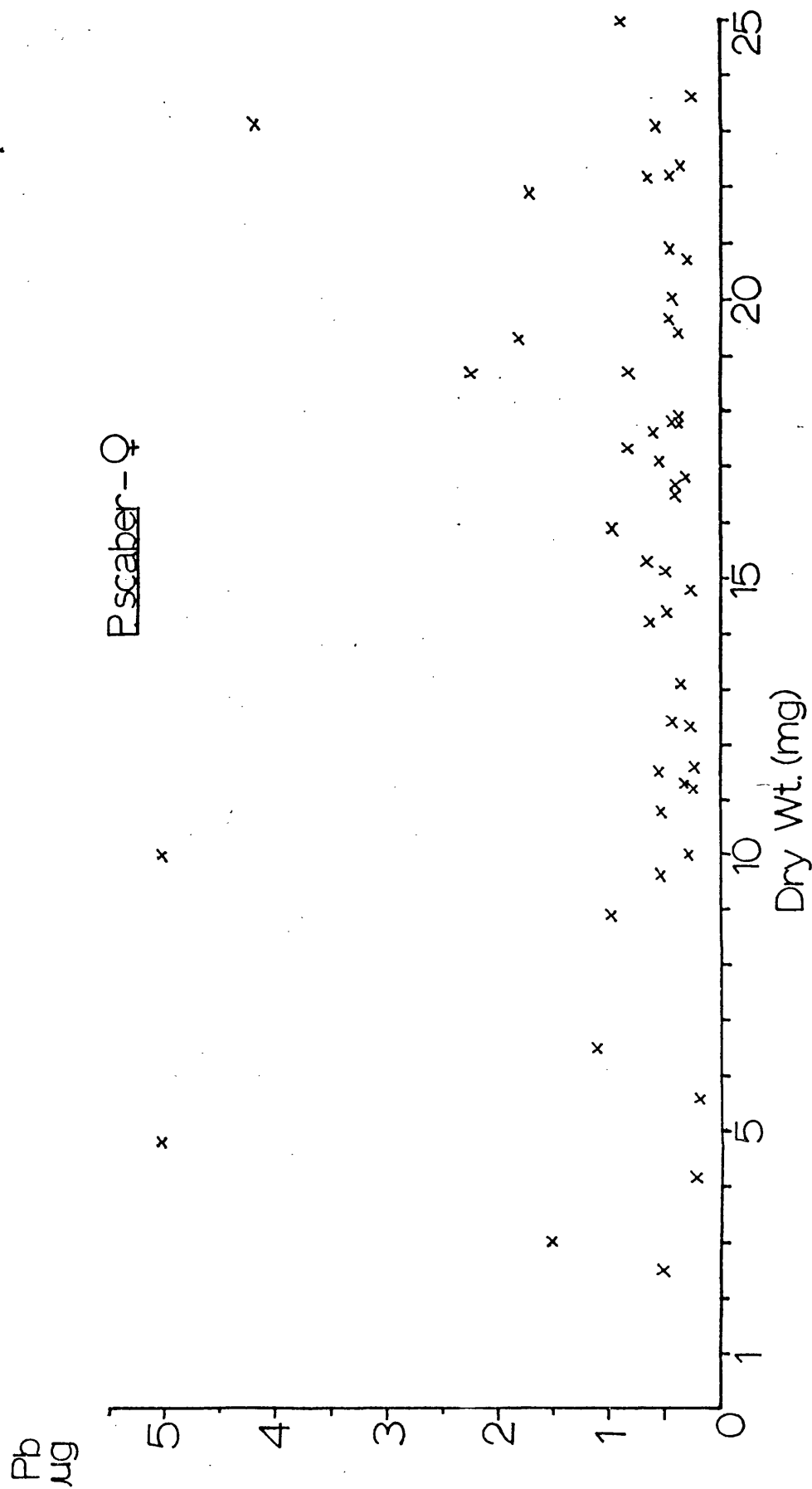


Fig. 39

The Pb content of female P. scaber with dry weight.



levels in the culture, and so 50 animals of each sex were randomly taken, starved, killed and analysed for Pb and Ca (cf. Methods V.iii). A linear regression of dry weight against Ca content was derived for each sex (Fig. 36 and 37), and which were shown to be significantly different ( $\delta b = 0.118$ ,  $\text{♀ } b = 0.102$ ,  $t = 3.54$ ,  $P < 0.001$ ). The mean weight of the females was greater than that of the males, but the latter had a higher weight-specific Ca level. Thus, the average Ca concentration of the males increased more rapidly with size.

A linear regression of dry weight against Pb content was also calculated for each sex, but only in the males was there a significant correlation ( $r = 0.5732$ ,  $P < 0.001$ : Fig. 38). The females all have roughly the same Pb level (Fig. 39), irrespective of weight, though these are much higher than those of the males ( $\text{♀ mean} = 1.06\mu\text{g}$ ,  $\text{♂ mean} = 0.26\mu\text{g}$ ). This may be related to their relative differences in weight: Al Dabbagh (1976) notes that the metabolic rate decreases with increasing size in Armadillidium vulgare. This was also found to be true of P. scaber (cf. section 4.5). In this case, the heavier females may have a slower turnover in both Pb and Ca.

These levels probably represent the background Pb inherent in the culture, and would have to be accounted for in the Pb dosing experiments. Generally, this means that most individuals, prior to dosing, would have a Pb concentration of about 10 ppm.

#### 4.3 Sexual differences in the uptake of lead and calcium in P. scaber

The background levels of Pb and Ca have been shown to have clear sexual differences. Consequently, an experiment was conducted to investigate the possibility of a similar difference in the rate of uptake of these two metals.

Three doses of Pb were prepared in a potato food (as described in Methods IV.i), producing 0.0, 50 and 500  $\mu\text{g/g}$  of Pb. No Ca was added, though the potato had a natural level of 2 mg/g. 30 woodlice of one sex were allocated, 10 at a time, to one of three petri-dishes, and allowed to feed on Pb-free carrot for two weeks. After this period, the carrot was removed and one of the three doses presented to the woodlice. Again, they fed for two weeks, before returning to the carrot for a final two weeks. They were then killed and analysed in the normal way; the results are presented in Fig. 40 and 41.

No significant differences between the sexes in their Pb levels are found for any of the doses, using both an analysis of variance and a non-parametric test (Mann-Whitney U test). Fig. 40, however, would suggest greater Pb uptake in both sexes with increasing dose, and this appears at its greatest in the males with the 500  $\mu\text{g/g}$  dose. In fact, the only significant differences are in the females, between the control and 50  $\mu\text{g/g}$  dose ( $t = 4.6550^{**}$ ), and between the control and 500  $\mu\text{g/g}$  dose ( $t = 9.7666^{***}$ ). The lack of any significant differences between doses in the males is due to the extremely high variability within treatments, which is not easily accounted for. Part of the variability of all the results may be due to the two week period on the Pb-free carrot at the end of the experiment, allowing a long time for the elimination of Pb in the faeces.

The Ca levels of the two highest Pb doses are significantly different between sexes (50 $\mu\text{g}$  - VR = 12.94\*\*, 500 $\mu\text{g}$  - VR = 10.516\*\*), though the levels in the control woodlice cannot be distinguished. Thus the Ca levels in the males are again shown to be higher. These results would also suggest that Ca uptake increases in both sexes with a high Pb diet.

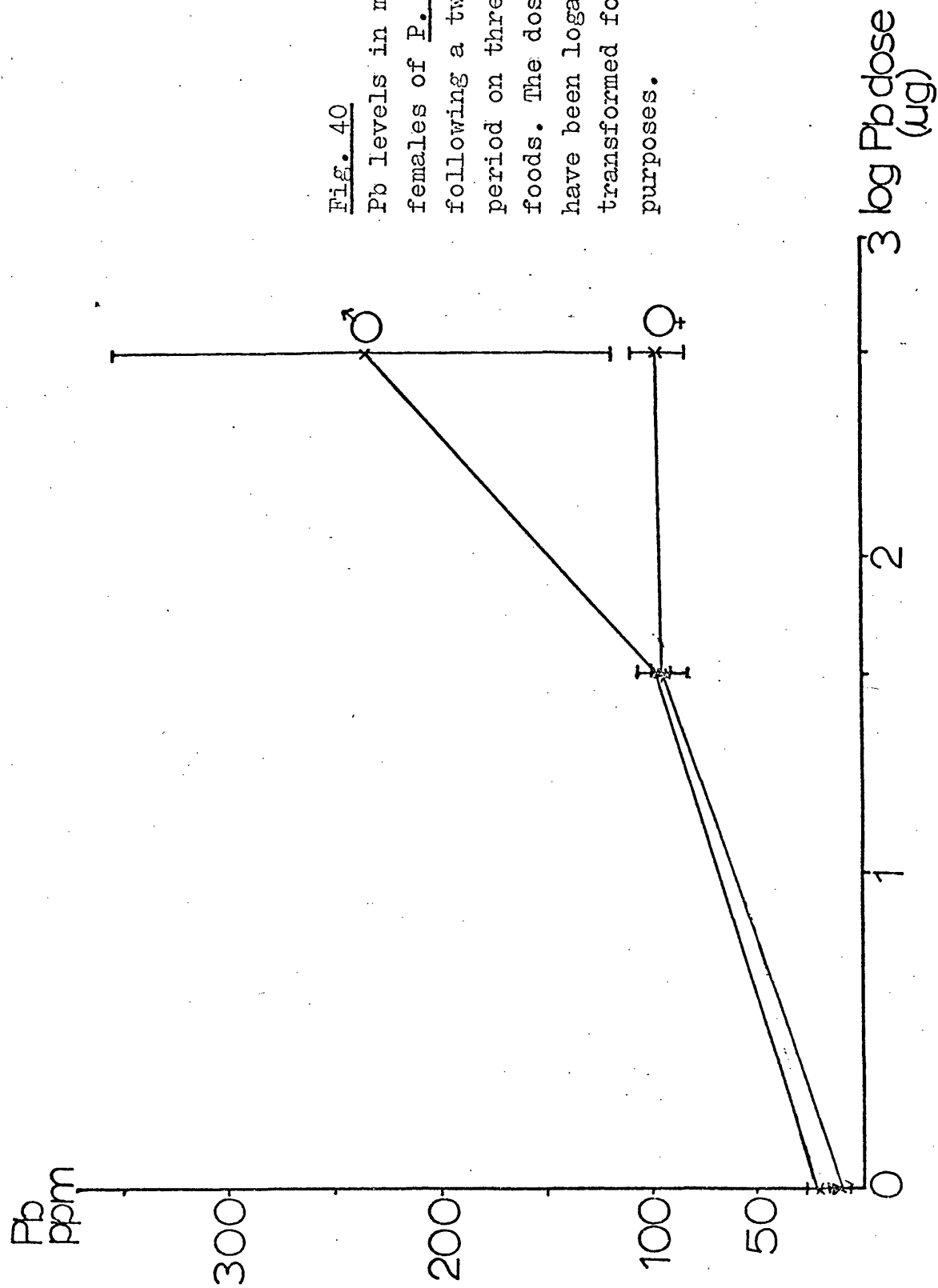


Fig. 40  
Pb levels in males and females of *P. scaber*, following a two-week period on three dosed foods. The dose levels have been logarithmically transformed for presentation purposes.

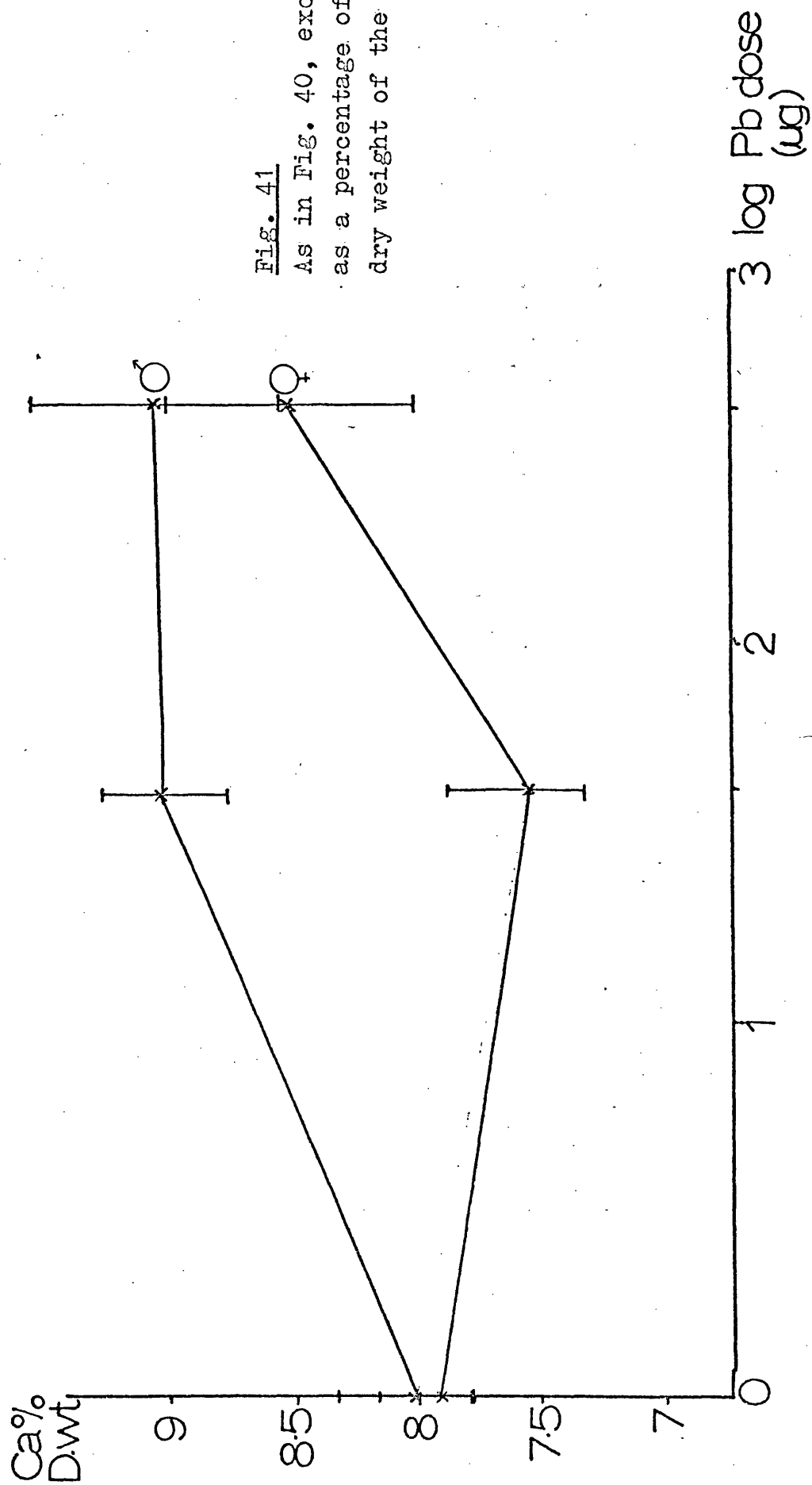


Fig. 41

As in Fig. 40, except Ca.  
as a percentage of the  
dry weight of the woodlice.

Although there is no statistical difference between the sexes, there is some suggestion of increased Pb uptake by the males, and this is correlated to their greater Ca assimilation ( $r = 0.4746^*$ ). No such relationship is found in the females. From the previous section, the correlation between Pb content and dry weight in the males and their greater weight-specific Ca level might suggest a more rapid turnover of both metals in the males, related to their metabolic rate (which, in turn, is related to their dry weight). With the development of a reliable technique for estimating Pb and Ca levels in the faeces of woodlice, this question might be resolved.

#### 4.4 The interaction of lead and calcium uptake in *P. scaber*

The possibility of a Pb/Ca interaction in the uptake of these metals was tested using a  $3^2$  factorial design feeding experiment. No distinction was made between the sexes, as this experiment took place before the differences between males and females had been noted.

Three levels of  $\text{Pb}(\text{NO}_3)_2$  (0.0, 0.1 and 1.0mM) and three of  $\text{Ca}(\text{NO}_3)_2$  (0.0, 1.0 and 10mM) were added as 10cc doses to 20g portions of boiled and sieved potato (cf. Methods IV.i). Deionised water was used as a blank in both cases, and a matrix of all nine possible doses was made. This gave the following levels of Pb and Ca in the potato:

Applied Pb dose	0.0	0.1	1.0mM
Resulting Pb in potato	0.0	0.206	2.06 mg/g dry wt.
Applied Ca dose	0.0	1.0	10mM
Resulting Ca in potato	2.0	8.0	32 mg/g dry wt.

About 0.5g (dry weight) were presented to each group of woodlice, while two samples of the remainder of each dose were used to estimate mean water content and Pb and Ca levels (cf. Methods IV.i).

Eight woodlice were randomly allocated to a petri-dish containing Pb-free carrot. After two weeks, they were transferred to a second petri-dish containing one of the dosed foods. All woodlice were between 8.0 and 20.0 mg dry weight. Their faeces were collected and removed daily

(to prevent coprophagy) and the food turned with clean stainless steel forceps: this was necessary to prevent the development of fungal conidiophores. After two weeks on the dosed food, the woodlice were returned to the Pb-free diet for a final two weeks, after which they were killed and analysed (cf. Methods V.iii).

The results are illustrated in Fig. 42 and 43, which show the total amount of Pb and Ca recovered from all of the eight woodlice given one treatment. This represents what has been assimilated by the woodlice plus their background level. The mean Pb levels for each treatment are presented in Table 13, and the mean Ca levels in Table 16; the analysis of variance of the Pb levels is given in Table 14 and for Ca in Table 17. The data for the Pb levels in the woodlice was also examined with an analysis of covariance, according to the technique described by Wishart (1950). Here, the variance of the dry weight of the woodlice in each treatment is calculated, and the effect of this on their Pb levels estimated. The mean Pb levels for each treatment are then adjusted accordingly, but in fact, these were not significantly different from the original values. The adjusted means are given in Table 15. The amount of Pb and Ca ingested from the food were calculated from the amount of food consumed, and these results are given for each dose in Table 18. By comparing the weight of Pb and Ca recovered from each treatment (minus a constant background figure, derived from controls, of  $9\mu\text{g/l}$ ), with the Pb consumed, the amount of Pb assimilated was estimated. These figures are also given in Table 18.

Woodlice not fed on a Pb-enhanced diet had measurable amounts of Pb (Table 13), comparable to those recorded with the lowest Pb dose. These levels are also lower than the mean

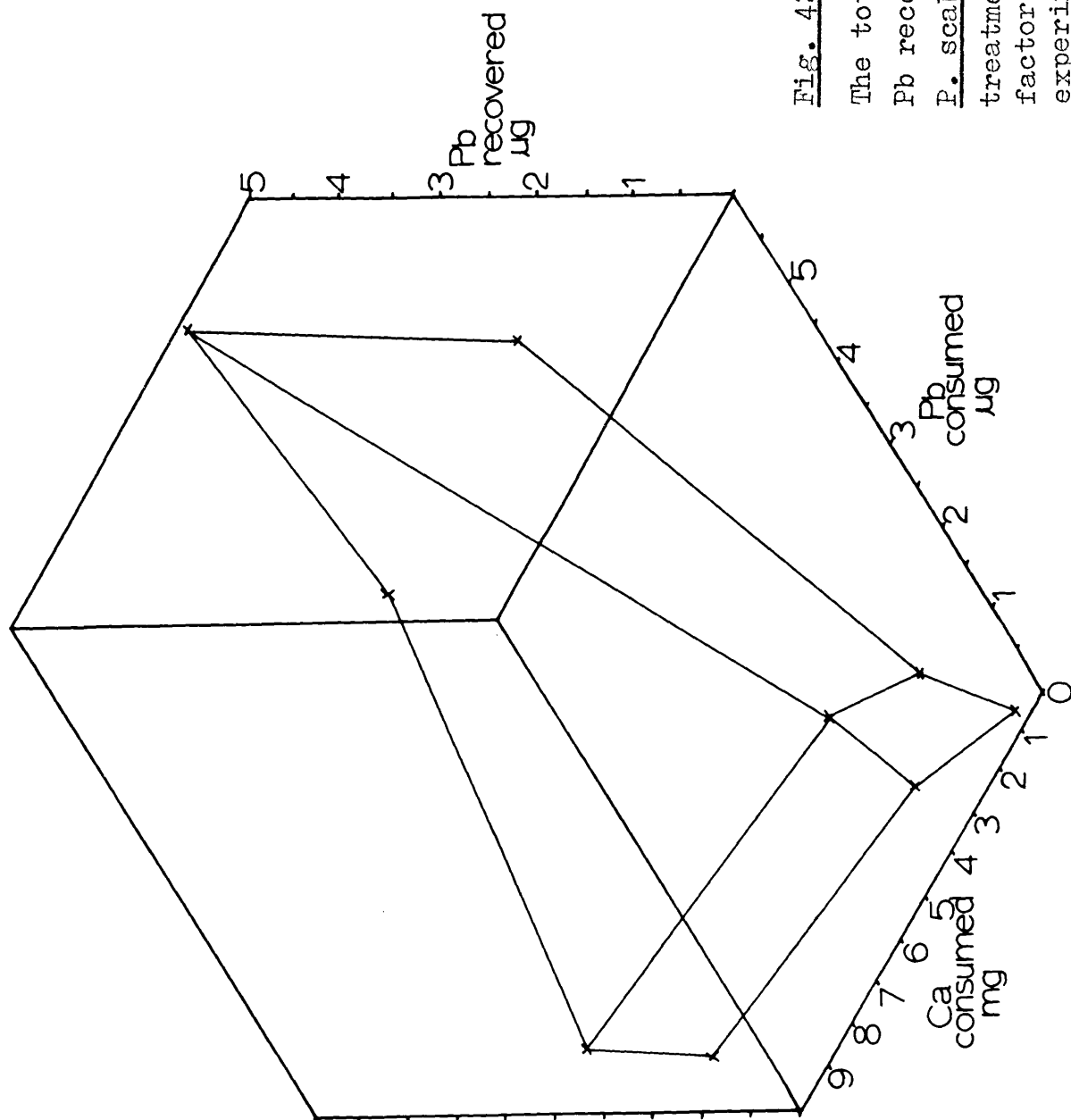


Fig. 42

The total amounts of Pb recovered from eight P. scaber, for each treatment in the  $^{32}$  factorial feeding experiment.



