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STUDIES ON THE EFFECT OF LEAD POLLUTION FROM  
VEHICLE EXHAUST ON THE ROADSIDE ECOSYSTEM

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Submitted as part of the requirements for the  
degree of M.Sc. (Advanced Course in Ecology) in  
the University of Durham,  
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## INTRODUCTION

### General

Every gallon of petrol sold in Britain today contains around 2 grams of lead in an organic form, T.E.L. or tetra-ethyl lead. It is there as an anti-knock agent to prevent spontaneous ignition of the fuel mixture under compression; motor vehicles run more smoothly because of it and fuel consumption is consequently reduced. To produce petrol of equivalent octane rating without the additive would involve an additional expense of 1-2 p/gallon. When the petrol is burnt, some lead is retained in exhaust system deposits and some is discarded during changes of lubricating oil and filters, but the greatest part, 75% or more, is emitted as an aerosol of fine particles of inorganic lead compounds. Detectable amounts of organic lead are also present in the exhaust gases.

As a consequence there are elevated levels of lead in air, water, soil, vegetation and our bodies. It is unlikely there is any part of the surface of the earth that is unaffected; measurable quantities of lead in Polar snows can be shown to be wholly due to the activities of man.

Lead is one of the most insidiously toxic of the heavy metals, a cumulative poison that interferes with virtually all metabolic processes and is especially harmful in its organic form. Since it acts in a number of different ways, there is no agreement over what may be considered the toxic threshold of lead for the human body. Monier-Williams (1949), a toxicologist, writes:

"Consideration of the toxic limits so far as these are defined by the appearance of symptoms of poisoning, is beside the point and tends to obscure the real question. What we would like to know is not so much the toxic limit as the safe limit, if indeed any limit, however small, for a cumulative poison can be regarded as safe." Concern has increased with the conclusion of Patterson (1965) that "the average resident of the United States is being subjected to severe chronic lead insult," and the work of Bryce-Smith (1971) associating environmental lead pollution in Britain with increased admissions to mental hospitals.

Almost nothing is known of lead levels in animals, other than man, and of their ecological implications. The N.E.R.C. Report (1969) on the deaths of sea birds in the Irish Sea found abnormally high levels of lead, as well as mercury, arsenic and poly-chlorinated-bi-phenyls (P.C.B.'s), in the tissues of corpses recovered but was unable to say which single pollutant, if any alone, was responsible for the disaster.

#### Sources of lead pollution

Lead may be present in the atmosphere due to either natural or man-made causes. Patterson (1965) lists the six most important natural sources as: silicate dusts from natural soils; volcanic halogen aerosols; volcanic silicate smokes; forest fire smoke; aerosolic sea salts and meteoritic smokes. An estimate of their combined effects accounts for only 1% of the lead present in rural air and less than 0.01% of that in city air.

The present annual production and consumption of lead in the world is around 3 million tons, of which the U.S.A. consumes 40% (approx. 15 lb. per capita). American usage of lead doubled between 1925 and 1964; increased use in anti-knock

compounds and for storage batteries more than offset decreased use in paints, pigments, building materials and insecticides. Lead in petrol, at an average concentration of 1.9 g/gallon in the regular grades and 2.56 g/gallon premium (420 and 565 ppm respectively), accounted for 223,000 tons of lead in 1964, 16.5% of the U.S.A.'s consumption for that year.

Britain's petrol sales rose from 1,700 million gallons in 1960 to 3,100 million in 1970, which must have released some 7,000 tons of lead into our air last year. Since the advent of lead additives in 1923, it has been estimated that 2.3 million tons of lead in its organic form have been burnt in the northern hemisphere; if evenly distributed over that part of the earth's surface, and all brought down by rain, this would amount to 10 mg Pb/sq.m. (Chow and Johnstone, 1965).

A much smaller amount, and proportion, of lead is "lost" to the environment through other industrial and technological processes. The manufacture of lead paint and storage batteries releases into the atmosphere only 0.1-0.01% of total production (Caldin 1966) whilst the use of lead arsenates as pesticides has greatly declined. The burning of coal, which may contain 10-15 ppm of lead, will make an additional contribution, but in power stations, and other industrial uses, the majority of this will be collected by filters.

There has been no serious challenge to the conclusion of a number of workers that the principal source of environmental lead pollution is that added to petrol (e.g. Lagerweff, 1967; Danielson, 1970). Proof has recently been provided by the application of the 'isotopic ratio finger-printing technique' whereby the relative proportions of the lead isotopes  $^{204}$ ,

206, 207 and 208 are used to identify a particular lead ore or pollution source. The work of Chow (1970, 1971) has shown a close correlation between the isotopic lead ratios in petrol and those in the soil of roadside verges in California, and in the air or soil of ten major cities in Europe, America and Asia. The correlations are closer for air than for soil since there is a higher background of natural lead in the latter. Biological material has yet to have its source of contamination identified by this technique.

#### Nature