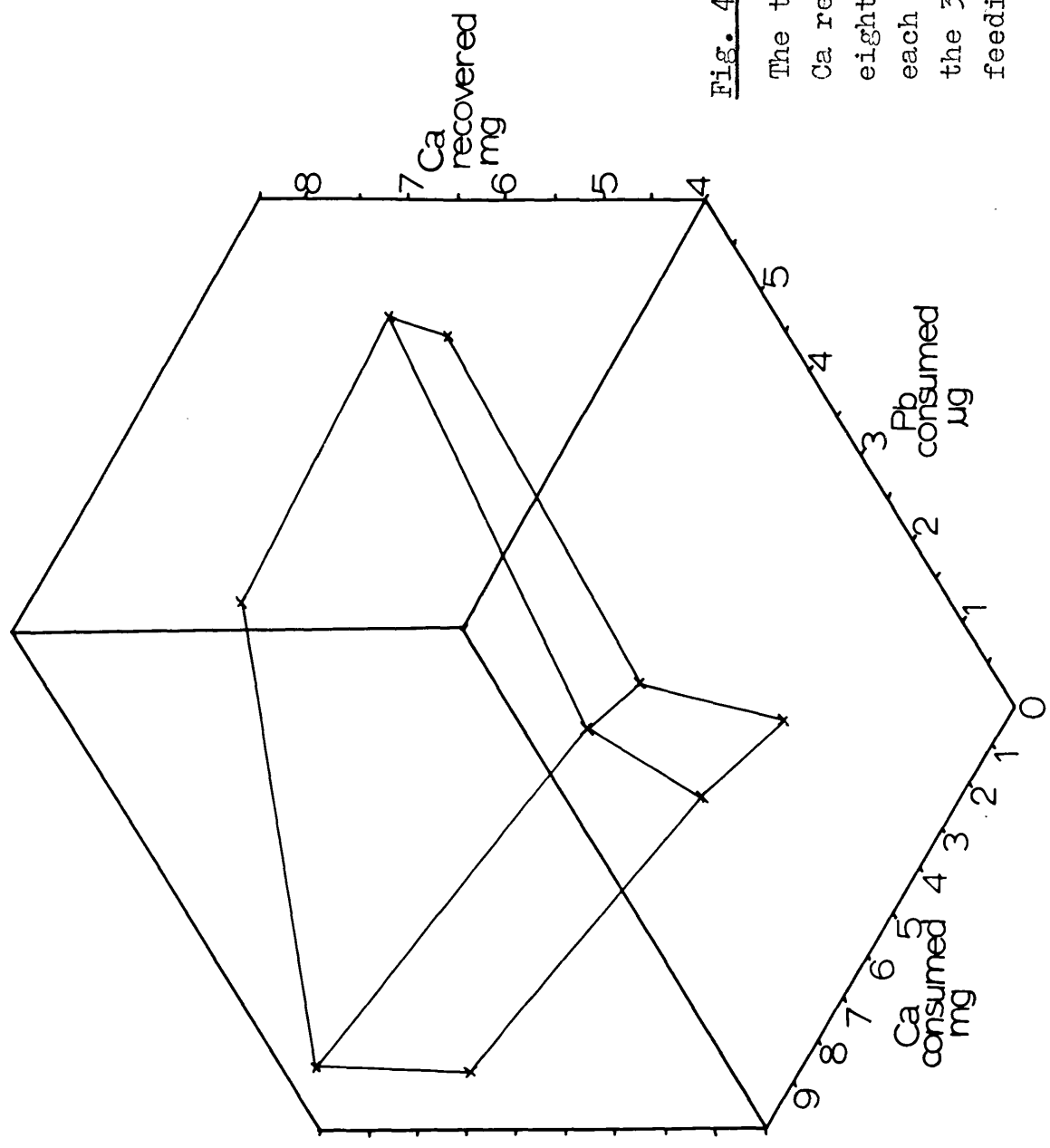


Fig. 43

The total amounts of Ca recovered from eight P. scaber, for each treatment in the  $3^2$  factorial feeding experiment.

		<u>Pb Dose</u>			$\bar{x}$
		0.0	0.1	1.0	
<u>Ca Dose</u>	0.0	0.2567	0.3562	0.5925	0.4018
	1.0	0.2017	0.3867	0.8262	0.4715
	10.0	0.3495	0.4452	0.4032	0.3995
	$\bar{x}$	0.2693	0.3960	0.6073	

Table 13 Mean Pb ( $\mu\text{g}$ ) per individual for each treatment (eight replicates) in a factorial dosing experiment. The means for each dose are also given.

<u>Items</u>	<u>df</u>	<u>MS</u>	<u>VR</u>
Pb'	1	2193.75	55.5239***
Pb''	1	45.67	1.1559 NS
Ca'	1	0.12	0.0037 NS
Ca''	1	129.59	3.2789 NS
Ca'Pb'	1	253.68	6.4206*
Ca'Pb''	1	787.18	19.9235***
Ca''Pb'	1	80.48	2.0369 NS
Ca''Pb''	1	92.36	2.3376 NS
Error	53	39.51	

Table 14 Results of the analysis of variance of the factorial experiment, (Pb,  $\mu\text{g}$ ). Standard probability notation.

		<u>Pb Dose</u>			$\bar{x}$
		0.0	0.1	1.0	
<u>Ca Dose</u>	0.0	0.2635	0.3345	0.5837	0.3939
	1.0	0.1867	0.3895	0.8397	0.4720
	10.0	0.3665	0.4317	0.4220	0.4067
	$\bar{x}$	0.2722	0.3853	0.6151	

Table 15 Adjusted means for Pb corrected for weight in the factorial experiment, by analysis of covariance. Standard error of a single mean is 0.052.

<u>Ca</u>	<u>Pb Dose</u>			$\bar{x}$
	0.0	0.1	1.0mM	
0.0	7.1161	7.3502	7.8072	7.6245
1.0	6.8014	8.9970	9.0656	8.2880
10.0	8.8858	8.6616	7.7072	8.4182
$\bar{x}$	7.8011	8.3362	8.1933	

Table 16

Mean levels of Ca, expressed as percentage of dry weight, for eight animals for each treatment.

<u>Items</u>	<u>df</u>	<u>MS</u>	<u>VR</u>
Pb'	1	2.0049	3.0335 NS
Pb''	1	1.8929	2.8641 NS
Ca'	1	7.4655	11.2959**
Ca''	1	1.1042	1.6707 NS
Ca'Pb'	1	3.6788	5.5663*
Ca''Pb'	1	20.6183	31.1973***
Ca'Pb''	1	1.9238	2.9108 NS
Ca''Pb''	1	4.1592	6.2932*
Error	53	0.6609	

Table 17

Analysis of variance for Ca % Dry weight, for Porcellio scaber following the factorial dosing experiment.

derived for the males and females of the laboratory culture, and this might indicate that the woodlice had lost Pb since the initial survey was taken, shortly after the culture had been collected. This means, of course, that only a slight elevation in their Pb levels has been achieved by any of the doses.

The Pb content of the woodlice show a strong linear response to applied Pb dose, but no direct relationship to Ca levels in the food (Table 14). There is also a linear response to increasing Pb and Ca dose, but the most significant interaction is a result of Pb levels rising linearly with increasing Ca dose, and quadratically with increasing Pb dose. This demonstrates that Pb uptake has consistently increased with the Ca content of the food, but also that the rate of Pb uptake tends to decline with the highest Pb dose.

Ca levels in P. scaber rise linearly with Ca dose, but again, there is no direct response to Pb dose alone. There is, however, a significant interaction with Ca levels rising linearly with the Pb content of the food and quadratically in response to Ca dose. This is the most significant response of the Ca levels and a reversal of the most significant interaction affecting the Pb levels of the woodlice - Ca uptake increases in a regular fashion with Pb dose, but tends to decline with the highest Ca dose. The net result is that Ca uptake and Pb uptake appear to be closely associated, with both declining at their highest respective doses. This might suggest that at these higher levels, selective absorption is taking place, with excess Ca and Pb remaining in the gut, and being lost with the faeces.

Actual Dose		Total Dry wt. Food consumed	Ca consumed	Pb consumed	Ca recover.	Pb recover.	Pb recover. % consumed
Ca	Pb						
1.0	0.0	0.2552	0.5104	----	6.1515	0.2969	----
4.0	0.0	0.2983	2.3860	----	6.5767	0.7736	----
16.0	0.0	0.2776	8.8832	----	7.2524	1.2424	----
1.0	0.103	0.1958	0.3916	40.57	7.4538	0.9464	2.23
4.0	0.103	0.2496	1.9968	51.72	7.4510	1.4648	2.83
16.0	0.103	0.3064	9.8048	63.48	8.2982	1.8480	2.91
1.0	1.03	0.2191	0.4382	453.99	7.2137	2.9256	0.64
4.0	1.03	0.2749	2.1992	569.61	6.7581	5.1024	0.89
16.0	1.03	0.2814	9.0048	583.09	5.4769	1.4768	0.25
mg	mg	g	mg	µg	mg	µg	

Table 18

Pb and Ca recovered from P. scaber and amounts consumed during a factorial feeding experiment, following the application of various doses of Pb and Ca. The Pb recovered values have been corrected for background Pb resulting from the extraction.

Generally, the amounts of Pb retained by the woodlice at the end of the experiment are very low (Table 18), and certainly less than would be expected if it was assimilated at the same rate as the food - Wieser (1965) gives a value of 36% assimilation of leaf litter by P. scaber. This would further suggest that Pb is not absorbed to any large degree from the gut, probably being related to the selective absorption of Ca. Notably, a degree of similarity is apparent between Fig. 42 and 43, the Pb and Ca levels in the woodlice, suggesting that Pb uptake rises sympathetically with increasing Ca uptake.

Much of the Pb ingested must be lost with the faeces, but whether it has always remained in the gut in its passage through the animal is not known. Hassall and Jennings (1975) note that in Philoscia muscorum any soluble nutrients are absorbed in the caeca, and they cite various authors who state that Fe and Cu are accumulated within the caecal cells. A further possibility therefore, is that Pb is absorbed by these cells at a relatively high rate (perhaps by the same mechanism by which Ca is taken up), but lost again with the caecal secretions to the hind-gut. The work of Ireland (1975) is of interest in this respect: he suspected a relationship between Pb and Ca in the oligochaete worm Dendrobaena rubida, and noted that the highest Ca level was found in the anterior gut, while Pb is mainly concentrated in the hind-gut. This cannot be directly applied to woodlice, but it does demonstrate the distribution of Pb in the gut which might be expected if there was some method of voiding toxic substances to the hind-gut.

#### 4.5 The effects of lead on the respiration rate of *P. scaber*

One possible sub-lethal effect of a high Pb concentration in the body may be an interference with the general metabolism, that correspondingly may affect the respiration rate of the animal. This would be detectable as a change in the rate of oxygen consumption.

Again, no work has been reported on the physiological effects of Pb on arthropods, and the only comparable work on invertebrates is that of Macinnes and Thurberg (1973), using heavy metals other than Pb. These authors found that Zn, Cd, Cu and Ag all lowered the oxygen consumption of the mud snail (Nassarius obsoletus). The following experiment was designed to test for any possible effect of Pb on the oxygen consumption of P. scaber.

As in section 4.3, ten animals of each sex were fed one of three dosed potato foods (0.0, 50 and 500 ppm Pb), with a uniform Ca level. The animals were kept at 20°C throughout the experiment. After the dosing period, the woodlice were returned to Pb-free carrot for three days, before their oxygen consumption was measured in a Gilson differential respirometer (according to the method given in Al Dabbagh, 1976), at 20°C for four hours.

Before being placed on the food, the woodlice had their backs marked with yellow paint (using 'Humbrol' model paint - this had been analysed and shown to be Pb-free). If this paint was lost during the experiment the animal had moulted, and this would result in a falsely high reading for the rate of oxygen consumption (Phillipson and Watson, 1965). Four animals of each sex did moult during the experiment, and their Pb levels were compared to other animals of their treatment.

The results are considered later in this section.

Sexing the woodlice was necessary since the females are of a higher mean weight, and oxygen consumption is known to decline proportionally to the weight of at least one species of woodlice, Armadillidium vulgare (Al Dabbagh, op. cit). For each individual, a regression of oxygen consumed against time was computed, and a mean value for  $\mu\text{l}$  of  $\text{O}_2/\text{mg}$  dry weight derived. An analysis of variance was used to compare these results, and also a regression of  $\text{O}_2$  consumed against Pb content tested any correlation between the two parameters. The results are given in Table 19, which shows the mean oxygen consumed and Pb levels for the woodlice of each treatment, and also the correlation coefficient for the regressions of Pb content against weight-specific oxygen consumption for each sex.

Fig. 44 shows the shows the weight-specific oxygen consumption of each woodlouse against dry weight, with the resultant regression equation for each sex. As was noted with A. vulgare (Al Dabbagh, op. cit), the rate of oxygen consumption declines with increasing weight. There is no significant difference between the lines derived for each sex, though the females are much more variable. Since females, on average, are heavier than males, their mean metabolic rate is likely to be less. As already noted, this may mean a less rapid turnover of metals in the females, and correspondingly account for the differences observed between the sexes in their Pb and Ca levels (cf. section 4.3).

The analysis of variance failed to show any difference between the mean oxygen consumption of the woodlice of either sex, for each dose ( $\delta$  VR = 2.6108,  $\eta$  VR = 0.3175, both NS).



Sex	Dose	Mean Pb ppm (+ SE) in individuals	Mean O <sub>2</sub> $\mu$ l/hr/mg Dry wt (+ SE) of individuals	Correlation Coefficient
♂	0.0	67.09 ( $\pm$ 24.12)	557.71 ( $\pm$ 56.63)	r = -0.1213 NS
	50.0	83.55 ( $\pm$ 45.51)	571.45 ( $\pm$ 46.85)	
	500.0	84.24 ( $\pm$ 6.66)	457.01 ( $\pm$ 52.26)	
♀	0.0	9.78 ( $\pm$ 5.40)	672.26 ( $\pm$ 89.20)	r = 0.1254 NS
	50.0	16.52 ( $\pm$ 5.63)	604.64 ( $\pm$ 67.93)	
	500.0	44.71 ( $\pm$ 7.69)	683.18 ( $\pm$ 79.11)	

Table 19

Oxygen consumption data for P. scaber following dosing. Regressions for both sexes of oxygen consumed against Pb level are shown, and are not significant.

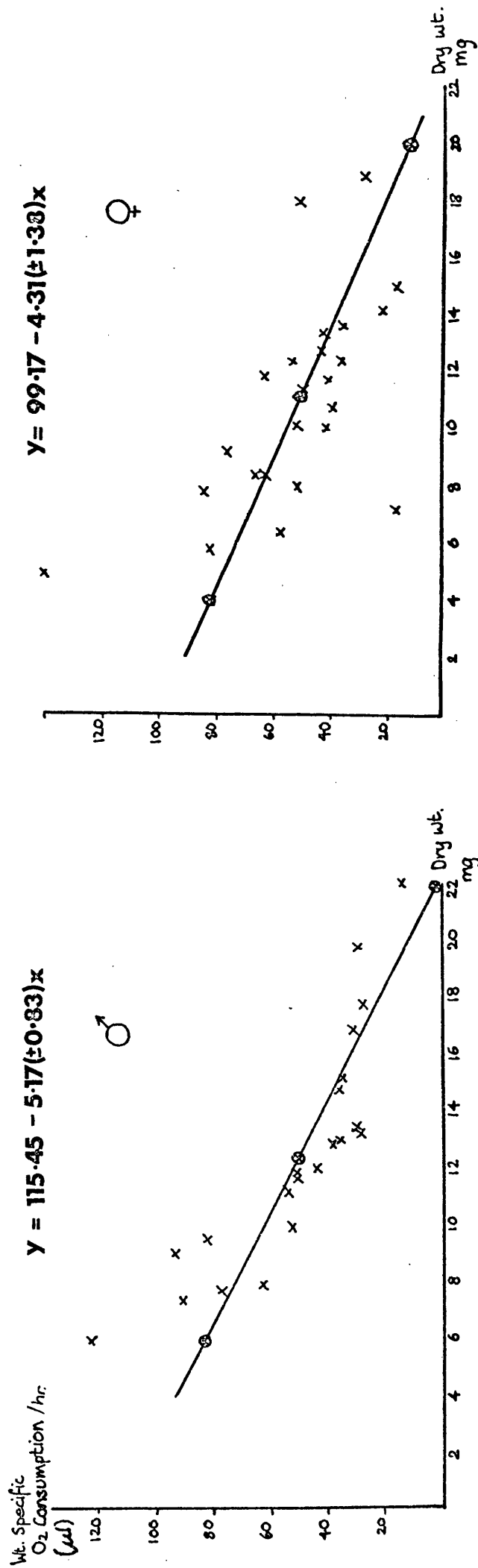


Fig. 44

Regressions of weight-specific oxygen consumption against dry weight for each sex of P. scaber. The two regressions are not significantly different.

No relationship was found between Pb level and rate of oxygen consumption of either sex (Table 19). This suggests that at the doses applied, the Pb content of P. scaber does not affect the animal's oxygen consumption.

An earlier experiment, conducted on Oniscus asellus (using grass dosed in the lead dosing chamber - p.12) also tested the effect of Pb on the oxygen consumption of woodlice. The mean results for males kept at 20°C are shown in Table 20. Again, no effect of Pb on the oxygen consumption of the woodlice can be found. The oxygen consumption of a large number of O. asellus were measured at this time (about 42), but the analysis of their Pb content was of rather dubious value. These were amongst the first animals to be analysed using flameless atomisation, after the installation of the carbon rod furnace, and there must remain some doubt over the accuracy of the results. The oxygen consumption, per unit dry weight, was relatively constant amongst all individuals of this species, and it is unlikely that the small variability can be attributed to Pb.

All the results follow a relatively short period on the dosed food, and these cannot be readily extrapolated to the long term. A longer dosing period is, however, likely to have more individuals moulting, reducing their feeding (and therefore their Pb consumption also) for several days. The net effect would be an even less homogeneous culture for each dose.

Moulting took place in four animals of each sex (Table 21) but because of the small number involved, it is not possible to test whether the Pb levels of these animals are significantly different from those non-moulted animals of the same dose. Numerically, at least, these levels are not greatly different, though more results would be required to confirm any change in the Pb content of the woodlice with the moult.

Pb Dose ppm	Mean Pb ppm Individuals	Mean O <sub>2</sub> /h/g dry wt. μl.
0.0	0.0	448.20 ± 96.78
25.0	108.2 ± 39.7	525.99 ± 68.35
160.0	127.1 ± 42.4	494.28 ± 44.78

Table 20

The mean levels of Pb and the oxygen consumption of males of Oniscus asellus, kept at 20°C. Each dose comprised fourteen animals, but the estimation of their Pb levels has to be treated tentatively (see text).

Sex	Dose	Pb ppm Moult Individuals	Mean (and SE) Pb ppm Non-moult Individuals
♂	0.0	59.32	67.87 ( $\pm 24.19$ )
	50.0	10.31 16.03	83.55 ( $\pm 45.51$ )
	500.0	96.82	84.24 ( $\pm 6.61$ )
♀	0.0	-----	9.78 ( $\pm 5.40$ )
	50.0	0.0 29.27 19.23	16.52 ( $\pm 5.63$ )
	500.0	88.61	44.78 ( $\pm 7.69$ )

Table 21

Levels of Pb in moult and non moult individuals of P. scaber, following a period on the doses shown. Four animals of each sex moulted, while there are ten replicates for each of the non-moult treatments.

#### 4.6 The effects of lead on the fecundity of *P. scaber*

One of the most direct ways in which Pb could affect woodlice numbers on the verge would be to impair their fecundity. This might happen in two ways: by reducing the number of live offspring produced, or, since woodlice retain their young in a brood-pouch, it may prolong 'gestation'.

All woodlice carry live young in a brood-pouch between the legs of the middle region of the body. Following copulation, a moult takes place in the female, producing the pouch as a series of extended oostegites (that overlap, but are not sealed) into which the eggs are shed (Sutton, 1972). Sutton notes that the pouch is fluid-filled, and he cites the work of Anderson (unpublished data) on the embryonic development of *P. scaber*. At 21°C, development takes about 30 days from oviposition to the young emerging from the pouch. In the present experiment, the animals were kept at 18°C and development is therefore likely to have taken longer.

Gravid females were identified by the presence of the brood-pouch, and were immediately presented with a Pb-dosed food. They were inspected every day, and the number of days before giving 'birth' was recorded from the time the brood-pouch was noted. Thus the number of days on the food varied from animal to animal.

The food consisted of grass (*Dactylis glomerata* - cf. Methods IV.ii) dosed with 100cc of either 1mM or 10mM  $\text{Pb}(\text{NO}_3)_2$  ('low' and 'high' load respectively) or deionised water (control). This produced levels of 2911ppm, 16483ppm and 0ppm respectively, and thus subjected the woodlice to extremely high levels of Pb. This was the first dosing technique to be used, and preceded the development of the potato dosing technique.

D. glomerata was also taken from two verge sites (1 and 5 - 'high' and 'low' verge) which had levels of 407 and 110 ppm, and was fed to the woodlice in the same manner as before. All grass was presented to the females in bundles of known dry weight. Both the artificially dosed grass and that taken from the verge enabled a wide range of doses to be given to the woodlice.

Only 5 animals could be fed on either the 'high' or 'low' verge doses as reproductive activity was ceasing in the culture, and no more gravid females were available. Because of these small numbers, no proper regression analysis could be carried out and so these results were amalgamated with those from the woodlice fed on the artificially loaded food. The former animals were presented with their dose towards the end of their 'gestation' period (unknowingly), and therefore had less time to feed. Part of the variability of the following results can be attributed to these animals.

Faeces were removed every day (to prevent coprophagy). When the young emerged, they were counted, and both parent and offspring were transferred to Pb-free carrot, until red faeces were produced. They were then killed and analysed for Pb and Ca in the normal way. The brood was analysed as a single extraction, as it was impossible to accurately analyse single individuals of such small weight.

Parents that died during 'gestation' were dissected and the eggs or embryos removed. The gut was also taken from the mother, to prevent its contents enhancing the Pb assay of the tissues. Mortalities were high amongst these females, but this could not be directly related to their Pb level (Table 22); it is more likely that they died from dessication (probably by excessive water loss from the brood-pouch). These deaths were termed 'unsuccesful pregnancies', and the results

Dose	No. Animals	Mean Fresh Wt. (mg)		Mean Dry Wt. (mg)		Mean No. Days on Load	Mean Grass consumed (mg)	
		Adult	Young	Adult	Young			
Control	7	41.21±6.12	10.73±2.65	12.05±1.52	2.36±0.57	15±3.58	13.02±3.69	(Successful)
High	7	42.10±8.27	5.35±1.17	12.05±1.40	2.41±0.47	15.28±2.98	11.28±2.59	( " )
Low	10	31.81±2.33	8.69±1.41	7.91±0.46	2.52±0.39	16.00±2.73	26.60±4.31	( " )
High Verge	5	45.22±5.64	7.08±2.19	9.86±0.68	2.50±0.58	7.80±0.37	5.42±1.20	( " )
Low Verge	5	34.52±4.57	2.58±0.72	7.96±1.00	0.90±0.16	8.00±0.31	7.58±1.34	( " )
Control	8	21.56±3.52	8.88±1.22	10.42±1.21	2.75±0.50	19.65±2.15	11.37±1.92	(unsuccessful)
High	8	21.86±2.87	13.53±2.86	8.78±0.94	2.80±0.54	20.25±1.60	13.47±1.17	( " )
Low	5	36.48±11.11	13.04±1.21	12.70±2.52	2.04±0.29	15.80±2.24	15.80±2.24	( " )

Table 22  
Fecundity data for *Porcellio scaber*  
on various loaded food.



Dose	Mean Grass consumed/Day mg	Mean Grass/Day/dry wt. mg	Mean Total Pb consumed ( $\mu\text{g}$ )	Mean Total Pb Burden (Adult + Young) ( $\mu\text{g}$ )
Control	$0.88 \pm 0.11$	$3.50 \pm 1.06$	$0.78 \pm 0.22$	$0.034 \pm 0.17$ (Successful)
High	$0.66 \pm 0.06$	$4.62 \pm 1.11$	$185.71 \pm 42.08$	$1.153 \pm 0.47$ ( " )
Low	$0.51 \pm 0.05$	$6.93 \pm 1.08$	$23.98 \pm 4.59$	$0.719 \pm 0.19$ ( " )
High Verge	$0.71 \pm 0.18$	$5.48 \pm 1.02$	$2.192 \pm 0.48$	$0.125 \pm 0.08$ ( " )
Low Verge	$0.95 \pm 0.16$	$9.69 \pm 1.49$	$0.83 \pm 0.14$	$0.045 \pm 0.03$ ( " )
Control	$0.68 \pm 0.10$	$1.70 \pm 0.32$	$0.66 \pm 0.12$	$0.054 \pm 0.02$ (Unsuccessful)
High	$0.67 \pm 0.04$	$1.80 \pm 0.19$	$221.7 \pm 19.55$	$2.212 \pm 0.55$ ( " )
Low	$0.90 \pm 0.02$	$1.68 \pm 0.26$	$37.60 \pm 3.76$	$2.092 \pm 0.75$ ( " )

Table 22

Fecundity data for Porcellio scaber  
on various loaded foods.

Dose	Mean No. Young	Mean Ca (mg)		Mean Pb (µg)		Mean Ca % Dry Wt.		Mean Pb ppm	
		Adult	Young	Adult	Young	Adult	Young	Adult	Young
Control	25±6.83	1.04±0.14	0.33±0.07	0.034±0.01	0.0	8.75±1.55	14.00±1.4	4.15±2.7	0.0
High	19.14±4.4	1.04±0.17	0.24±0.06	1.01±0.47	0.14±0.1	9.10±1.13	10.57±1.36	74.83±26.4	48.46±29.6
Low	26.60±4.3	0.76±0.04	0.29±0.04	0.66±0.17	0.05±0.02	10.07±0.3	12.14±0.71	90.79±22.9	19.41±10.3
High Verge	18.40±5.38	0.74±0.05	0.29±0.04	0.05±0.02	0.06±0.05	7.45±0.3	12.70±2.07	5.77±3.7	2.22±2.22
Low Verge	9.4±2.35	0.74±0.07	0.11±0.02	0.01±0.01	0.04±0.02	9.31±0.5	11.10±2.02	1.06±1.0	38.32±35.1
Control	19.65±2.15	1.16±0.10	0.13±0.05	0.03±0.01	0.03±0.02	11.49±0.6	4.99±1.39	1.82±0.8	8.66±8.66
High	30.12±2.36	0.93±0.17	0.17±0.05	1.99±0.41	0.22±0.14	11.14±0.92	5.53±1.62	221.6±37.5	109.5±82.4
Low	41.60±2.71	1.54±0.34	0.10±0.05	1.05±0.34	0.99±0.78	11.85±0.47	3.87±1.56	91.08±25	540.6±388.9

Table 22.

Fecundity data for Porcellio scaber

on various loaded foods. The upper doses are the successful pregnancies, the lower the unsuccessful.

	$x_1$	$x_2$	$y$	$r_{y1.2}$	$r_{y2.1}$	$R_{y.12}$
i.	No. days	Adult Dry wt.	Grass cons.	0.1299	-0.6341***	0.6423**
	Adult Dry wt	Total Ca	Grass cons.	-0.7245***	0.1943	0.7650**
ii.	No. days	Adult Ca	Adult Pb	-0.2113	-0.7589***	0.8231**
	Adult Dry wt	Total Ca	Adult Pb	-0.8027***	0.4184**	0.8028**
	Adult Dry wt	Total Ca	Total Pb	-0.7560***	0.3000	0.7795**
	Adult Dry wt	Grass cons.	Total Pb	-0.2566	-0.1427	0.3803
iii.	No. days	No. young	Young Pb	-0.6345***	-0.6346***	0.7600**
iv.	Adult Dry wt	Grass cons.	Total Ca	0.1943	-0.7241***	0.7650**
	Adult Dry wt	No. days	Total Ca	0.5183**	-0.8241***	0.8275**
v.	Adult Ca	Adult Pb	Young Ca	-0.4719**	-0.1543	0.4875**
	Adult Ca	Total Pb	Young Ca	-0.8072***	-0.8038***	0.8114**
vi.	Adult Dry wt	Total Ca	No. young	-0.8023***	0.4749**	0.8162**
	No. days	Grass cons.	No. young	-0.4480**	-0.2321	0.6592**
	Total Ca	Total Pb	No. young	-0.4182*	-0.5083**	0.7218**

Table 24

The partial correlation coefficients of the significant regressions from the fecundity experiment. For explanation see text. Standard probability notation.

Adult Pb (ppm)		Young Pb (ppm)		Young Ca (% Dwt)		Adult Dry wt. (mg)		Grass consum. (mg)		Total Pb (ug)	
Young Pb	t=3.0996***	Young Pb	Adult Ca (% Dwt)	Young Ca (% Dwt)	No. Days	Adult Dry wt. (mg)	No. Young	Grass consum. (mg)	Total Pb (ug)		
Adult Ca	0.4681*	0.0919	Adult Ca (% Dwt)								
Young Ca	0.0050	0.0527	0.1387								
No. Days	0.8139***	0.5412**	0.4895*	-0.0301							
Adult Dry wt	-0.0083	-0.1747	----	-0.1064	0.1321						
No. Young	0.4249*	0.0138	0.2776	0.3302	0.5410**	0.2509					
Grass consum.	0.3400	0.0189	0.3063	0.1129	0.6344***	0.3562*	0.6340***				
Total Pb	-----	-----	0.1849	-0.9204***	0.5619***	0.2905	0.4897**	0.4890**			
Total Ca	0.3519*	-0.1095	-----	-----	0.5515**	0.7543***	0.6476***	0.6067***	0.5950***		

Table 23

Matrix of correlation coefficients for linear regressions of the fecundity data (except for Adult Pb - Young Pb which is a t test of the 'tau' statistic). No regressions have been performed where the x variable may not be independent. All significant correlations are denoted by asterisks, using standard probability notation.

treated separately. The mean results for each treatment are given in Table 22. The complete results for each animal are also given in Appendix III. All data was analysed initially with linear regression and rank correlation (Kendall's 'tau' test: Kendall, 1970). A matrix of all possible correlation coefficients between the nine parameters measured in the 'successful pregnancies' are given in Table 23. Where the x variable may not be independent of the y variable, no regression has been performed. From these results, the significant coefficients have been further analysed using partial correlation (Steel and Torrie, 1960). This gives the expected correlation between two variables ( $x_1$  and  $y$ ) when a third variable ( $x_2$ ) is held constant ( $r_{y1.2}$ ), and between  $x_2$  and  $y$  when  $x_1$  is fixed ( $r_{y2.1}$ ). A third coefficient is also derived which gives the correlation between observed  $y$ 's and their expected value ( $\hat{y}$ ) given by the regressions ( $R_{y.12}$ ). This gives a measure of the effects of  $x_1$  and  $x_2$  on the variability of  $y$ . Thus, these three coefficients isolate the significant correlations between the factors examined. The results are given in Table 24.

#### i. Grass consumed

The amount of food consumed by the females is negatively correlated with the number of days feeding when the dry weight is held constant (Table 24). Since the grass consumed is shown to be related to adult dry weight (Table 23), the former correlation reflects the effect of the number of days feeding on total consumption. There is a highly significant correlation between observed and expected grass consumption, suggesting that both adult dry weight and the number of days feeding (which are held constant) are the main factors governing the total grass consumption.

## ii. Adult Pb and total Pb

Adult Pb is inversely correlated with the number of days feeding, when adult Ca is fixed. Since adult Ca levels are closely associated with adult dry weight (cf. section 4.2), this probably reflects a relationship between adult Pb levels and the rate of food consumption. This is further demonstrated by the positive correlation shown for adult dry weight and adult Pb, when adult Ca is held constant. The expected adult Pb levels have a good correlation with the observed values when adult dry weight, adult Ca, total Ca and the number of days feeding all remain constant. This would imply that adult Pb levels are related to the number of days feeding on the food and the Ca levels of the gravid female (which are closely related to dry weight).

The total Pb burden (parent + young) is similarly related to adult dry weight and total Ca (Table 24).

## iii. Young Pb

The Pb level of the young is significantly correlated with the number of days feeding by the parent, when the actual number of young is fixed. This may be a reflection of the rate at which Pb is passed on to the brood-pouch by the mother, and this is supported by the simple correlation between grass consumed and young Pb level (Table 23).

The young Pb level is also correlated with the adult Pb burden by a rank correlation test ('tau' = 0.0771,  $t = 3.0996$ ,  $P < 0.01$ ). This would suggest that the Pb assimilated by the young is not a direct function of the parental Pb level, but rather that the tendency to pass Pb on to the brood-pouch increases as the adult Pb content increases.

## iv. Adult Ca and total Ca

Total Ca (parent + young) is inversely correlated with

adult dry weight, both when grass consumed and the number of days is fixed. This was to be expected from the results of section 4.2, since the contribution of the adult to total Ca is by far the major proportion.

Total Ca is positively correlated with total Pb, and again, this is simply a product of the adult Pb and Ca relationship (Table 23). This is the first time adult Pb and Ca have been significantly correlated in the females, and is probably the result of active uptake of Ca during 'gestation'. At this time, Ca is likely to be required for the production of eggs, and may be possibly added to the fluid in the brood-pouch.

#### v. Young Ca

The Ca levels of the young can only be significantly correlated with the adult Ca content if total Pb is held constant (Table 24). This is a product of the highly significant, but negative correlation between total Pb and young Ca levels (Table 23). Further, total Pb is shown to be significantly related to adult Ca, if the Ca levels of the young are fixed. This suggests that both adult Ca and total Pb are the main factors determining young Ca levels.

#### vi. Number of young

The adult dry weight has a significant relationship to the number of live young, with a constant total Ca level. The expected number of young is also correlated with the observed number with a fixed adult dry weight and total Ca level. This is also true with a constant number of days and amount of grass consumed, and with total Ca and total Pb levels. Thus, the size of the brood is probably determined by the size of the parent and the length of the 'gestation' period. The apparent correlation between brood-size and total Ca is probably a result of the close association of these parameters with the dry

weight of the parent. Certainly, there is no indication that there is any decline in the number of young with increasing Pb content of the adult or the young, or in total levels.

From the results of the 'successful pregnancies', it is therefore concluded that:

(a) the Pb levels assimilated by P. scaber in this experiment do not affect 'gestation'.

(b) the Pb level of the gravid female is related to the animal's Ca level and the number of days feeding on the dosed food. This may result from the active uptake of Ca during the 'gestation' period.

(c) the young Pb level is not directly related to parental Pb, though the higher the adult Pb content, the greater the tendency for Pb to be passed on to the young.

(d) total Pb (parent + young) appears to be closely related to the Ca levels of the adult and the brood, and this factor may determine the amounts of Ca assimilated by the young during 'gestation'.

The results from the 'unsuccessful pregnancies' are less clear, and it is not surprising that certain relationships mentioned with the 'successful pregnancies' will not hold good.

Grass consumed ( $y$ ) is a function of the number of days feeding ( $x_1$ ) and the dry weight of the parent ( $x_2$ ) (multiple regression,  $VR = 6.347$ ,  $P < 0.05$ ). The Pb level of the offspring can again be related to the parental Pb by rank correlation ('tau' = 0.0503,  $t = 2.469$ ,  $P < 0.05$ ), and total Pb correlates with total Ca ( $r = 0.421$ ,  $P < 0.05$ ). Adult Pb however, is not correlated with adult Ca in these animals, though it can be related to grass consumed ( $r = 0.462$ ,  $P < 0.05$ ).

Since many of these deaths were probably due to



dessication, it follows that the animal was under physiological stress. Under these conditions, it is not surprising that the metallic ion balance does not correspond to that in a healthy female.

Finally, it is notable that the extremely high levels of Pb consumed by the woodlice (Table 22) could not be related to either parent mortality or the success of the 'pregnancy'. In the short term at least, gravid females appear unaffected by such high levels.

#### 4.7 Discussion

With any toxicological experiment on animals, it is not sufficient to dose the subject with the poison until it dies. This is particularly true of Pb - it would be very surprising if Pb did not kill woodlice, if given in sufficient quantities. Even so, 16500 ppm of Pb in the diet of P. scaber, over a period of 3 - 4 weeks ( and during 'gestation', potentially a more vulnerable time) could not be shown to be responsible for any increase in mortality. However, rather than finding the level most toxic to the animal, it is more informative to note the possible effects of the levels most commonly encountered in woodlice on their basic functions. In the case of P. scaber, these sub-lethal doses appear to have few deleterious consequences.

Most notably, there has been shown to be an association between Pb and Ca uptake in P. scaber, which also applies to the adult and young during 'gestation'. Generally, a high rate of Ca uptake is associated with high Pb uptake, and it has been found that a high Pb content of the diet increases Ca uptake. Ireland (1975) has suggested that high Ca uptake in such circumstances may be an attempt to counteract the toxic effects

of Pb, at least in earthworms.

Two possible routes for the passage of Pb through the woodlice have been suggested. Firstly, most of the Pb may remain in the gut and be absorbed selectively, probably with Ca (and according to Ca demand). Secondly, it may be absorbed at a relatively high rate by the caecal cells, again possibly with Ca, but lost to the hind-gut with the caecal secretions. Certainly, only a small proportion of the Pb ingested is actually assimilated (normally less than 5%), though there appear to be sexual differences in the rate of uptake of Pb and Ca. This is probably a function of the greater weight of the females - metabolic activity has been shown to decline in proportion to the dry weight of the individual, and this might mean a slower rate of turnover of both Pb and Ca in the females.

The low rates of assimilation undoubtedly contribute to the apparent ineffectiveness in disrupting the functions examined. Neither the respiration rate or the fecundity of P. scaber could be shown to be disrupted by the Pb levels attained in these experiments. Thus, despite the relatively high levels of Pb in woodlice compared to other roadside invertebrates, P. scaber does not seem to be as vulnerable to Pb as might initially appear.

## 5. Lead toxicology experiments on *Helix aspersa* Muller.

### 5.1 Introduction

Gastropods were one of the few groups of invertebrates found in large numbers in the pitfall traps of the major sites. The snails appeared to show a preference for areas of low pollution, while slugs were more evenly distributed. Snails have a greater Ca demand than do slugs because this metal is a major constituent of the shell (cf. Boycott, 1934). If Pb interferes with Ca metabolism, it is conceivable that snails would either be repelled or be killed by a diet rich in Pb.

Much of the previous work on the effects of Pb on molluscs has followed the concern for mercury levels in sea creatures used for food. Bivalves were of particular interest since they concentrated heavy metals from particulate matter (Brooks et al, 1965). Pringle et al (1968) showed that Pb was accumulated in the gills and were able to relate these levels to ambient temperature and the "physiological status" of the animal. Pb accumulation generally doubled as environmental levels doubled, though Oysters were observed to lose Pb in proportion to their "biochemical turnover". These authors also assayed the main tissues of several bivalves, providing some basis for comparison with terrestrial Gastropods.

Phillips (1976a) related Pb levels in the common mussel (*Mytilus edulis*) to the distance of the animal from the source of Pb input into a bay, and also described the environmental factors affecting uptake, essentially temperature and salinity. He also noted (Phillips, 1976a; 1976b) that Pb uptake is not affected by the presence of Zn, Cd or Cu, though Pb uptake did increase with lower salinity.

Jones (1940) in his faunal survey of the River Melindwr

contaminated with Pb from spoil-heaps, recorded the absence of all species of freshwater molluscs from the most highly polluted stretches of the river.

Little work has been reported on the effects of Pb on terrestrial molluscs. Williamson and Evans (1972) recorded high levels in snails from roadside verges in Co. Durham, while Coughtry and Martin (1977) analysed Helix aspersa for its capacity to accumulate Pb (amongst other heavy metals) originally derived from a smelter. Although the Zn, Cd and Cu of the wet tissues all increased in a linear fashion with ambient levels, no such reaction was found with Pb, though no attempt was made to partition the animal into its various organs, as was noted to have been necessary in the Oyster (Pringle et al, op. cit).

Although molluscs were common at all sites in the present study, no one species occurred in any large numbers. A survey of the Pb content of three taxa of gastropods collected from beneath the tiles of six sites (cf. section 3.5) is given in Table 25. Of the slugs, Arion spp. were the most common group and their Pb levels at each site roughly follow the same order for the respective vegetation samples (cf. section 2.5). Discus rotundatus was the most common snail, but this had much more variable Pb levels.

Attempts were made to demonstrate any preference by snails (using H. aspersa) for a low-Pb substrate. Filter papers were soaked in 10cc of solutions of various concentrations of  $Pb(NO_3)_2$  and used to simulate surfaces with different Pb levels in a sequence of preference tests. The results were inconclusive since it was found that the pH of the solution varied with the concentration of  $Pb(NO_3)_2$ . When acidic (at the lower concentrations) the snails reacted by exuding a frothing liquid and retreating into the shell. No

Species

Site	Arion spp.	Cepaea nemoralis	Discus rotundatus
1 } (2)	21.12	----	1.98
∞	35.73 ± 10.4	70.96*	20.21 ± 8.6
5 (5)	8.23 ± 7.5	----	0.775
A46 (3)	25.30 ± 0.9	----	----
A50 (1)	43.66 ± 1.5	----	----
A47 (4)	53.22	----	----

Table 25

Mean levels of Pb in three species of molluscs collected from six roadside verges, between April 1976 and September 1976. Where less than three individuals were collected no standard error is given. The sites have been ranked in order of greatest Pb contamination (values in brackets). \*Without shell.

such reaction occurred at the higher Pb levels. It was impossible to control these changes in pH without including other factors which would complicate the experiment further, though obviously, this is one possible cause of the observed distribution on the verges.

Since no one species occurred in sufficiently large numbers on the verges, H. aspersa were collected from a suburban garden and used to form a laboratory culture. As they were taken from a heavily-trafficked part of Leicester, they were likely to have measurable quantities of Pb in their tissues. H. aspersa is both herbivorous and detritivorous, and, since it is heavily shelled, requires Ca-rich food (Boycott, op. cit). Although it was never found on the verges, it is likely to be comparable to other species of snail found, particularly the larger varieties, such as Cepaea nemoralis. The culture was kept in a perspex aquarium with sterilised soil (about 20cm deep to facilitate egg-laying. cf. Methods III.ii).

## 5.2 The lead content of the adult tissues

The adult snails were fed on Pb-free cabbage leaves, and allowed to breed. After laying eggs (which were collected and used in a subsequent experiment), they were fed for a further month, before being induced to aestivate by placing the culture in a constant temperature of 4°C. This was to ensure a gut devoid of Pb, and by virtue of the reduced metabolic rate, minimise the mobility (and possible elimination) of Pb between tissues. After four months, ten animals were killed by placing in boiled, deionised water. They were then dissected into 7 component 'tissues': Digestive system, Reproductive system, Dart sac, Cerebral ganglia, Digestive gland, Shell and the remainder of the body (or 'Soma' - essentially the muscular tissues of the foot). These were then dried, weighed

and ashed in a muffle furnace at 500°C before extraction and analysis (cf. Methods V.iv). Interference, due to severe non-atomic absorption, meant that no reliable estimate of the Pb content of the shells could be made: levels of about 15 ppm were recorded in the shells, but this could not be successfully distinguished from the non-atomic background. As yet, there appears to be no simple solution to the problem. The Pb and Ca concentrations in the other tissues are shown in Table 26; the absolute amounts of each metal are also given for each tissue in the ten animals in Tables 27 and 28.

The large standard error terms attached to the mean Pb concentrations of each tissue (Table 26) are the result of high variability between individuals. The animals were induced to aestivate to ensure little loss of Pb from the tissues, but following dissection, it was noticeable that the gonads of some of the animals were more developed than others, suggesting that such individuals were close to 'awakening'. This is shown by the large standard error of the mean dry weight of the reproductive system (Table 26). If this does signify the end of aestivation, it may mean that increased physiological activity might make the Pb and Ca more mobile within the tissues, and thereby account for the high variability observed.

Significantly, the digestive system and digestive gland have the highest concentrations of Pb. Pringle et al (op. cit) obtained similar results from marine bivalves, and this would suggest that the food is the main source of Pb in both groups. A very large fraction of the total Pb burden is found in the digestive gland (Table 27), and this is probably a result of the cycles of digestion and absorption which take place within this organ (Purchon, 1968). The gonads have the next highest concentration of Pb (Table 26), and

Tissue	Dry Weight (mg) mean $\pm$ SE	Pb (ppm) mean $\pm$ SE	Ca (% Dry Wt.) mean $\pm$ SE
Soma	266.78 $\pm$ 31.02	11.43 $\pm$ 7.01	1.65 $\pm$ 0.13
Digestive system	34.03 $\pm$ 4.91	61.79 $\pm$ 56.86	0.69 $\pm$ 0.21
Reproductive system	134.84 $\pm$ 35.52	44.93 $\pm$ 42.24	0.83 $\pm$ 0.20
Cerebral ganglia	1.45 $\pm$ 0.12	16.89 $\pm$ 10.66	1.04 $\pm$ 0.29
Digestive gland	166.09 $\pm$ 11.43	90.47 $\pm$ 10.80	1.91 $\pm$ 0.35
Dart Sac	25.42 $\pm$ 2.23	2.62 $\pm$ 2.16	3.56 $\pm$ 0.63
Shell	921.72 $\pm$ 69.88	-----	12.30 $\pm$ 0.66

Table 26

Pb and Ca content of tissues of adult Helix aspersa.

Means of ten animals, dissected immediately after aestivation.



Animal No.	1	2	3	4	5	6	7	8	9	10	Mean $\pm$ SE
Soma	0.0	1.13	0.43	0.31	13.85	0.0	0.0	0.19	0.0	7.71	2.36 $\pm$ 1.47
Digestive system	0.0	0.47	0.0	0.0	0.0	0.33	0.0	0.03	0.41	----	0.15 $\pm$ 0.07
Reproductive system	0.92	0.0	0.96	0.91	0.20	----	0.42	0.0	0.0	0.0	0.37 $\pm$ 0.14
Cerebral ganglia	0.51	0.0	0.06	0.0	0.0	0.0	0.0	0.0	0.03	0.0	0.02 $\pm$ 0.02
Digestive gland	31.43	13.92	17.90	14.01	7.65	9.77	21.09	10.83	8.41	13.93	14.43 $\pm$ 2.10
Dart sac	0.0	0.0	0.0	0.03	0.0	0.0	0.06	0.0	0.0	0.22	0.03 $\pm$ 0.02
Shell*	---	---	---	---	---	---	---	---	---	---	
Total	32.86	15.52	19.35	15.26	21.70	10.10	21.57	11.05	8.85	21.86	

Table 27

Absolute amounts of Pb ( $\mu$ g) in various tissues of

H. aspersa. \*Shell estimates were not made (see text).

Animal No.	1	2	3	4	5	6	7	8	9	10	Mean $\pm$ SE
Soma	5.99	5.39	18.66	8.59	4.40	3.99	2.99	2.59	2.43	1.89	5.70 $\pm$ 1.57
Digest. system	0.26	0.19	0.04	3.09	0.19	0.21	0.09	0.24	0.08	0.19	0.46 $\pm$ 0.29
Reprod. system	1.94	0.24	1.49	0.93	0.94	0.63	0.36	1.46	0.67	0.65	0.87 $\pm$ 0.19
Cerebral ganglia	0.0009	0.0009	0.030	0.0008	0.020	0.023	0.010	0.006	0.034	0.010	0.014 $\pm$ 0.003
Digest. gland	1.62	1.47	1.26	2.34	3.19	2.67	5.39	2.99	5.39	2.92	2.93 $\pm$ 0.46
Dart sac	1.00	0.26	0.95	1.04	1.07	1.91	1.45	0.61	0.82	0.16	0.93 $\pm$ 0.16
Shell	152.39	77.49	113.79	110.79	118.19	204.99	60.99	72.80	137.99	105.59	115.52 $\pm$ 13.44
Total	163.20	85.04	136.22	126.78	128.00	214.42	71.28	80.69	147.47	111.41	

Table 28 Absolute amounts (mg) of

Ca in the tissues of adult Helix aspersa

this ranking of the organs is the same (ignoring the gills) as that found in marine bivalves (Pringle et al, op. cit).

Disregarding the shell, there is no simple correlation between the Pb and Ca content of the various organs: the dart sac has the highest Ca as a percentage of its dry weight, but the lowest concentration of Pb. Pb may be deposited in the shell, but without a more successful technique for measuring this, no correlation between total Ca and total Pb can be calculated.

Since these analyses followed the end of aestivation, these results represent the condition after a period of a reduced metabolic rate, and therefore are the proportions between the tissues when no Pb is being consumed or lost.

Boyden (1974) analysed whole bodies (including the shell, though the technique is not discussed) of several marine and estuarine molluscs and demonstrated a relationship between total Pb content and the dry weight, by means of a log.-log. regression. This could not be shown for the present data ( $r = 0.0192$  (log. data) NS;  $r = 0.0595$  (untransformed data) NS). However, Ca (mg) could be correlated with dry weight (g) ( $r = 0.7336$ ,  $P < 0.01$ , untransformed data). A partial correlation was used to examine any relationship between these three variables (cf. section 4.6), using untransformed data. The total body weight (including the shell) and the total Pb burden ( $\mu\text{g}$ ) could be significantly correlated if the Ca level is held constant ( $r_{y1.2} = -0.7378$ ,  $P < 0.01$ ). The closeness of the regression plane to the observed values for total Pb, when dry weight and total Ca is held constant, is highly significant ( $R_{y.12} = 0.7389$ ,  $P < 0.01$ ). This suggests that the combined effects of dry weight and Ca content are of importance in determining the Pb levels in H. aspersa.

Although there is no simple relationship between Pb and body weight in H. aspersa as described for marine bivalves and gastropods (Boyden, op. cit), total Pb burden appears to be a product both of the dry weight and the Ca level of the individual. This is evidence for a Pb/Ca interaction in snails, but this would have to be tested on animals where analysis of the shell was also possible.

### 5.3 The interaction of Pb and Ca uptake in young H. aspersa

The possibility of an interaction in the uptake of Pb and Ca was tested in young H. aspersa, grown from eggs laid by the adults in the soil of the laboratory culture. The eggs were collected and placed in a petri-dish with moist filter paper to hatch. They were then fed for two weeks on the flour from sieved rolled oats, before being randomly allocated, nine at a time, to a petri-dish containing a dosed food. All cultures had a total wet weight of around 330mg.

The doses were prepared with 1g of the sieved flour from the rolled oats, to which were added five weights of  $\text{PbCO}_3$  (0.0, 0.1, 0.2, 0.5 and 1.0g) and three of  $\text{CaCO}_3$  (0.0, 0.25 and 0.5g). producing all 15 possible combinations. Thus, each group of snails recieved a different weight of food, and these are shown in the table below (g):

	<u>Pb dose</u>				
<u>Ca dose</u>	0.0	0.1	0.2	0.5	1.0g
0.0	1.0	1.1	1.2	1.5	2.0
0.25	1.25	1.35	1.45	1.75	2.25
0.5	1.5	1.6	1.7	2.0	2.5

The oats in fact, had a low level of Pb prior to the dosing, and of course, also had a natural 'background' level of Ca. Thus, the actual amounts of Pb and Ca presented to the snails were somewhat larger than the stated dose. The

table below gives the corrected values for each metal. These have also been converted to the absolute amounts of Pb and Ca (rather than  $\text{PbCO}_3$  or  $\text{CaCO}_3$ ):

Pb dose

Wt. of $\text{PbCO}_3$ presented	0.0	0.1	0.2	0.5	1.0 g
Actual Pb level	0.14	77.7	155.3	387.9	775.8 mg

Ca dose

Wt. of $\text{CaCO}_3$ presented	0.0	0.25	0.5 g
Actual Ca level	1.03	101.14	201.26 mg

The snails were kept on dosed food for four weeks, after which they were starved for a further two weeks. They were then killed, dried, weighed and analysed (cf. Methods V.iii). The food remaining was dried and reweighed, and the amount that had been consumed was calculated (Table 29); this last was also expressed as a percentage of the original wet weight of each culture of snails. From the proportions of Pb and Ca in each dose, the total weight of each metal consumed was also derived, and these are given in Table 29.

The mean Ca and Pb levels for the snails of each treatment are given in Table 30. These results were highly variable and were therefore logarithmically transformed (to reduce within sample variability) prior to statistical analysis. An analysis of variance was performed on both the Pb and Ca levels, and the linear responses to the doses of each metal were extracted (Table 31). These data were also analysed using a curvilinear regression (untransformed data) by correlating the amount of each metal consumed for each treatment (x) with the level of that metal in the snails given that dose (y). The significance of these results were tested with an analysis of variance (Tables 32 and 33). Where the linear component of the analysis alone was significant,

Ca Dose	<u>Pb Dose</u>				
	0.0	0.1	0.2	0.5	1.0 g
0.0	0.76	0.65	0.62	0.70	0.67
0.25	0.85	0.64	0.72	0.65	0.65
0.5	0.91	0.72	0.75	0.83	0.68

A. Amounts of food consumed (g, dry weight) by nine juvenile Helix aspersa in each treatment, over a four week period.

Ca Dose	<u>Pb Dose</u>				
	0.0	0.1	0.2	0.5	1.0 g
0.0	206.8	176.1	164.6	170.6	154.1
0.25	227.1	177.8	182.6	146.6	159.4
0.5	242.1	201.6	193.3	230.7	184.9

B. Food consumed expressed as a percentage of the weight of the snails of each treatment.

Ca Dose	<u>Pb Dose</u>				
	0.0	0.1	0.2	0.5	1.0 g
0.0	0.1	45.9	80.2	181.0	260.0
0.25	0.1	36.8	77.1	141.1	224.0
0.5	0.1	35.0	68.5	161.0	211.0

C. Amounts of Pb consumed by the snails of each treatment. These values are calculated from the amounts of food consumed and the proportion of Pb in the food. (mg)

Ca Dose	<u>Pb Dose</u>				
	0.0	0.1	0.2	0.5	1.0 g
0.0	0.78	0.61	0.53	0.48	0.34
0.25	68.77	47.94	50.22	37.57	29.22
0.5	122.10	90.57	88.79	83.52	54.74

D. Amounts of Ca consumed by the snails of each treatment. (mg)

Table 29 Pb, Ca and food consumption data for juvenile Helix aspersa, on the various Pb and Ca doses shown.

Dose (g)		Ca % Dry wt.		Pb ppm		Dry weight	
Pb	Ca	mean $\pm$ SE		mean $\pm$ SE		mean $\pm$ SE	
0.0	0.0	9.97 $\pm$ 0.68		2549.43 $\pm$ 579.19		6.12 $\pm$ 0.43	
0.1	0.0	12.52 $\pm$ 0.68		5059.01 $\pm$ 368.43		4.82 $\pm$ 0.33	
0.2	0.0	10.97 $\pm$ 0.33		5551.13 $\pm$ 390.03		5.33 $\pm$ 0.30	
0.5	0.0	12.98 $\pm$ 0.56		11069.02 $\pm$ 1661.29		5.38 $\pm$ 0.38	
1.0	0.0	14.47 $\pm$ 0.54		24069.40 $\pm$ 8254.65		4.42 $\pm$ 0.23	
0.0	0.25	15.41 $\pm$ 0.81		66.29 $\pm$ 10.83		6.25 $\pm$ 0.44	
0.1	0.25	16.47 $\pm$ 2.05		7599.94 $\pm$ 1120.47		3.76 $\pm$ 0.36	
0.2	0.25	15.68 $\pm$ 1.40		8904.92 $\pm$ 2044.46		4.36 $\pm$ 0.44	
0.5	0.25	14.21 $\pm$ 1.36		11382.19 $\pm$ 1937.66		4.63 $\pm$ 0.53	
1.0	0.25	14.89 $\pm$ 1.26		18157.72 $\pm$ 4186.70		4.08 $\pm$ 0.49	
0.0	0.5	16.81 $\pm$ 0.57		22.14 $\pm$ 2.35		10.11 $\pm$ 1.07	
0.1	0.5	15.14 $\pm$ 1.07		5083.80 $\pm$ 684.41		4.83 $\pm$ 0.35	
0.2	0.5	12.87 $\pm$ 0.73		7702.40 $\pm$ 729.48		5.88 $\pm$ 0.51	
0.5	0.5	14.76 $\pm$ 1.27		15295.70 $\pm$ 7182.91		4.22 $\pm$ 0.26	
1.0	0.5	12.70 $\pm$ 0.74		19942.46 $\pm$ 5773.23		5.21 $\pm$ 0.34	

Table 30

Dry weight, Pb and Ca content of juvenile Helix aspersa following four weeks on the doses of Pb and Ca shown.

### Analyses of Variance

#### 1. Pb (ppm) - log. transformed

<u>Item</u>	<u>VR</u>
Pb'	1372.248***
Ca'	78.986***
Pb'Ca'	231.129***

#### 2. Ca (% Dry weight) - log. transformed.

<u>Item</u>	<u>VR</u>
Pb'	0.0132 NS
Ca'	16.6431***
Pb'Ca'	27.5636***

Table 31

Analysis of variance for the Pb and Ca content of young H. aspersa following a dosing period. Only the linear components and interactions were extracted (see text).



Curvilinear Regression

Linear Regression

Ca dose	Linear component		Quadratic component		Error		
	b <sub>1</sub>	SS	VR	b <sub>2</sub>	SS	VR	MS
0.0	-1.6229	2233396570	14.74***	0.2887	158130654	1.04NS	151512975
0.25	-138.26	1323384408	10.43**	0.8727	586345325	4.62*	126882247
0.5	113.06	2241793428	17.08***	-0.1101	9014518.4	0.07NS	134454819
				b	r	r <sup>2</sup>	
				74.62	0.505***	0.251	
				69.79	0.467**	0.218	
				89.46	0.533***	0.284	

Table 32

Curvilinear and linear regression analysis of Pb levels in young H. aspersa

following dosing with five levels of Pb and three doses of Ca. Each of the

Ca doses have been analysed separately by regressing the amount of Pb

consumed (x) against Pb level (y). The sum of squares for both the linear

and quadratic components of the curvilinear regression were extracted and

tested with a variance ratio test. Both had 1 df, and the error term 42.

The simple linear regression was tested with the r value, and the r<sup>2</sup> gives

a measure of the amount of variability in the Pb levels accounted for by

the linear component (42 df.).

Curvilinear Regression

Linear Regression\*

<u>Pb dose</u>	<u>Linear component</u>			<u>Quadratic component</u>			<u>Error</u>		<u>Linear Regression*</u>		
	<u>b<sub>1</sub></u>	<u>SS</u>	<u>VR</u>	<u>b<sub>2</sub></u>	<u>SS</u>	<u>VR</u>	<u>MS</u>		<u>b</u>	<u>r</u>	<u>r<sup>2</sup></u>
0.0	0.1135	151.78	69.63***	0.0004	14.55	3.86NS	3.6157		0.0615	0.844***	0.713
0.1	0.1422	34.45	1.65NS	-1.2332	37.12	1.78NS	20.8090		-----	-----	-----
0.2	-1.1433	23.21	2.92NS	-0.0019	80.21	16.09***	7.9543		-----	-----	-----
0.5	0.0387	12.15	1.05NS	-0.0002	0.85	0.74NS	11.5438		-----	-----	-----
1.0	-0.6974	0.03	0.01NS	0.0116	66.07	5.38*	12.2716		-----	-----	-----

Table 33

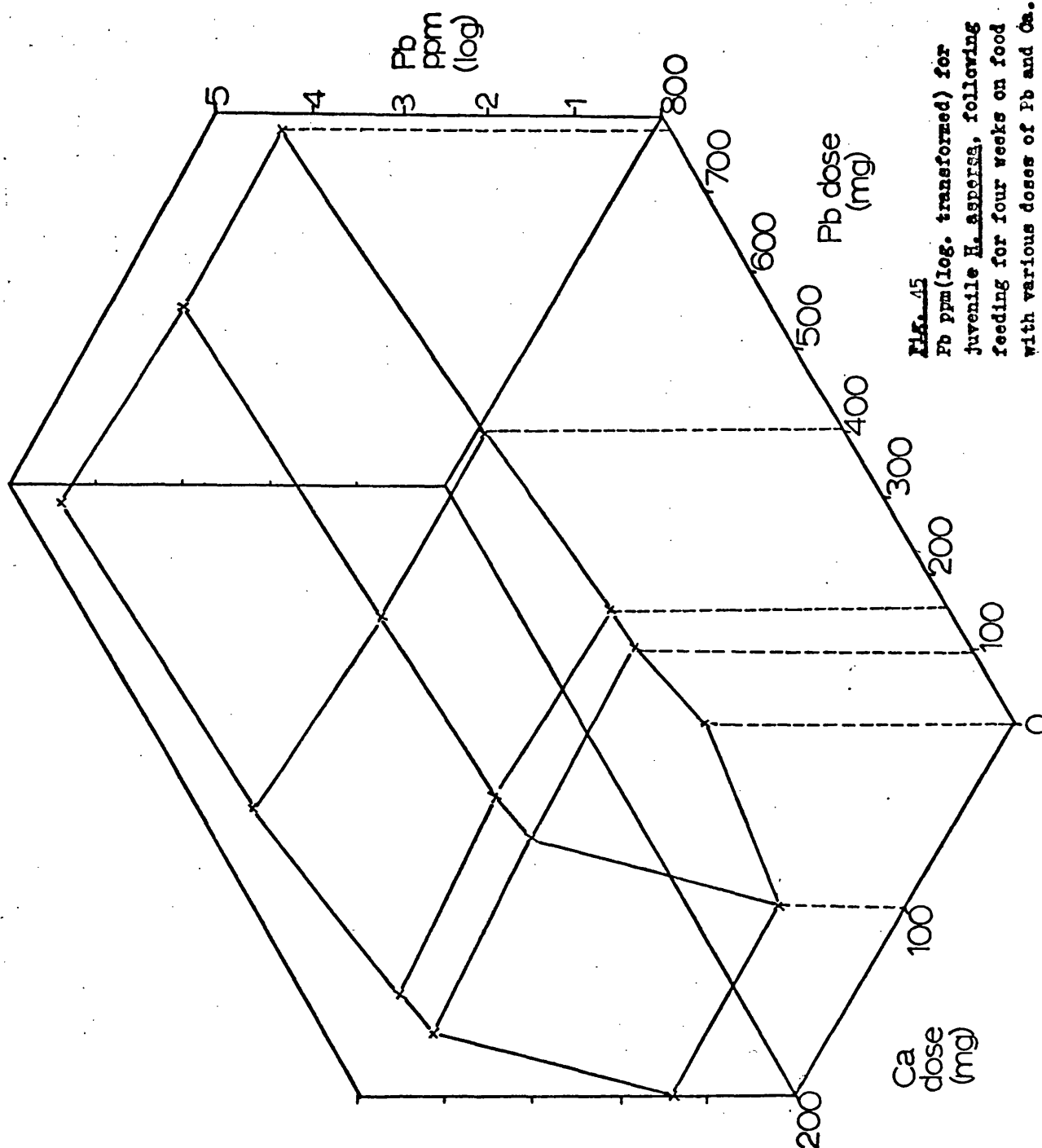
Curvilinear and simple regression analysis of the Ca levels in young H. aspersa, as in Table 32, except the amount of Ca consumed (x) has been regressed against Ca levels (y) for each dose.

\*The linear regression analysis only applies where the linear component of the curvilinear regression is found to be significant. In this case, this was only the 0.0 Pb dose. All regressions had 1 degree of freedom ( therefore SS = MS), and the error term of the curvilinear regression had 24 df.

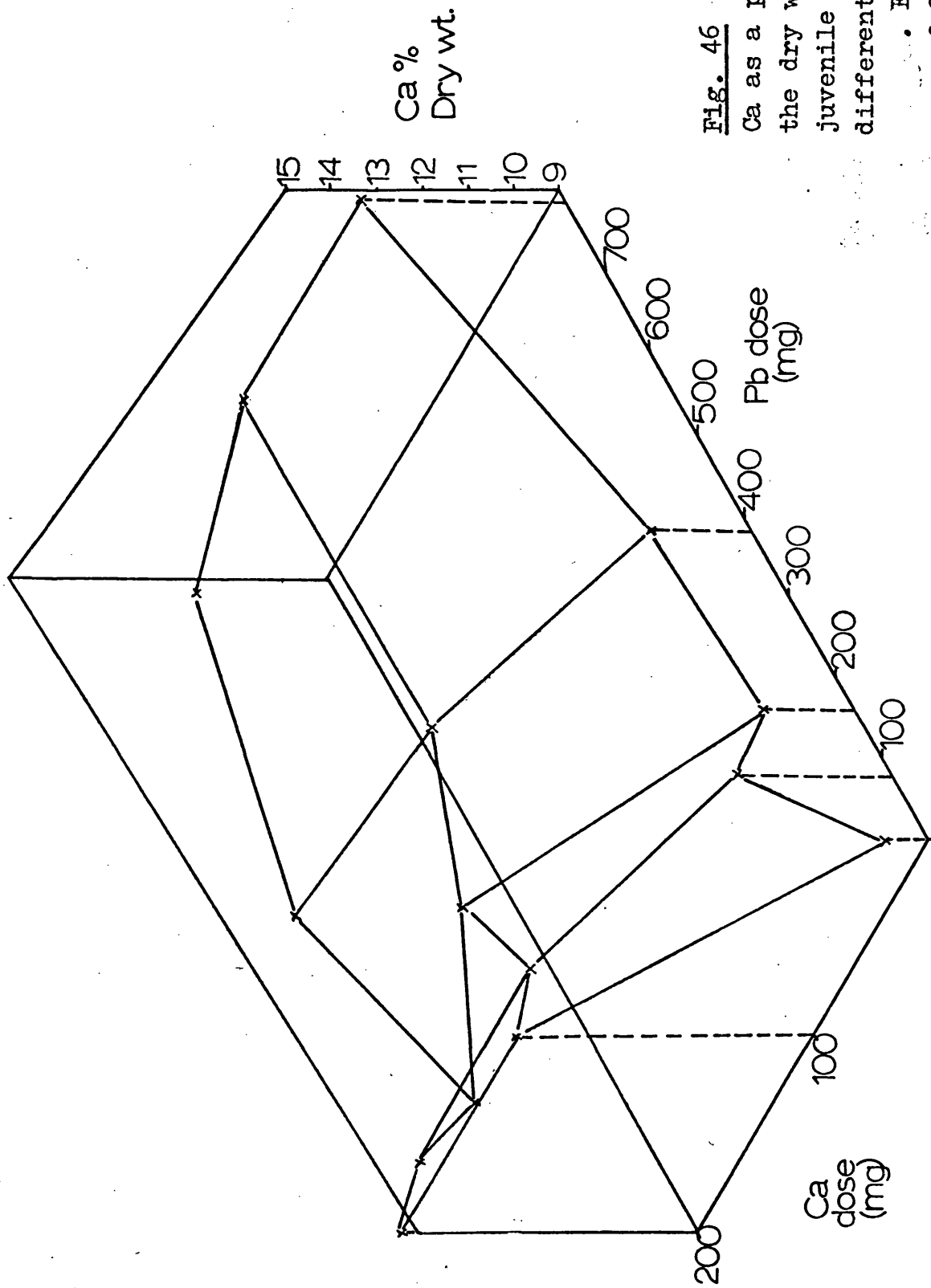
the data were further examined by a simple linear regression, and tested with a correlation coefficient (Tables 32 and 33). In this way, the  $r^2$  value gave a measure of the amount of variation accounted for by the linear regression alone. Because of the very high Pb levels attained by the snails, the mean of the log. values for each treatment have been plotted as a three-dimensional graph (Fig. 45). The Ca levels, the mean dry weight of each culture, and the amount of food consumed have been plotted as untransformed data (Fig. 46 - 48).

As a log. plot, the Pb levels are seen to reach a 'plateau' with the highest Pb doses, while at the lower doses, the Pb accumulation decreases with Ca dose. The analysis of variance shows a significant linear rise in Pb levels as a response to the Ca level of the food (Table 31), as well as to applied Pb. Notably, there is also an interaction whereby Pb levels rise linearly both with increasing Pb and Ca doses.

The curvilinear regression analysis of the Pb content of the snails (Table 32) shows that the significance of the linear or quadratic components varies according to Ca consumed, though there is only a slight quadratic response. Generally, only the linear component is significant, which, with the highest Ca dose, accounts for nearly 30% of the variability in the Pb levels. With lower Ca doses, this is reduced to around 20%, but is still significant. This would suggest that the Pb level of the snails is mainly increasing as a linear function of Pb consumed. In an attempt to estimate the effect of Ca dose on the snail Pb levels, the deviations of the actual Pb content from the expected levels given by the curvilinear regression ( $\hat{y}$ ) were further regressed against Ca consumed ( $x$ ), testing whether such variation was due to Ca. No significant correlation was

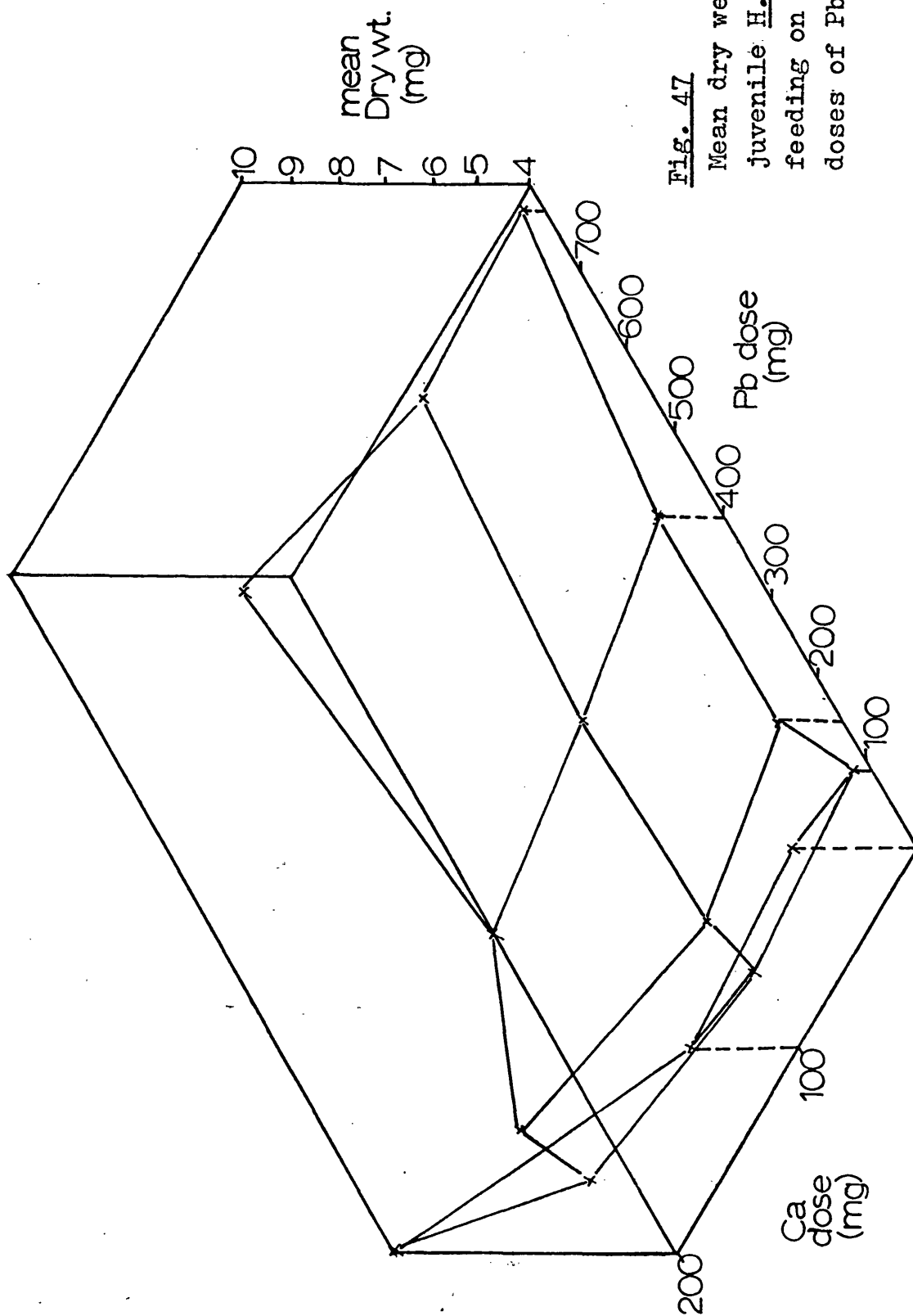


**Fig. 45**  
Pb ppm (log. transformed) for  
juvenile *H. aspersa*, following  
feeding for four weeks on food  
with various doses of Pb and Ca.  
Each value is the mean of nine  
animals.



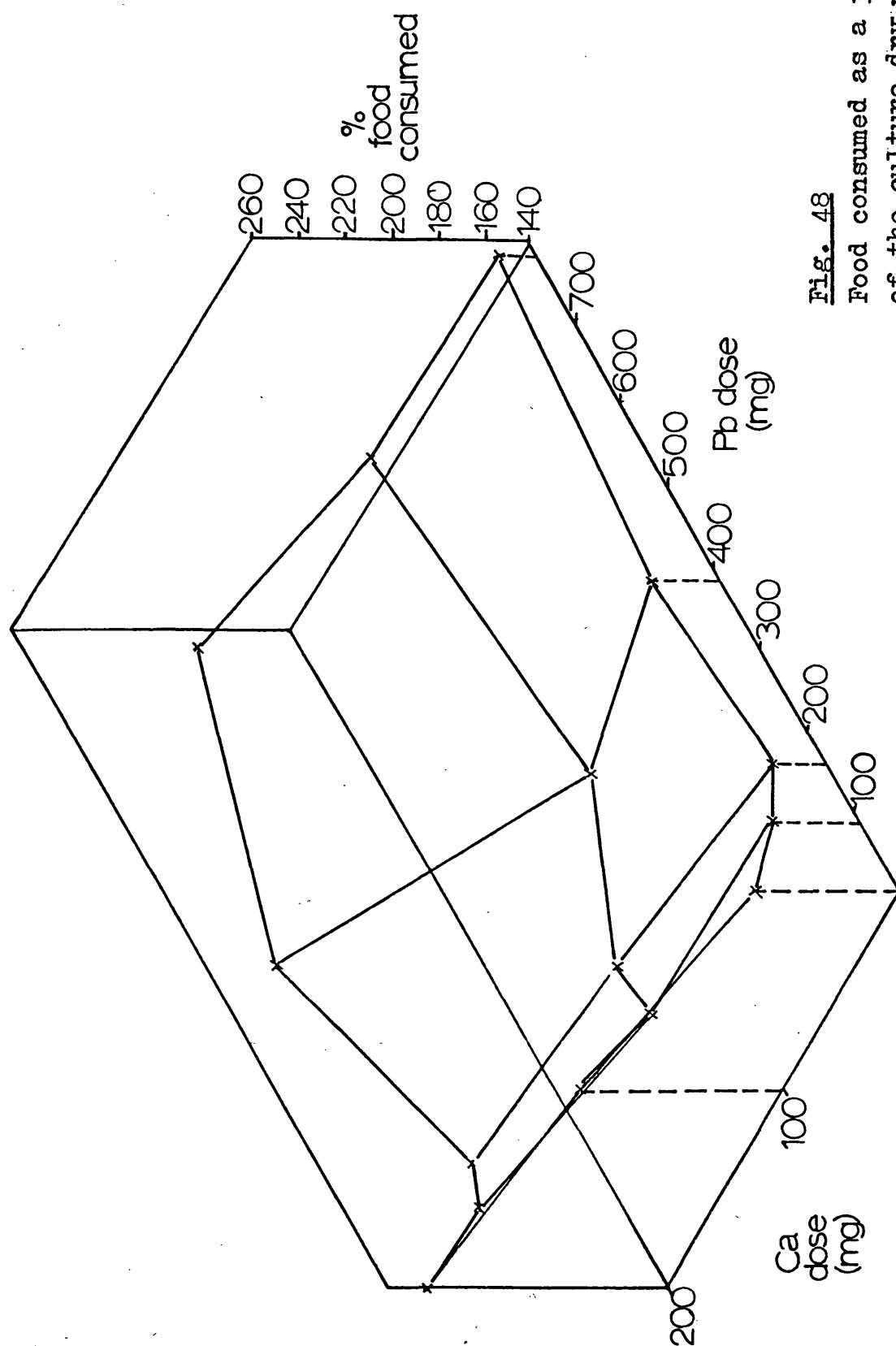
**Fig. 46**

Ca as a percentage of the dry weight of juvenile H. aspersa, at different doses of Pb. Each plot is the mean of 9 animals.



**Fig. 47**

Mean dry weight of 9 juvenile H. aspersa after feeding on different doses of Pb and Ca.



**Fig. 48**

Food consumed as a percentage of the culture dry weight (9 individuals), at different Pb and Ca doses, in young *H. aspersa*.

found ( $r = -0.1384$ , NS). The Ca consumption certainly affects Pb uptake, but over the range of doses applied, there appears to be no consistent relationship between the two.

The Pb content of the food does not directly affect Ca uptake, though this does increase in a linear fashion with applied Ca dose (Table 31, Fig. 46). There is, however, a significant interaction with linearly rising Ca levels as both Pb and Ca content of the food rises.

The curvilinear analysis of the Ca levels show that the most significant response to Ca consumed is only quadratic at the higher Pb doses (Table 33). Only when no Pb is applied is there a significant linear response: simple regression <sup>a</sup>analysis demonstrates that here the linear component accounts for 71% of the variability in the Ca levels of the snails. The fact that this <sup>does</sup> not occur in those animals dosed with Pb would suggest that the presence of Pb is disrupting Ca assimilation. The deviations from the expected Ca level ( $\hat{y}$ ) however, show no significant relationship to Pb consumed ( $r = 0.1262$ , NS).

The analyses of variance suggest that Pb uptake is affected by the Ca content of the food, though the results of the curvilinear regression do not show any simple relationship between the two. The relationship between Ca uptake and the Pb content of the food is also unclear, with Ca levels assimilated appearing to fall off with increasing Pb dose (Fig. 46). Nevertheless, they do rise in a linear fashion as a response to both the Pb and Ca doses increasing.

The food consumed (expressed as a percentage of the original wet weight of each group of snails - Fig. 48) generally rises with Ca dose and declines with Pb dose: the highest food consumption is when there is no applied Pb dose.



This pattern of food consumption is very similar to that produced by the mean dry weight for each group of snails (Fig. 47), and the two can be correlated by linear regression ( $r = 0.7558$ ,  $P < 0.001$ ), suggesting that dry weight is a product of food consumed. Thus, where no Pb is given, more food is consumed, more Ca assimilated and a greater dry weight results.

#### 5.4 Discussion

The proportion of Pb in the tissues of adult H. aspersa closely follows the results of Pringle et al (op. cit), suggesting that the mode of uptake in terrestrial gastropods is similar to that in marine bivalves. Whether Pb is lost in relation to the 'biochemical turnover' in H. aspersa as these authors report in the Oyster is not known, but the high levels of Pb in the digestive gland might suggest that Pb uptake is closely associated with the internal chemistry of the snail. This does not seem to be directly associated with the body weight of the adult snails, as has been reported for marine species, but it is probably a function of both body weight and the total Ca level of the snail. This might be clarified if effective measurements of the Pb content of the shell were possible.

The interaction between Pb and Ca in the juvenile snails is also confused, and there appears to be no simple relationship between the two. Pb uptake rises as the Ca content of the food increases, but Ca uptake cannot be directly related to the Pb content of the food. The presence of Pb in the food certainly reduces food consumption, and this, in turn, appears to reduce Ca assimilation. Ca is probably actively absorbed in these animals to reinforce the shell, and as with Porcellio scaber, there is some indication that Pb may be absorbed in association with Ca.

This absorption is likely to take place in the digestive gland, where the highest concentration of Pb was found in the adults.

As juvenile H. aspersa consume less food if it is contaminated with Pb, it may be that the snails on the verge are congregating in areas where the Pb content of their food is less. In this case, both herbivorous and detritivorous snails may be expected in their largest numbers at the back of the verge, or their range may be limited totally to sites with low traffic frequencies. Other factors would have to be taken into account to confirm this conclusively, most particularly the effects of different vegetation heights and the effects of road salt.

In the short period covered by the dosing experiment on the snails (6 weeks) the high levels of Pb assimilated did not appear to cause any increase in mortality. This does not necessarily apply to the long term and to a more continuous exposure, albeit with lower levels of Pb consumption. Nevertheless, there is evidence that Pb may slow their rate of growth, if only by reducing their food consumption.

## 6. Discussion

There are about 500,000 acres of verge in Britain (Way, 1967), which, with their associated roads, ramify many of the country's natural regions. For this reason, verges are of ecological interest in their own right, though owing to the variability of their biotic components, they are essentially defined by their morphology rather than their biology.

The verge is bordered on one side by the road and on the opposite side usually by a hedgerow and/or a ditch. In general, the verge itself is a fairly flat 'grassland' area, perhaps with occasional trees (Way, 1973). The road and hedge demarcate the verge 'unit' and to some degree, isolate it. The verge is floristically quite different from the surrounding land and very often more diverse: in comparison with most rural areas under cultivation, a greater variety of plants can be expected on adjacent verges, and Way (1970) has suggested that this may also be true of the fauna.

How clearly the biotic components of the verge habitat are isolated from those of the immediate vicinity depends on the effectiveness of the boundaries. Thus, a water-filled ditch and a perpetually busy road may well prevent large-scale movements of ground-living animals in and out of the system, though there is no apparent barrier to aerial movements of plants and animals. The verge may be therefore considered as a distinct habitat into which there are quantifiable inputs and outputs, both physical and biotic. Given the morphological distinction of the verge, these factors are potentially measurable and might allow a relatively complete description of the verge system.

Previous work has either treated the verge as an area with potential conservation value (Way, 1973), or as a zone

subject to the pollutants associated with the road. In this latter respect, most of the attention has centered on the Pb emitted by motor vehicles. Recently, the input of salt to the verge has also been considered (Davison, 1971), mainly with regard to <sup>its</sup> effects on plants. These two substances are generally regarded as the most important pollutants affecting verge ecology, though there has been little evaluation of other emissions, such as carbon monoxide, oil droplets and other metals. The present work has concentrated on Pb for two main reasons - it is known to impair many biological processes, and secondly, because it is one pollutant which may need to be regulated or even eliminated altogether.

Chow (1970) established that most of the Pb in roadside soil and vegetation originated from passing vehicles, and with other authors, (Daines et al, 1970; Hopkinson et al, 1972) showed that Pb apparently declined as an exponential function of distance from the road (Ward et al, 1975). Some workers have regarded the road as a line-source for Pb on the verge, with the absolute amount deposited being dependent on traffic frequency (Motto et al, 1970). Daines et al (op. cit) note that the amount of airborne Pb at the roadside was also a product of traffic frequency, and that this again declined in an exponential pattern (Ward et al, op. cit). This pattern was attributed to a particle-size distribution: between half and two-thirds of the Pb emitted by motor vehicles is less than 5 $\mu$ m in diameter (Hirschler and Gilbert, 1964) and is not thought to be removed by precipitation, remaining suspended as an aerosol (Hirschler et al, op. cit). The rest is of larger particles, and will fall out by gravity or be removed by precipitation (Hammond et al, 1972).

Even though Ter Haar et al (1967) and Atkins (1969) had shown that rainfall can increase the deposition of Pb, no work

has yet looked at its effects on Pb accumulation in roadside soil and vegetation. Wind movements have received more attention in this respect and Ward et al (1974) and Fidora (1972) had noted that prevailing wind direction could alter the pattern of Pb deposition on roadside vegetation. Daines et al (op. cit) attribute seasonal changes in the concentration of Pb in the air to variations in the degree of atmospheric mixing: the winter maximum observed is due to little airborne Pb being transported away from the site of its emission by the wind. This may have been responsible for the seasonal fluctuations in the Pb content of Avena fatua, recorded by Rains (1971), but although there is a general winter high and summer low, the author does not attribute this to climate. Mitchell and Reith (1966) report similar results for grass growing in areas remote from towns, though here the seasonal range is very low. These authors thought this was due to an increased mobility of Pb within the plant at senescence, but as Rains (op. cit) points out, there is little evidence of this from other work.

In the present study, most of the investigations into roadside Pb deposition has centered on a verge on the A46 dual-carriageway north of Leicester. This was created in 1968, as a result of modifications to the road, about 8 years prior to this study. In virtually all respects, this is a typical verge for a road of this type, and was selected for this reason. The present studies have revealed that the existing picture of Pb deposition and accumulation on verges has to be modified to take into account meteorological factors, and most especially, precipitation.

Pb deposition in dry conditions is probably very regular: in the very dry summer of 1976, the Pb content of the Hawthorn on the A46 rose in a uniform fashion (Fig. 24, section 2.4), which, in the absence of rainfall, could only reflect accretion

with standing time of the leaves. With increasing precipitation, Pb deposition increases, and this is a major contributory factor to the seasonal changes in the Pb content of roadside grasses.

The high winter Pb content of grass is a product both of the persistent wet weather and longer standing time of the grass (section 2.4). It was noted that the lower temperatures of this season reduce the rates of decay of dead grass and the production of live leaves (this is confirmed by Williamson (1976)), so that there is little turnover of either. In the spring, the higher temperatures bring about an increase in the growth of live grass and the decay of dead, producing a characteristic fall in Pb levels. These begin to rise again toward the autumn and winter as both standing time and precipitation increase. Within a year, this may produce ranges of 100 to 1000 ppm of Pb in both live and dead grass, at a  $\frac{1}{2}$ m from the road.

The absolute amount of Pb deposited per  $m^2$  of vegetation also has a large seasonal range - from 19.5mg in the summer to 280mg in the winter ( $\frac{1}{2}$ m distance). These values show that there is a real increase in the deposition of Pb in the winter, rather than just the effect of increased standing time of the grass. If the winter maxima in airborne Pb levels described by Daines et al (op. cit) for New Jersey, U.S.A. also applies to the present site, then there is a combined effect of increased precipitation and airborne Pb.

The similarity of these seasonal changes to those described by Rains (op. cit) and Mitchell and Reith (op. cit) suggest that there is a common cause. As at the present site, the areas studied by these authors (San Francisco and N.E. Scotland respectively) there is a winter high in rainfall, and the corresponding peak in Pb levels is probably attributable

to this. Since it is now generally accepted that Pb is not mobile within grasses (Höll and Hampp, 1972) the theory of Mitchell and Reith that Pb is translocated at senescence is not valid. These changes are a product of climatic conditions and also the death of the grass: the present work has demonstrated the significance of the state of the grass, showing that dead grass accumulates more Pb than live.

Previous work has failed to distinguish between live and dead and this makes the results of the deposition of Pb on verges difficult to interpret. No absolute measurement of Pb levels in roadside grass is possible unless this disparity between the binding capacities of the two states is taken into account. Since the proportions of live and dead grass change with season, this will contribute to the variability of an unsorted sample. Other workers have either collected at one time (Daines *et al*, *op. cit*) or have replicated the samples many times (Chow, *op. cit*): for this reason, the pattern of the distribution of Pb across the verge is the same in an unsorted sample as it is in live and dead grass.

Results from experiments using both roadside grass and artificially loaded Arrhenatherum elatius (section 2.2) suggest that most of the Pb is bound from solution, with only a small fraction held as surface particulate material. This component probably corresponds to the 10% insoluble particulate Pb in rainfall reported by Ter Haar *et al* (*op. cit*). Most workers accept the cell wall as the main site of Pb-binding in plants (Höll *et al*, *op. cit*) and thus, the real difference between live and dead grass is probably the differences in accessibility of the interior of the plant to Pb-rich solutions. The intact cuticle and epidermis of live grass prevents the solution infiltrating the intercellular spaces, whereas the surface of

dead grass is normally fractured and 'wetable'. The total binding capacity of the two states is probably very similar, but the fact that little or no Pb is translocated past the roots (Höll et al, op. cit; Arvik and Zimdahl, 1974; Jones and Clement, 1966) means that the condition of the cuticle is responsible for the actual differences between live and dead grass.

The failure of both Rains (op. cit) and Mitchell and Reith (op. cit) to distinguish between live and dead grass has no doubt contributed to their observed seasonal ranges: there is generally a predominance of dead grass during the winter. Both the increased standing time and precipitation of the winter would contribute to the greater deposition of Pb and which would be readily retained by the dead grass of this season. With the results of Mitchell and Reith (op. cit) the very small seasonal changes (generally less than 20 ppm) are almost certainly due to aerosol Pb being derived from a more trafficked area, rather than the region of sampling.

Precipitation has also been shown by the present work to play an important role in determining the distribution of Pb across the verge, and particularly in generating the typical 'exponential decline' described by other authors. With precipitation is included the spray generated by passing vehicles and which is splashed onto the verge. Here, Pb directly from the exhaust pipe is mixed with the spray produced during wet weather (which itself <sup>may</sup> contain Pb from the road surface), and is then deposited on the verge. The large input of Pb at the road margin compared to the rest of the verge (the 'exponential' decline) is partly a consequence of this spray. Certainly, more spray falls immediately next to the road in the zone of the most contaminated air (Daines



et al, op. cit), causing localised removal of Pb from the air at this distance. Pb deposits at the road margin may be as high as  $90\text{mg}/\text{m}^2/24\text{h}$ , yet virtually zero at the 7m distance, for the same period. This, combined with the gradation of Pb deposition in dry conditions (owing to particle diffusion in the air from the road) are probably the main causes of the pattern of contamination which has been observed so frequently. The Pb aerosol probably contributes little to this pattern, and, for the most part, is blown away from the verge, probably for considerable distances (Atkins, op. cit; Hirschler et al, op. cit).

The rate of Pb deposition also varies with the weather conditions and this may be a product of the 'spray' effect as well. Rates of deposition with melting snow over 24 hours were shown to be greater than intermittent rain for the same period - at the  $\frac{1}{2}\text{m}$  distance this was  $90\text{mg}/\text{m}^2/24\text{h}$  and  $6.6\text{mg}/\text{m}^2/24\text{h}$  respectively. This is most likely to be due to the snow continually wetting the road with meltwater, and over the year, is probably exceptional. The more persistent the spray, the greater the period in which Pb is being removed from the air. Ter Haar et al (op. cit) suggested that showers were the least effective 'type' of rainfall for depositing Pb, probably because of their duration and characteristically large droplet-size. This is further supported by results from the lead dosing chamber, which suggest that more Pb is deposited on grass if there is a fine 'drizzle' of water - exactly the conditions produced behind a moving vehicle. In this case, wetness of the road may be the main factor responsible for the correlation between Pb levels in 'live' and 'dead' unwashed grass and rainfall, rather than direct precipitation alone. To resolve whether splash or direct rainfall was the main factor affecting Pb deposition at the road margin, future

experiments might involve counting the number of days of wet roads, and relating this to the Pb content of the grass.

The pattern of Pb deposition on roadside vegetation is also affected by the aerodynamics of the verge. Measurements of air movements at various sites demonstrated a 'shadowing' effect of the hedgerow, creating a volume of 'stagnant' air around its base. At the road margin (under prevailing wind conditions) the air is most rapidly moved, both by natural forces and turbulence from passing vehicles. Pb-rich air would be quickly replenished at this distance with air from the road, helping to generate higher levels of Pb compared to that in the unmoving air at the back of the verge. It seems likely, therefore, that both the pattern of Pb in the air and the vegetation is affected by the aspect and morphology, and that this cannot be simply<sup>1</sup> attributed to a particle-size distribution. This is shown at sites with a different aspect to the prevailing wind direction, where this pattern is not produced. In these conditions, airborne Pb is probably blown directly away from the verge or at various angles from it, effectively destroying the exponential pattern in the air. From the resulting patterns produced in the vegetation of these sites, it would seem that both wet and dry deposition at the road margin is altered. If this is the case, the real difference between wet and dry deposition is simply one of degree.

Taken together, this work now presents a revised picture of the nature of Pb contamination of roadside vegetation. It appears that only particles greater than about 5µm in diameter (the non-aerosol component) are deposited on the verge, either by gravity or by precipitation and splash. The typical exponential decline of Pb in the vegetation is a product of both this spray and the particle-size distribution in the

air above the verge. This pattern is modified at sites not in the lee of the predominant winds. The verge morphology may also contribute to this, most particularly the hedgerow. Pb is probably bound in solution at the cell wall of the plants, and in this respect, the condition of the grass is important. The fracturing of the cuticle in dead grass may increase its binding capacity by over ten times compared to live. As the dead vegetation will eventually form the litter layer, so its exposure to Pb-rich solutions on the ground surface increases. Levels of up to 1600 ppm have been recorded in the litter. This is eventually incorporated into the soil, though it probably remains bound to organic material, or more specifically the clay-humus particles (Hildebrand and Blum, 1975; Olson and Skogerboe, 1975).

This means that little Pb is leached down the soil profile, remaining, as with other cations, in the upper layers. As Olson et al (op. cit) point out, most of the Pb salts occurring in soil are highly insoluble, and only in acidic conditions does any appreciable amount of Pb become soluble, though even at low pH's, this is less than other cations.

This retention of Pb in the upper layers of the soil led Goodman and Roberts (1971) to suggest that soils might be useful as integrators of heavy metal pollution. This technique was used in the present study, by estimating the total 'excess' Pb (Pb content above a background level - cf. section 2.2) in the soil of the A46 site, which had been undisturbed for 8 years. This assumes, of course, that the traffic frequency has remained relatively constant since the verge was made up, and that the net effects of climatic variations between years tended to cancel each other out. Several methods were used to compare the value obtained - by analysing the yearly input from the vegetation, and by measuring the amount of Pb

deposited in 24 hours under wet conditions. A mean estimate of  $5.2 \text{ g/m}^2/\text{year}$  was obtained at the  $\frac{1}{2}\text{m}$  distance from the road, on a site with 7,000 vehicles/16h day ( $2\frac{1}{2}$  - 3 million vehicles/year). On this basis, the passage of one vehicle is contributing around  $1.7 - 2.1 \text{ } \mu\text{g Pb/m}^2$  at this distance, and it would be useful to compare these figures with different verges under different traffic conditions.

The same calculations were performed for the 7m distance from the road, and a result of  $2\text{g Pb/m}^2/\text{year}$  obtained. Here the input to the soil is higher than the typical exponential decline (when compared to the input at the road margin) would have indicated. This is almost certainly due to the unmown vegetation at this distance: not only was this dead material for long periods (thereby increasing its binding capacity), with a longer standing time, but it also had an increased trapping efficiency due to its height and thickness.

There are no studies to which these results can be directly compared. Ward et al (1973) only sampled the first 6 cm of the soil profile, while in the present study, 'excess' Pb was found below 12 cm, after only 8 years. The former results also refer to deposition at 6m from the road for a length of verge (rather than a unit area). Ter Haar et al (op. cit) working in a semi-rural area, some distance from any major road, estimated the yearly input as  $0.01 \text{ } \mu\text{g Pb/m}^2/\text{year}$ , but this may represent additions from the Pb aerosol alone. It would be preferable to <sup>compare</sup> roadside situations with different traffic frequencies on a  $\text{m}^2$  basis, at a standard distance from the road.

The present results of this work show that a complete picture of Pb deposition on roadside verges cannot be obtained by sampling the vegetation alone: the shallow

decline of Pb input from these results suggest that the whole pattern of Pb deposition is more even than the distribution in the vegetation would suggest. This is probably a long-term feature, while the pattern in the vegetation is of a shorter time-scale, and is certainly more variable.

This work on Pb deposition on roadside vegetation and its subsequent incorporation into the soil has concentrated on the parameters affecting Pb input to the verge, and has not considered the effects on the plants themselves. In fact, little work has been reported on the plant communities of roadside verges, and this has mainly concentrated on the effects of road-salt in the soil (Davison, 1971; Westing, 1969). Some work has been done on the effects of artificially adding Pb to the soil with certain plants (Baumhardt and Welch, 1972; Arvik and Zimdahl, 1974). The general conclusion is that the immobility of Pb in plant tissues prevent the metal from having any serious effects. In a survey of several heavy metals and metabolism in Sunflower, Pb was the least able to alter the photosynthetic rate (Bazzaz et al, 1974). It could be shown to have no effect on the growth of maize (Baumhardt et al, op. cit) even when added as a highly soluble salt, lead acetate, to levels of 3200 kg/Ha; only small amounts of Pb were absorbed by the plants. Pb uptake is greatest when the soil is acid (Arvik et al, op. cit), and is probably passive, with little Pb being translocated past the roots (Jones and Clement, 1974; Arvik et al, op. cit).

Nevertheless, plants are known to become tolerant to Pb (Jowett 1958; 1964; Wilkins, 1957). Wilkins (op. cit) suggests that the tolerant genotypes will be selected for when the acetic acid extractable level of Pb in the soil ranges from 30 - 100 ppm (this extractive technique measures the amount of Pb in the soil which may be available for uptake

by the plants), though Jowett (1964) noted that there appear to be degrees of tolerance to Pb in Agrostis tenuis.

Comparison of these results to the soil levels on the verges studied here would suggest that tolerant genotypes may already be present at the roadsides. Assuming 10% availability as an estimate of the acetic acid-extractable component (section 2.6), this would mean that a total soil level of 300 - 1000 ppm might select for Pb tolerance in roadside grasses. This range would encompass all the sites studied here (at the  $\frac{1}{2}$ m distance) except for the two country lanes. Given the present Pb content of the soil at the site where  $2\text{g Pb/m}^2$  is being added each year, and assuming that this effectively raises the concentration of Pb by about 150 ppm, then this site may be selecting for tolerant genotypes by about 1982, 7 years after the measurements were taken. This will depend on how quickly the existing grasses are replaced, since selection for tolerance probably occurs at the seedling stage. Thus, new seedlings growing in such levels of soil will have to be tolerant when the soil level reaches 1000 ppm, though existing grasses (with well-developed root systems below the highly contaminated upper layer), may not need to be. Obviously, this assumes a great deal, but since Pb is not lost from the soil profile, it does point out that eventually, most verges on major roads may be colonised totally by Pb tolerant plants.

This will come most quickly at road margins: tolerant genotypes of Festuca rubra have been found on roadsides in Leicester (Ratcliffe, pers. comm.). At most sites, the exponential pattern of Pb deposition will accentuate the differences between the road margin and the back of the verge. This  $\frac{1}{2}$ m distance at all the main road sites studied

here is already noticeably different from the rest of the verge, and the indication is that this will become more apparent with time. The sparsity of grass at the roadside may be a product also of road-salt, which has been shown to have a similar exponential pattern to that of Pb. Dust, oil and grit from the road quickly discolours the vegetation, particularly after rainfall, and this is likely to reduce photosynthesis. Carbon monoxide, a toxic gas emitted in the exhaust fumes, may also affect the roadside grasses. Further work might consider this in more detail, and decide whether the bare ground developing on verges is a product of high Pb levels, high salt levels, or the aerial gases and deposits.

The major differences in Pb deposition - that is, according to proximity to the road, between sites of different traffic frequencies and between seasons - present three possible 'dimensions' in which the effects of Pb on invertebrate communities could be measured. Gish and Christensen (1973) used the first dimension to examine Pb levels in earthworms, and could demonstrate an exponential decline in their Pb content according to distance from the road, comparable to the pattern found in the soil. Maurer (1974) also used this approach in his examination of the diversity of Carabid and Staphylinid beetles near roads. Several species of rove beetle and Lycosid spiders had reduced numbers at the roadside, though the work examined relatively large roadside 'meadows'. With areas larger than verges there is likely to be a greater range of habitats encouraging a wider diversity of invertebrates; consequently, the chances of detecting a sensitive species is increased. Two species of ground beetle showed higher Pb levels at the road margin (Carabus auratus and Pterostichus cupreus) compared to

individuals caught 30m from the road, though no such differences were found in Ocypus olens.

By using the second dimension - comparisons between sites - Williamson and Evans (1972) carried out the only comprehensive survey of the Pb content of all invertebrate types found on roadside verges. Using pitfall traps, they sorted the samples into orders (insects) or classes (the rest), and analysed the whole bodies of each group from two sites. They noted that animals with calcaereous exoskeletons, namely snails, woodlice and millipedes, had a higher Pb content than the other types. Examining the garden snail Helix aspersa, Coughtrey and Martin (1977) concluded that it was unable to accumulate Pb in proportion to levels in its food, although this was the case with other heavy metals (Zn, Cd, and Cu). Martin, Coughtrey and Young (1976) concluded that the same was true of the woodlouse, Oniscus asellus, even when the Pb available (in woodland leaf litter) was measured using 5% acetic acid.

A similar technique was used in the present study, though by measuring the extractable component with 10% acetic acid (the lower acid concentration of the previous work may well underestimate the Pb available), a maximum available Pb level could be derived. On average, this suggests that only 10% of the total Pb bound by dead grass is available to detritivores - the actual quantity depending on traffic frequency, season and distance from the road. With herbivores, the amount will be correspondingly less by virtue of the lower Pb accumulation by live grass.

In terms of seasonal changes, the spring 'crash' in the Pb content of both live and dead grass is likely to coincide with the flush of invertebrate life at this time, reducing the effective threat still further. In addition, most food



consumption will take place during the summer when there are low Pb levels in the grass. This would mean that if Pb were to affect the diversity of the invertebrates, any consistent change is likely to appear between verges or toward the road, rather than between seasons. For this reason, measurements of the diversity were examined in these first two dimensions.

Pb may affect the animal populations of the verge in several ways. By contaminating the food of herbivores and detritivores, it may limit the range of these groups, or it may reduce the numbers of a sensitive species. Such changes (without prior knowledge of which species are sensitive) can be detected most obviously by plotting the numbers of different species in the two dimensions of this study, though this would require a large period of time to replicate and fully analyse the data. If species were disappearing, the composition of the invertebrate community would change, both in terms of the number of individuals and the number of species. Together, these two components define the concept of diversity, and it may be that if diversity could be accurately measured, the depletion of certain species could be detected as a decline in diversity. Using an index of diversity reduces the effects of samples of varying size (different numbers of individuals), and thereby clarifies any effect due to Pb. In this respect therefore, using an index of diversity within one habitat is an attempt to measure the effects of Pb, enabling the detection of sensitive species. The validity of this approach depends most simply on its effectiveness.

Generally, no decline in diversity could be shown, though seasonal changes were clearly demonstrated, as was an increase

in diversity with the greater vegetation height following the cessation of mowing. On this latter point, further work would be required to show whether this was a long-term increase.

The measurements of diversity were subject to several sources of error. Firstly, it may be that two apparently comparable verges are not in reality very similar, masking any effect due to Pb. Indeed, there is some evidence that the diversity was increased at the site where the ditch was most shallow. This may be due to migrations from the hedgerow, and would suggest that the ditch is only an effective boundary where its depth is greater.

The levels to which the animals were identified were important in two respects: this determines the degree of accuracy with which the diversity is measured, and also the amount of time required to analyse the sample. Certainly, a large proportion of the duration of this study was spent identifying pitfall and Tullgren samples, even though some groups were not taken to species.

Thirdly, such a study relies heavily on the sampling technique - in the present case, sampling was centered upon ground-living invertebrates since it seemed likely that these were subject to the highest ambient levels of Pb. Only two sampling techniques were used - the pitfall trap and Tullgren extraction, which between them do not necessarily sample equally all varieties of soil and ground-living animals. Without more time it would be impossible to sample accurately all groups, involving a large number of other techniques.

Finally, the study also presumes that the index of diversity used (Shannon-Weiner Information Statistic,  $H'$ ) may be sufficiently sensitive to detect an effect due to Pb.

Maurer (op. cit) used the same index and was able to show a decline in diversity of spiders and beetles toward the road. However, as Bullock (1971) points out,  $H'$  is insensitive to species with small numbers of individuals, - a rather important weakness, since any decline in diversity is likely to be the result of a reduction of numbers of a sensitive species. This problem may be overcome by comparing the results with those from another index (but which is likely to have other weaknesses) or by looking at the components of diversity themselves - the numbers of individuals and the numbers of species.

Using the Tullgren data, the mean number of individuals extracted per soil core for each site are not significantly different, nor is there a significant increase with distance from the road. Using the mean number of species per core, there is also no significant differences in these two dimensions. In both cases, this is due to the very small differences between samples. The unmown site appears to have greater numbers of individuals and species (though they cannot be statistically distinguished from the adjacent mown site), which would support the results from the pitfall traps that show a greater diversity for this site.

With the pitfall data, several taxa were examined individually, but only snails and Sminthurid collembolans appear to be avoiding areas of high Pb contamination. Woodlice, with low numbers at all sites may be excluded totally from the most polluted site (A50). It was these two groups which Williamson and Evans (op. cit) had shown to have higher Pb levels than other roadside invertebrates. These authors analysed specimens caught in 2% formalin and then washed in 70% ethanol, solutions which may remove Pb from the tissues, particularly the exposed wet tissues of snails, yet

this was not tested. Time did not allow for the Pb analysis of the animals caught in the pitfall traps of the present study. This may be worthwhile in the future, but experiments would have to be performed to show that the Pb content of the animals was not affected by the collecting fluids.

The most obvious connection between these otherwise dissimilar groups is their high Ca demand. Notably, the shell-less slugs with their reduced Ca requirements (Boycott, 1934) have a more even distribution and show no such preferences.

This association between Pb and Ca has appeared before. There is a recurring implication of increased Pb uptake in young vertebrates with a high Ca demand (Clegg and Rylands, 1966; Jacobson and Snowdon, 1976), while Pb is known to interfere with Ca-mediated neuromuscular transmission (Kober and Cooper, 1976). Thus, the connection of Pb with Ca seems to be one of general biological significance, yet no work has demonstrated a definite link between the two metals.

With invertebrates, Ireland (1975a) and Ireland and Wootton (1977) has noted that Ca often appears to be associated with Pb assimilation and suggested that Ca is absorbed in the earthworm Dendrobaena rubida to nullify the toxic effects of Pb. This would not explain why only woodlice and snails had elevated Pb levels, compared to other invertebrates (Williamson et al, op. cit). Rather, the results of this latter work suggest that Pb is only assimilated in large quantities by animals with a high Ca requirement. Perhaps the simplest answer is that such animals cannot distinguish between Pb and Ca physiologically, possibly because of the 'chemical similarity' of the two elements (Venugopal and Luckey, 1975).

In the present study, an interaction in the uptake of Pb

and Ca has been established in Porcellio scaber and juvenile Helix aspersa. The pattern of Pb and Ca uptake and their interaction is not comparable between the two species, but certain indirect evidence would link the two processes in both animals. The physiology of Pb absorption in woodlice or snails has not been directly examined in this work, but two possible mechanisms for Pb uptake in woodlice are suggested. Firstly, Pb may be absorbed according to the animal's Ca requirement - that is, by selective absorption. Any excess Pb is lost with the faeces. The low rates of Pb assimilation observed (generally less than 6%) do not necessarily contradict this possibility, since Ca is only likely to be required to any large degree during the moult-cycle, or possibly when the female is gravid. On this last point, it was notable that assimilation rates for Pb were around 10% in gravid females compared to less than 2% in non-gravid, but unsexed, animals. This may indicate that Pb uptake may be associated with the metabolism of woodlice.

The second possibility is based on the observation of Hassall and Jennings (1975) that most soluble nutrients are absorbed in the caecal epithelium of the hepato-pancreas of woodlice, soon after ingestion. As these same cells mature they enter a secretory phase, producing enzymes to be discharged into the posterior hind-gut (the secretions by-pass the anterior hind-gut by the typhlosole channels, before being discharged into the papillate region of the hind-gut). Thus any Pb absorbed in the hepato-pancreas may, with time, be discharged into the hind-gut with these secretions.

The possible association of Pb with the hepato-pancreas active in both absorption and secretion, is also suggested by the results of the Pb assay of the tissues of adult H. aspersa.

Here, the highest levels of Pb were consistently found in the digestive gland, which fulfills a similar physiological role to the hepato-pancreas of woodlice. These results are comparable to those of Ireland and Wootton (op. cit) who worked on two marine molluscs, Thais lapillus and Littorina littorea. As these authors suggest, it may simply be that Pb is being bound to the enzymes within the gland, particularly the amylases which contain Ca, though further work would be needed to confirm this. Either way, if Pb was accumulated in the digestive diverticula and lost during a secretory phase, it would be an effective method of isolating and voiding toxic substances, not necessarily confined to Pb. Unfortunately, little is known about Ca absorption in woodlice, though this would almost certainly give some indication of how Pb is absorbed.

Evidence that Pb uptake may be related to the metabolism is provided by the different Pb levels in each sex of P. scaber. The Pb content of male P. scaber can be related to dry weight, though these levels are significantly lower than those of the females, where there is no correlation with weight. However, the Ca content of males of a given weight is, on average, higher than that of a comparable female, and there is some indication that the sexes differ in their uptake of the two metals. These differences are obviously physiological. Females are usually heavier, and since the metabolic rate has been shown to decline with increasing dry weight, it may be that there is a reduced turnover rate of Pb and Ca in the females. This could account for the apparent spring rise in the Pb and Ca content of Philoscia muscorum (using animals collected from the roadside), though these results have to be treated tentatively because of the very small numbers involved.

During the spring the woodlice begin their reproductive activity with its associated sequence of moults - Phillipson and Watson (1965) have shown that the metabolic rate changes both during moulting and sexual activity. If more Ca is absorbed at this time, Pb assimilation may also increase. No firm conclusions can be reached about such changes since such variations would have to be distinguished from the concurrent changes in the Pb content of their food.

The experiments in the present work have concentrated on the sub-lethal effects of Pb on woodlice, mostly because these are the doses likely to occur on the verges examined. The experiments were also short-term and therefore not strictly comparable to the verge situation. There is little evidence that such doses of Pb over these periods affect the metabolic rate of the woodlice, since there was no detectable effect on the respiration rate. Nor could it be shown to impair the fecundity of P. scaber, or more precisely, the numbers of young emerging from the brood-pouch. This happened even though Pb was applied in its most soluble form,  $\text{Pb}(\text{NO}_3)_2$ . Very low rates of assimilation meant that about 6% of the Pb consumed was actually retained by the woodlice. This no doubt served to protect the animal from any possible toxic effects of a high body concentration, particularly with the gravid females which consumed food with 16500 ppm of Pb, without apparent effect.

Such results have to be compared to those from the verges, and more especially the site where no woodlice were found, and where the highest Pb level in the vegetation only reached 1000 ppm. If Pb is really excluding woodlice from this site, the fact that gravid females were apparently unaffected on a diet with over 16 times as much Pb has to be explained. Further work would be needed to resolve this, but if it is a real result, one possible cause may be the effects of Pb on

juvenile woodlice. No results are available on whether Pb has any effect on young woodlice, but it may be that the high Ca requirement which would be expected during this stage may induce high Pb uptake. The young may be more susceptible to any toxic effects of Pb, possibly during the rapid sequence of moults in early life. It may simply be that the young cannot survive on the high Pb diet of this most contaminated site, and therefore the verge cannot sustain a population of woodlice.

Snails are also lacking in any large numbers at this site, though slugs were frequently found. It is worth noting here that the food consumption of juvenile H. aspersa was observed to decline with increasing Pb content of the food. This may account for their preference for Pb-free areas of the verge. Woodlice may be able to make a similar distinction - Dallinger (1977) has shown them to prefer Cu-rich food, and there is some indirect evidence from the present study that woodlice may chose a low-Pb food. It may be that both groups are concentrated where the Pb content of the roadside vegetation is least. Any future attempts to demonstrate this would have to distinguish between Pb and other pollutants.

Whether the Pb retained by these invertebrates is actually concentrated up food chains has yet to be demonstrated conclusively. Ireland (1975a) has shown that the Pb assimilated by earthworms increases the amount available to carnivores, and the same is likely to be true of woodlice and snails. Previous attempts to show the concentration of Pb up invertebrate trophic levels (Price et al, 1974; Giles et al, 1973) have failed to distinguish between Pb consumed and Pb absorbed in the air tracts. With the large aerosol component



of airborne Pb near the verge, this latter source may well be significant.

The same problem confounds studies on roadside vertebrates. Indeed, Welch and Dick (1975) suggest that in roadside mice, the main route of Pb absorption is through the lungs. On the other hand, Quarles et al (1974) report that levels assimilated depend on metabolic rate and diet; in the event, they conclude that Pb is not limiting numbers of small mammals on the verge. Mierau and Favara (1975) reached the same conclusion with Deer Mice.

Both woodlice and snails are part of the diet of a wide range of predators, both vertebrate and invertebrate. For the most part, woodlice will be consumed whole and therefore all the Pb in the tissues is potentially available for absorption; with Philoscia muscorum collected from one site, this could be as high as 3  $\mu\text{g}$ . With a predator consuming the wet tissues of Cepaea nemoralis this would, on average, also be 3  $\mu\text{g}$ , but in adult H. aspersa it could rise to 32  $\mu\text{g}$ . These estimates exclude Pb in the shell which would not be consumed. These levels would be tempered to some degree by the assimilation efficiency of the predator, but even so, quite large amounts of Pb are potentially available.

The simplest way to decide whether Pb is actually moving up trophic levels would be to examine the animal populations on Pb-rich spoil-heaps removed from any source of airborne Pb. These sites are normally rich in other heavy metals (particularly Zn), and these would have to be taken into account.

Generally, this work and other studies suggest few deleterious effects of Pb on the biotic components of roadside verges. This may be due to the insolubility of most Pb salts, and the capacity of organic material to bind it readily, effectively removing it from solution. For the most part, Pb

may only be re-dissolved under acidic conditions, and it may be that only when Ca is mobilized is Pb mobilized also. In vertebrates, most Pb is 'stored' in the bones (Hammond et al, op. cit) and only enters the blood stream when Ca is again mobilized from the long bones (Hammond et al, op. cit). It is at this time that the overt symptoms of Pb poisoning can be observed. The same chemical parameters may also apply to the soil, where high levels of Ca may ameliorate the effects of Pb on root-growth (Jowett, 1964).

The deleterious effects of high Pb levels in the soil on plants seem few. The selection for Pb-tolerant genotypes is probably the major effect and the indication is that further investigation might successfully find Pb-tolerant grasses at the road margin of most well-trafficked roads. In the invertebrates, the snails and woodlice may be confined to low Pb areas, and future work might investigate any preference in these groups to low-Pb food, considering possible differences between species. In this way, they may be evaluated as biological monitors of Pb pollution, with different species being sensitive to different degrees of contamination.

Both morphologically and biologically, the verge is a habitat distinct from its surroundings and in this preliminary study, the investigations have been confined to the effects of Pb in these areas. Future work might decide how effectively the verge is isolated by its boundaries and whether it can be legitimately treated as a unit. In this case, it should attempt to show whether these 'boundaries' are selective for particular animals (and possibly plants). For example, the effectiveness of the road as a boundary might be tested by looking at the fauna of the central reservations - a 'verge' with road boundaries on two faces. Also, the effect

of deepening the ditch may give some indication of its role as a boundary. As a convenient unit available in most parts of the country, the verge could provide a habitat for intensive study. Incidental to this, such work would also provide valuable information on the conservation value of verges.

Way (1969) has considered this aspect of verge ecology and proposes that the verge should only be mown during the winter when the invertebrate fauna is likely to be less vulnerable. Mowing, ostensibly to improve the visibility of the roads, is probably necessary to prevent the encroachment of tall-standing grasses, such as Arrhenatherum elatius, and thereby encourage a wider diversity of plants. As Way points out (1973), this is likely to encourage a greater diversity in the invertebrate fauna, and the faunal lists in Appendix II go some way to recording what may be expected at such sites.

Mowing is not a practical possibility in the wet weather of the winter, since the grass is usually flattened. The machinery is also likely to break up the soil and vegetation. Perhaps the best policy would be a single mowing in early spring, leaving the last 2 - 3 metres unmown. This would provide a range of vegetation heights offering a wider choice of habitats: notably, in the unmown area of some sites there is evidence of the presence of small mammals.

The value of roadside verges extends further than their possible conservation role: in most rural areas they serve as a 'buffer' between the road and its pollutants and agricultural land. In several areas of Leicestershire, verges and hedgerows have been removed completely, and such crops as wheat and barley have been growing right up to the road's edge.

These crops will be subject to the large Pb deposition in the first few metres of the road, though modern processing techniques probably ensure that little Pb actually reaches the consumer.

The study of roadside verges as ecosystems must cover wider aspects of their ecology than just the pollution they suffer. Even so, as a relatively well-defined 'unit' with an identifiable source of pollution entering at one end of the habitat, they afford a very useful opportunity for examining the effects of these pollutants in a more or less 'fixed' system.

This work can only be regarded as a preliminary study into one of these pollutants and suffers many weaknesses, primarily due to the lack of time and the need to establish exactly the conditions determining the Pb threat to the life of the verge. A more comprehensive analysis of the pitfall and Tullgren data would be desirable, as would further experiments on the physiology of Pb uptake in both woodlice and snails. Some of the experiments have suffered from a large variability in results, and although this seems to be a common feature of toxicology experiments with Pb, some of the variation can be attributed to sampling error and the analytical technique. More details are required on the optimum operating conditions for the carbon rod furnace, particularly with regard to the atomisation of animal tissues, in the hope of reducing this variability.

Perhaps a closer examination of the floral components of the verge, allied to the animal studies, would have helped to distinguish real effects of Pb on both plants and animals. Finally, more extensive use could have been made of the lead dosing chamber, particularly with regard to the parameters

affecting Pb deposition.

The recommendations for future work have centered around the verge as a unit ecosystem, as a possible area for conservation, yet the simple truth is that verges are habitats created by man and which are managed to suit his convenience. A real possibility is that we might make better use of them if we knew more about them.

## Summary

1. The pattern of the Pb contamination of roadside vegetation from nine verges in rural Leicestershire is described. The rapid decline of Pb concentration with distance from the road reported as typical by other workers is only found at certain sites. This 'exponential decline' in Pb is attributed to proximity to traffic, reactions of airborne Pb with time and predominant wind characteristics. Splash from passing vehicles enhances Pb levels next to the road.
2. Sites not in the lee of the predominant winds do not have the 'exponential' decline in Pb levels. The size of the hedgerow may also be important in determining this pattern.
3. A major difference between live and dead grass in their capacities to bind Pb is described, and is attributed to the condition of the cuticle and the ability of Pb-rich solution to penetrate the intercellular spaces. Little Pb is moved through the transpiration stream.
4. Most of the Pb bound by grass is from solution, and only a small fraction is probably surface-held or particulate material. This is true of both live and dead grass.
5. Seasonal variations in the Pb content of roadside grass are described over a two year period. This comprises a winter maximum in both live and dead grass, which falls rapidly to a spring low. These changes can be correlated with precipitation, and are also considered in terms of the standing time of the vegetation.
6. Several estimates are made of the net input of Pb into the soil, and a mean of  $5.2\text{g/m}^2/\text{year}$  is derived for the  $\frac{1}{2}\text{m}$  distance from the road. This probably represents a yearly increase of about 14%, at the moment.
7. The litter of various roadside verges was analysed for Pb, and the maximum proportion likely to be available to

detritivores was estimated using a 10% acetic acid extraction. On average, this was around 10%.

8. The invertebrate communities of six sites were analysed using pitfall traps and Tullgren extraction sampling techniques. The diversity was measured with the information statistic,  $H'$ . Samples were taken throughout the year (1974/75). No decline in diversity could be attributed to Pb, though a greater diversity was apparent at a site which had purposely been left unmown.

9. Snails and sminthurid collembolans are the only groups which appear to avoid areas with high Pb contamination. Woodlice numbers were low at all sites, and none were found at the most heavily polluted site (A50).

10. Evidence is given to suggest that there are seasonal changes in the Pb and Ca content in the woodlouse Philoscia muscorum. There is a spring maximum in Ca levels and possibly a slightly earlier peak in Pb levels.

11. The sexes of Porcellio scaber are shown to differ both in their Pb and Ca levels; males have a higher Ca content per unit dry weight, and their Pb levels can be correlated to their dry weight. Females tend to be heavier, with lower Ca levels, but higher Pb levels (which show no correlation to dry weight). It is speculated that this may be due to differences in the turnover of these metals between the sexes, perhaps related to their metabolic rate (which, on average will be lower in females).

12. An interaction between Pb and Ca uptake is demonstrated in P. scaber and juvenile Helix aspersa. In the former, Pb and Ca assimilation appear to rise sympathetically, but there is no such simple relationship in the snails. These results are compared to previous work, and it is suggested that Pb may be absorbed in the hepato-pancreas or digestive

diverticula, and lost with the secretions to the hind-gut.

13. Toxicological dosing of P. scaber with Pb showed that Pb does not affect

i. the rate of oxygen consumption

ii. the fecundity

at the doses applied. A diet of 16500 ppm Pb did not increase the mortality of gravid females.

14. The distribution of Pb in the tissues of adult H. aspersa is described, and shown to correspond to that found in marine molluscs. A correlation between total Ca levels and total Pb is demonstrated when body weight is also taken into account.



Appendix IFloral Components of the Major SitesSite1m from road: *Taraxacum officinale**Dactylis glomerata**Poa* sp.*Agropyron* sp.*Lolium* sp.*Festuca rubra**Plantago major**Crepis capillaris**Ranunculus repens**Potentilla reptans*

3m

*Arrhenatherum* sp.*Dactylis glomerata**Taraxacum officinale**Ranunculus repens**Deschampsia caespitosa**Anthriscus sylvestris**Potentilla reptans**Festuca rubra* (trace)*Agropyron* sp.

5m

*Holcus lanatus**Heracleum sphondylium**Arrhenatherum* sp.*Taraxacum officinale**Anthriscus sylvestris**Epilobium hirsutum**Deschampsia caespitosa*Site 11m: *Ranunculus repens**Taraxacum officinale**Lolium* sp.*Agropyron* sp.*Plantago lanceolata*

3m: as above

5m: as for site , 5m.

Floral Components (continued)Site 2

1m: Taraxacum officinale  
 Dactylis glomerata  
 Agropyron sp.  
 Atriplex sp.  
 Poa sp.  
 Sonchus arvensis  
 Polygonum auriculare  
 Plantago major  
 Lolium sp.  
 Trifolium repens (trace)  
 Arrhenatherum sp. (trace)  
 Tussilago farfara

3m: Achillea millefolium  
 Ranunculus repens  
 Agropyron sp.  
 Cirsium pratense  
 Cerastium sp.  
 Anthriscus sylvestris  
 Crepis capillaris

5m: as above

Site 3

1m: Sonchus arvensis  
 Plantago major  
 Taraxacum officinale  
 Polygonum auriculare  
 Matricaria matricariodes (trace)  
 Atriplex sp.  
 Dactylis glomerata  
 Lolium sp.  
 Holcus lanatus (trace)  
 Poa sp.  
 Anthriscus sylvestris  
 Agrostis stolonifera  
 Arrhenatherum sp. (trace)  
 Festuca rubra

3m: Deschampsia caespitosa  
 Cirsium pratense  
 Arrhenatherum sp.  
 Dactylis glomerata  
 Lolium sp.  
 Festuca rubra  
 Anthriscus sylvestris  
 Taraxacum officinale  
 Rumex acetosa  
 Urtica dioica  
 Phleum pratense

7m: Arrhenatherum sp.  
 Rumex acetosa  
 Urtica dioica  
 Dactylis glomerata (trace)  
 Deschampsia caespitosa  
 Epilobium hirsutum

Floral Components (continued)Site 5

1m: Lolium sp.

Plantago major

Poa annua

Polygonum auriculare

Festuca rubra

Taraxacum officinale

Agrostis tenuis

Trifolium repens

Ranunculus repens

Cirsium pratense

Poa sp.

Potentilla reptans

Arrhenatherum sp.

Rubus sp.

3m: Ranunculus acris

Phleum pratense

Holcus lanatus

Sanguisorba officianalis

Potentilla reptans

5m: Rubus sp.

Various mosses

Site 4

1m: Lolium sp.

Plantago major

Poa annua

Festuca rubra

3m: as above

Identifications by Dr. D. Ratcliffe

APPENDIX IIFauna of the Major Sites - First Series Pitfalls

<u>Taxon</u>	<u>Sites</u>					
	1	2	3	4	5	
Mollusca:						
<u>Gastropoda</u>						
snails	+	+	+	+	+	+
slugs	+	+	+	+	+	+
Annelida:						
<u>Oligochaeta</u>	+	+	+	+	+	+
Arthropoda:						
<u>Isopoda</u>						
Porcellio scaber	+	+	-	+	+	+
Oniscus asellus	-	-	+	+	-	-
Philoscia muscorum	+	+	+	+	-	+
<u>Myriapoda</u>						
Diplopoda, Julidae	+	-	+	+	-	-
Chilopoda, Lithobiidae	+	+	+	+	-	+
Arachnida, <u>Acari</u> :						
Prostigmata	+	+	+	+	+	+
Cryptostigmata	+	+	+	+	+	+
Mesostigmata	+	+	+	+	+	+
<u>Aranea</u> :						
<u>Linyphiidae</u>						
Bathyphantes concolor	-	-	-	+	-	-
B. dorsalis	-	-	-	-	+	-
B. setiger	-	-	-	-	+	-
Lepthyphantes zimmermani	+	-	+	+	+	+
L. cristatus	+	-	-	-	-	-
L. flavipes	-	-	+	-	-	-
L. tenuis	-	-	-	-	+	-
Centromerita bicolor	+	-	+	+	+	+
C. concinna	+	-	+	+	+	+
Oedothorax retusus	-	-	-	-	+	-
Meioneta saxatilis	+	-	+	-	+	-
M. mollis	+	-	+	+	-	-
M. rurestris	+	-	-	-	+	-
Erigone dentipalpis	+	-	+	-	+	-
Walckenaera acuminata	-	-	-	+	-	+

	1	$\alpha$	2	3	4	5
<i>Erigonidium graminicola</i>	-	-	+	-	-	-
<i>Micargus herbigradus</i>	+	-	+	+	+	-
<i>Gonatium rubens</i>	-	-	-	-	+	+
<i>Agyneta decora</i>	+	-	-	-	-	-
<i>Asthenargus paganus</i>	-	-	-	-	+	-
<i>Robertus lividus</i>	-	-	-	-	+	-
<i>Dycimbium nigrum</i>	+	-	-	+	-	-
<i>Hypelistes jacksoni</i>	-	-	-	+	-	-
<i>Leptorhoptum robustum</i>	-	-	-	+	-	-
<i>Lessertia denticelis</i>	+	-	-	-	-	-
<b>Tetragnathidae</b>						
<i>Pachygnatha listeei</i>	+	-	-	-	-	-
<i>P. clerckii</i>	+	-	+	-	-	+
<i>P. degeeri</i>	+	-	+	-	+	-
<b>Clubionidae</b>						
<i>Clubiona</i> sp.	-	-	+	-	-	-
<b><u>Opiliones:</u></b>						
<i>Leiobunum blackwalli</i>	-	+	-	-	-	-
<i>L. rotundrum</i>	+	-	+	+	-	+
<i>Oligolophus agrestis</i>	+	+	+	+	+	+
<i>O. tridens</i>	+	+	-	+	+	-
<i>Platybunus triangularis</i>	+	+	+	+	+	+
<i>Phalangium opilio</i>	-	+	-	-	-	-
<i>Nemastoma bimaculatum</i>	+	+	+	+	+	+
<b>Insecta</b>						
<b><u>Collembola:</u></b>						
<i>Arthropleona</i>	+	+	+	+	+	+
<i>Sminthuridae</i>	+	+	+	+	+	+
<b><u>Thysanoptera:</u></b>						
<i>Thripidae</i>	+	+	+	+	+	+
<i>Aeleohipidae</i>	+	+	+	+	+	+
<b><u>Psocoptera:</u></b>						
<i>Epipsocidae</i>	+	-	-	+	-	-
<b><u>Dermaptera:</u></b>						
<i>Forficula auricularia</i>	-	-	+	-	-	+

	1	2	3	4	5
<u>Hemiptera:</u>					
Miridae	+	-	-	-	-
Cicadellidae	+	+	+	-	-
Cercopidae	+	+	+	+	+
Nabidae	+	+	+	-	-
Reduviidae	+	+	+	-	-
Aphidae	+	+	+	+	+
Jassidae	+	-	+	-	-
<u>Hymenoptera:</u>					
Platygasteridae	+	+	+	+	+
Pteromalidae	+	+	+	+	+
Agriotypidae	-	-	+	-	-
Dryinidae	+	-	-	-	-
Trigonalidae	+	-	-	-	-
Formicidae	+	+	+	+	+
Apoidea	+	-	+	-	+
Mymaridae	+	+	+	+	+
Ichneumonidae	+	+	+	-	+
Braconidae	+	+	+	+	+
Mutillidae	-	-	+	-	-
Tenthredinidae	-	-	+	-	-
Cynipidae	+	+	+	+	+
Sclerogibbidae	+	-	-	-	-
Proctotrupidae	-	+	+	+	-
Torymidae	-	+	+	+	-
<u>Siphonaptera:</u>					
Histrichopsyllidae	+	-	-	-	-
Ceratophyllidae	+	-	-	-	-
<u>Coleoptera:</u>					
<u>Staphylinidae</u>					
Xylodromus concinnus	-	-	-	+	-
X. depressus	-	-	+	+	-
Quedius boops	-	+	-	-	-
Q. mesomelinus	-	-	+	-	-
Q. fuliginosus	-	-	+	-	-
Tachyporus fasciatus	+	-	-	-	-
T. hypnorum	-	-	-	+	+
T. chrysomelinus	+	+	-	-	-
T. nitidulus	+	-	-	-	-
Staphylinus caesarius	-	+	-	-	-

	1	2	3	4	5
<i>S. fuscatus</i>	-	+	-	-	-
<i>S. sculus</i>	-	-	-	-	+
<i>Philonthus immundus</i>	-	-	-	+	-
<i>P. splendens</i>	+	+	-	-	-
<i>P. longicornis</i>	+	-	+	-	+
<i>Micropeplus caelatus</i>	-	-	-	+	-
<i>M. staphylinoides</i>	+	+	+	+	+
<i>Acrulia inflata</i>	-	-	-	+	-
<i>Lesteva heeri</i>	-	-	-	+	-
<i>L. punctata</i>	+	-	-	-	-
<i>Othius myrmecophilus</i>	+	+	+	+	+
<i>O. laevisculus</i>	-	-	-	-	+
<i>O. punctulatus</i>	+	+	+	+	+
<i>Phyllodrepoidea crenata</i>	+	-	-	-	-
<i>Xantholinus tricolor</i>	-	-	+	-	-
<i>X. lineatus</i>	-	-	+	-	-
<i>Oxytelus rugosus</i>	-	-	+	-	-
<i>O. sculpturatus</i>	-	-	+	-	-
<i>Bledius femoralis</i>	-	+	-	-	-
<i>Omalius excavatus</i>	-	+	+	-	-
<i>Coryphium augusticolle</i>	+	-	-	-	-
<i>Pseudopsis suleata</i>	+	-	-	-	-
<i>Ischnoglossa corticina</i>	+	-	-	-	-
<i>Medon fuscus</i>	-	+	-	-	+
<i>M. propinquus</i>	-	-	-	+	-
<i>Mycetoporus splendens</i>	-	+	+	-	-
<i>M. clavicornis</i>	-	+	-	-	-
<i>Aleochara ruficornis</i>	-	+	-	-	-
<i>A. moesta</i>	+	+	-	-	-
<i>Trichophya pilicornis</i>	-	-	-	-	+
<i>Oxypoda lentula</i>	-	-	-	+	-
<i>Leptacius bathycrus</i>	-	-	-	+	-
<i>Microglossa marginalis</i>	-	-	-	+	-
<i>Cratarea suturalis</i>	-	-	+	-	-
<i>Gabrieus bishopi</i>	+	-	-	-	+
<i>Mycetoporus angularis</i>	-	+	+	-	-
<b>Carabidae</b>					
<i>Platyderus ruficollis</i>	+	+	+	-	-
<i>Dromius linearis</i>	+	+	+	-	+
<i>Trachus obtusus</i>	-	-	-	+	-
<i>Stomis pumicatus</i>	-	+	-	-	-

	1	2	3	4	5
Byrrhidae:					
Simplocaria semistriata	-	-	-	-	+
Curculionidae:					
Balanobius salicivorous	-	-	-	+	-
Sitona puncticollis	-	-	-	-	+
Apion difforme	-	-	+	-	-
A. immune	-	+	-	-	-
Helophoridae:					
Helophorus affinis	-	+	+	+	+
H. aequalis	-	+	-	+	-
Sphaeridiidae:					
Cercyon quiquilis	-	-	+	-	-
Coelostoma orbiculara	-	-	-	+	-
Ptillidae:					
Aerotricha grandicollis	-	+	-	-	-
Chrysomelidae:					
Chrysolina staphylea	+	-	-	-	-
Cantharidae:					
Rhagonycha lutea	+	-	-	-	-
Cholevidae:					
Bathyscia wollastoni	+	-	+	+	-
Nargus velox	-	-	+	-	-
Choleva jeanneli	-	+	-	-	-
Colon brunneum	+	+	-	-	+
Haliplidae:					
Cremidotus caesus	-	-	+	-	-
Cateretidae:					
Cateretes rufilabris	-	-	+	-	-
Buprestidae:					
Trachys pumila	+	-	-	-	-
Silphidae:					
Silpha tyrolensis	+	-	-	-	-
Antribidae:					
Anthribus variegatus	-	+	-	-	-
Curcujidae:					
Paediacus dermestoides	+	-	-	-	-



## APPENDIX II - Part 2

Fauna of the Major Sites - Second Series Pitfalls

The following records are in addition to those made for the First Series pitfalls.

<u>Taxon</u>	<u>Sites</u>					
	1	2	3	4	5	
Mollusca						
<u>Gastropoda:</u>						
Agriolimax reticulatus	+	+	+	+	+	
Arion ater ater	+	+	+	+	-	+
A. circumscriptus	+	+	+	+	+	+
A. hortensis	+	+	+	+	-	+
A. intermedius	+	+	-	-	+	+
Helicella caperata	-	-	+	-	-	-
Discus rotundatus	+	+	+	+	+	+
Euconulus fulvus	-	-	-	+	+	-
Cepaea nemoralis	-	-	-	+	+	+
Cochlicopa sp.	-	+	+	+	-	-
Vitrina pellucida	+	+	+	+	+	+
Vallonia sp.	-	-	+	-	-	-
Vitrea crystallina	+	+	+	+	-	+
Retinella pura	-	-	-	+	-	+
R. nitidula	+	+	+	+	+	+
Vitrea contracta	+	+	+	+	+	-
Monacha cantiana	-	-	-	-	-	+
Hygromia hispida	+	+	+	-	+	+
Punctum pygmaeum	+	+	-	+	+	+
Arthropoda, Insecta						
<u>Coleoptera:</u>						
Carabidae	+	+	+	+	+	+
Helophoridae	+	+	+	+	+	-
Coccinellidae	-	+	+	+	-	-
Chrysomelidae	+	+	+	+	-	+
Scydmaenidae	+	-	-	-	-	-
Lathridiidae	+	+	+	+	-	+
Cholevidae	+	+	-	-	-	-
Cryptophagidae	+	+	+	+	+	+
Curculionidae	+	+	+	+	-	-
Sphaeridiidae	+	-	-	+	-	-
Hydrophilidae	+	-	-	-	-	-
Ptilidae	-	+	+	+	+	+

	1	$\alpha$	2	3	4	5
Georissidae	-	-	-	-	+	-
Silphidae	+	-	-	+	-	-
Bostrichidae	-	-	-	-	+	-
Cleridae	+	-	-	-	-	-
Meloidae	-	-	+	-	-	-
Cebrionidae	+	-	-	+	+	+
Helodidae	-	-	-	+	-	+
Elateridae	-	+	+	+	+	+
Byrrhidae	-	+	-	-	-	+

LOAD	No.	Fresh Wt.		Dry Wt.		Days On Load	No. Young	Grass Consumed	Ca Level		Pb Level		Total Pb Burden
		Parent	Young	Parent	Young				Parent	Young	Parent	Young	
CON.	2	64.6	18.3	14.0	4.3	28	52	27.5	1.32	0.56	3.0	0.0	3.0
	4	34.3	17.3	12.6	3.3	23	32	26.5	1.47	0.47	0.0	0.0	0.0
	11	56.4	6--	17.5	--	7	--	8.0	1.25	--	0.0	--	0.0
	12	38.4	7.3	10.5	1.7	7	17	6.5	0.79	0.22	0.0	0.0	0.0
	13	32.6	8.2	9.3	1.4	8	27	3.7	0.78	0.29	0.0	0.0	0.0
	15	46.3	1.2	15.1	0.5	8	2	8.7	1.17	0.05	2.3	0.0	2.3
	14	15.9	12.1	5.4	3.0	24	20	10.3	0.50	0.40	4.3	0.0	4.3
	1	37.1	3.0	7.7	2.4	24	30	19.9	1.21	0.26	59.1	4.4	63.5
	3	89.6	10.6	24.0	4.0	27	37	19.6	1.98	0.58	147.8	0.0	147.8
	11	40.9	6.7	11.0	2.2	8	24	5.5	1.00	0.18	3.9	0.0	3.9
	12	42.5	1.2	11.5	0.6	7	7	5.9	1.00	0.09	19.5	1.0	20.5
	13	26.6	4.8	9.8	3.0	16	11	14.7	0.76	0.18	21.6	3.8	25.4
	14	27.2	7.3	10.0	3.6	16	19	10.1	0.72	0.26	21.7	31.7	53.4
	15	30.8	3.9	10.4	1.1	9	6	3.3	0.67	0.13	8.5	0.0	8.5

# SUCCESSFUL BIRTHS

## APPENDIX III

Fecundity data for P. scaber on various doses of Pb.

LEAD	No.	Fresh Wt.		Dry Wt.		Days on Load	No. Young	Gross Consumed	Ca Level		Pb level		Total Pb Burden
		Parent	Young	Parent	Young				Parent	Young	Parent	Young	
	3	45.5	7.0	9.0	3.8	21	48	9.8	0.94	0.47	38.8	0.0	38.8
	5	31.7	4.7	8.0	1.7	33	9	10.8	0.86	0.12	25.6	1.1	26.7
	6	25.3	13.8	7.9	2.3	21	32	11.9	0.93	0.30	76.0	9.2	85.2
	7	38.2	12.1	7.2	3.6	21	38	13.0	0.73	0.42	36.7	0.0	36.7
LOW	9	37.7	14.0	8.5	3.8	21	34	17.2	0.79	0.39	37.7	0.0	37.7
	11	28.3	9.1	9.5	2.7	8	32	2.7	0.86	0.33	14.8	4.5	19.3
	12	24.9	7.0	7.8	1.7	8	17	2.7	0.69	0.25	4.0	0.0	4.0
	13	23.9	1.2	5.5	0.6	9	7	4.5	0.64	0.08	2.4	0.0	2.4
	14	25.8	4.8	5.7	1.0	7	15	5.6	0.50	0.15	11.3	0.0	11.3
	15	36.8	13.2	10.0	4.0	11	34	4.2	0.98	0.48	18.2	7.3	25.5
		mg	mg	mg	mg			mg	mm	mm	ug/l	ug/l	ug/l

SUCCESSFUL BIRTHS

LOAD	No.	Fresh Wt. Parent Young		Dry Wt. Parent Young		Days On Load	No. Young	Grass Consumed	Ca level Parent Young		Pb level Parent Young		Total Pb Burden
High Verge	1	61.2	4.8	10.7	2.8	7	17	3.9	0.87	0.23	0.0	2.6	2.6
	2	42.5	6.2	11.2	1.4	7	15	10.2	0.83	0.20	9.0	0.0	0.0
	3	52.2	13.6	10.4	4.0	8	35	4.0	0.67	0.41	4.3	0.0	4.3
	4	27.3	0.8	7.3	--	9	2	4.7	0.60	--	0.0	0.0	0.0
	5	42.9	10.0	9.7	1.8	8	23	4.3	0.71	0.32	7.2	10.8	18.0
Low Verge	1	27.4	4.0	5.7	0.7	8	16	8.4	0.56	0.18	0.0	5.0	5.0
	2	28.4	1.2	6.5	0.5	7	4	5.3	0.73	0.05	1.4	2.6	4.0
	3	52.4	4.6	11.5	1.5	8	11	12.4	0.99	0.15	0.0	0.0	0.0
	4	32.6	1.1	8.6	0.9	8	4	5.0	0.68	0.05	0.0	0.0	0.0
	5	31.8	2.0	7.5	0.9	9	12	6.8	0.67	0.11	0.0	0.0	0.0

SUCCESSFUL BIRTHS

LOAD No.	Total Pb Burden	Ca% Dry Wt. Parent Young		Gross Consumed/Day	Gross Consumed/Day % Dry Wt.	Pb P.P.M. Parent Young		Total Pb consumed
2	3.0	9.42	13.02	0.98	1.57	5.35	0.0	1.7
4	0.0	11.66	14.24	1.15	1.96	0.0	0.0	1.6
11	0.0	7.17	---	1.14	2.02	0.0	0.0	0.5
12	0.0	7.55	12.88	0.93	2.42	0.0	0.0	0.4
13	0.0	8.38	20.57	0.46	1.41	0.0	0.0	0.2
15	2.3	7.72	10.00	1.09	7.22	3.8	0.0	0.5
14	4.3	9.38	13.33	0.43	7.96	19.9	0.0	0.6
1	63.5	15.71	10.83	0.82	1.81	191.8	45.8	328
3	147.8	8.25	14.50	0.72	0.68	153.9	0.0	323
HIGH 11	3.9	9.00	8.27	0.68	6.18	8.86	0.0	90
12	20.5	8.64	15.50	0.51	4.43	42.39	41.66	97
13	25.4	7.75	6.03	0.92	9.40	55.12	31.66	242
14	53.4	7.16	7.25	0.63	6.30	54.25	220.13	166
15	8.5	7.21	11.63	0.37	3.56	17.50	0.0	54
	ug/l.			mg				ug

SUCCESSFUL BIRTHS

LOAD No.	Total Pb Burden	Ca % Dry Wt.		Grass Consumed/Day	Grass consumed/Day % Dry Wt.		Pb ppm		Total Pb consumed
		Parent	Young				Parent	Young	
3	38.8	10.41	12.47	0.46	5.10	116.76	0.0		28.52
5	26.7	10.75	7.05	0.32	4.00	148.83	16.17		31.43
6	85.2	11.79	13.04	0.56	7.09	240.50	100.00		34.64
7	36.7	10.15	11.55	0.62	8.61	127.43	0.0		37.84
9	37.7	9.28	10.31	0.82	9.64	116.76	0.0		50.06
LOW									
11	19.3	9.05	12.22	0.34	3.57	38.54	32.40		7.85
12	4.0	8.94	14.70	0.34	4.35	12.80	0.0		7.85
13	2.4	11.61	13.33	0.50	9.07	10.90	0.0		13.09
14	11.3	8.87	14.80	0.80	14.09	49.56	0.0		16.30
15	25.5	9.85	11.95	0.38	3.80	45.50	45.60		12.22
	ug/l			mg					ug

SUCCESSFUL BIRTHS

LOAD No.	Total Pb Burden	Ca % Dry Wt. Parent Young		Grass Consumed/Day	Grass Consumed/day % Dry Wt.	Pb P.P.M. Parent Young		Total Pb consumed
1	2.6	8.05	8.35	0.56	3.64	0.0	0.0	1.58
2	0.0	7.38	14.42	1.46	9.10	0.0	0.0	4.10
3	4.3	6.32	10.37	0.50	3.84	10.3	0.0	1.62
High Verge 4	0.0	8.23	--	0.52	6.43	0.0	0.0	1.91
5	17.2	7.30	17.66	0.538	4.43	18.55	11.11	1.75
1	5.0	9.84	17.50	1.050	14.73	0.0	178.5	0.92
2	4.0	11.18	10.20	0.76	8.15	5.3	13.00	0.58
Low 3	0.0	8.64	9.93	1.55	10.78	0.0	0.0	1.36
Low Verge 4	0.0	7.96	5.11	0.63	5.81	0.0	0.0	0.55
5	0.0	8.94	12.77	0.76	9.06	0.0	0.0	0.74
	ug/l			mg				ug

SUCCESSFUL BIRTHS



LOAF No.	<u>Fresh Wt.</u> Parent Young		<u>Dry Wt.</u> Parent Young		Days on Load	No. Young	Gross Consumed	<u>Ca level</u> Parent Young		<u>Pb level</u> Parent Young		Total Pb Burden
1	14.8	9.0	7.6	2.3	22	30	10.1	1.00	0.25	4.6	0.0	4.6
3	29.2	10.1	14.3	3.3	9	40	6.1	1.79	0.03	0.0	9.15	9.15
5	35.6	5.5	16.6	2.9	22	26	4.1	1.45	0.02	0.0	0.0	0.0
6	34.6	6.6	8.8	0.5	7	20	7.3	1.01	0.04	0.7	0.0	0.7
CON. 7	13.1	7.8	9.0	3.0	21	32	11.2	1.04	0.09	0.0	0.0	0.0
8	15.6	5.6	6.3	1.1	18	20	17.3	0.90	0.01	0.0	0.0	0.0
9	18.4	16.1	10.1	4.1	21	48	17.4	1.09	0.32	2.18	0.0	2.18
10	11.2	10.4	10.7	4.8	21	44	17.5	1.00	0.35	0.90	0.0	0.90
2	37.4	19.5	10.4	1.8	26	30	19.6	0.88	0.22	181.1	48.9	230.0
4	15.7	6.8	9.4	1.3	13	26	10.2	0.81	0.03	49.1	5.3	54.4
5	29.5	9.8	11.0	4.9	26	26	10.9	1.07	0.36	81.1	8.8	89.9
6	14.0	6.3	4.2	0.9	16	20	10.1	0.68	0.007	21.8	0.0	21.8
HIGH 7	25.0	30.6	6.7	3.5	21	40	16.0	0.78	0.41	88.0	7.0	95.0
8	17.0	12.6	10.4	4.2	21	36	14.5	1.05	0.08	87.4	0.0	87.4
9	19.4	13.7	11.7	4.1	21	36	14.5	1.26	0.26	80.0	0.0	80.0
10	16.9	9.0	6.5	1.7	18	27	12.0	0.88	0.02	49.5	0.0	49.5

UNSUCCESSFUL PREGNANCIES

LOAD	No.	Fresh Wt.		Dry Wt.		Days On Load	N <sub>2</sub> mg	Grass Consumed	Ca level		Pb level		Total Pb Burden
		Parent	Young	Parent	Young				Parent	Young	Parent	Young	
LOW	1	44.3	16.9	14.7	1.0	15	48	13.7	1.84	0.03	95.6	20.0	115.6
	2	76.5	10.9	21.4	2.0	11	40	9.5	2.74	0.03	20.8	164.4	185.2
	4	24.3	12.0	11.6	2.4	11	48	10.9	1.21	0.10	20.1	10.6	30.7
	8	15.8	10.6	8.3	2.0	21	36	15.9	1.03	0.02	37.0	2.3	39.3
	10	21.5	14.8	7.5	2.8	21	36	15.7	0.86	0.27	35.6	1.0	36.6
		mg	mg	mg	mg			mg	mM	mM	ug/l	ug/l	ug/l

UNSUCCESSFUL PREGNANCIES

LOAD No.	Total Pb Burden	Ca % Dry Wt. Parent Young		Gross Consumed/Day	Gross Consumed/Day % Dry Wt.		Pb p.p.m. Parent Young		Total Pb consumed
1	4.6	13.15	10.86	0.45	1.31		5.13	0.0	0.6
3	9.15	12.51	0.91	0.67	1.42		0.0	69.3	0.3
5	0.0	8.73	0.68	0.18	0.35		0.0	0.0	0.2
6	0.7	11.47	8.00	1.04	2.86		1.94	0.0	0.4
CON. 7	0.0	11.52	3.13	0.53	1.07		0.0	0.0	0.7
8	0.0	14.31	1.27	0.96	3.11		0.0	0.0	1.0
9	2.18	10.96	7.68	0.83	1.71		5.39	0.0	1.0
10	0.90	9.33	7.39	0.83	1.84		2.10	0.0	1.1
2	230.0	8.46	12.22	0.75	1.41		435.33	679.16	323
4	54.4	8.61	2.30	0.78	2.46		130.58	101.92	168
5	89.9	9.68	7.34	0.41	1.09		184.31	44.89	179
6	21.8	16.14	0.77	0.63	2.67		129.76	0.0	166
HIGH 7	95.0	11.70	11.68	0.76	1.94		328.26	50.0	263
8	87.4	10.14	1.95	0.69	1.78		201.09	0.0	239
9	80.0	10.83	6.43	0.69	1.28		173.07	0.0	239
10	49.5	13.58	1.58	0.66	1.83		190.38	0.0	197
	ug			mg					ug

UNSUCCESSFUL PREGNANCIES

LOAD No.	Total Pb Burden	Ca % Dry Wt. Parent Young	Grass Consumed/day	Grass Consumed/day % Dry Wt.	Pb P.D.M. Parent Young	Total Pb consumed
1	115.6	12.51 3.00	0.91	1.40	162.25 500.0	39
2	189.2	12.80 1.50	0.86	0.88	24.29 2055.0	27
4	30.7	10.43 4.16	0.99	2.40	43.31 110.41	31
8	39.3	12.49 1.00	0.94	1.97	111.43 28.75	46
10	36.6	11.03 9.71	0.82	1.71	114.10 8.92	45
	ug		mg			ug

UNSUCCESSFUL PREGNANCIES

Site	State	Mean Mg Conc.		Mean Ca Conc.		Mean K Conc.	
		mM		mM		mM	
1	Live	0.795		1.094		5.44	
	Dead	0.595		1.507		2.38	
2	Live	0.59		0.71		5.17	
	Dead	0.41		0.81		1.79	
3	Live	0.66		0.97		4.52	
	Dead	0.50		1.07		1.46	
4	Live	0.73		0.91		6.78	
	Dead	0.40		1.01		2.00	
5	Live	0.66		0.77		7.88	
	Dead	0.65		1.01		2.65	

#### APPENDIX IV

Mean levels of magnesium, calcium and potassium in samples of live and dead grass from the major sites. Two replicates, both  $\frac{1}{2}$  m from the road.

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AbstractLead in the Ecology of Roadside Verges

by

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The pattern of the deposition of lead (Pb) from motor exhausts on roadside vegetation from nine verges in rural Leicestershire is described. This is shown to be a function of proximity to traffic, reactions of airborne Pb with time and predominant wind characteristics. Splash from vehicles enhances Pb deposition next to the road.

Most Pb is bound by grass from solution. Live and dead grass are shown to have markedly different capacities to bind Pb, and this is attributed to the condition of the cuticle and the ability of Pb-rich solutions to penetrate intercellular spaces. The Pb content of both states of grass are shown to undergo seasonal changes, with a winter peak and summer low. This is a function of precipitation and standing time of the grass.

A mean estimate of  $5.2 \text{ g of Pb/m}^2/\text{year}$  is added to the soil on a site with 7000 vehicles/16h day,  $\frac{1}{2}\text{m}$  from the road. Input increases with precipitation and splash.

The invertebrate community of six verges were sampled with pitfall traps and Tullgren extractions, and their diversities compared. No decline in diversity could be attributed to Pb, though a greater diversity was apparent at a site which had been left unmown. Snails and Sminthurid collembolans may avoid areas of high Pb contamination, though woodlice numbers were low at all sites.

The sexes of the woodlouse Porcellio scaber are shown to differ in their Pb and Ca content, and an interaction in the uptake of the two metals is shown in both P. scaber and juveniles of the snail Helix aspersa. It is suggested that the digestive diverticula may be the main site of Pb and Ca absorption in both species. Toxicological experiments dosing P. scaber with sub-lethal levels of Pb could show no effect on the rate of oxygen consumption or fecundity. No increase in mortality in either P. scaber or H. aspersa could be attributed to a high Pb diet. The distribution of Pb in the tissues of adult H. aspersa is found to correspond to that of marine molluscs.

Few effects on the biotic components of roadside verges have been found, though it is suggested that Pb-tolerant plants may increasingly dominate the vegetation.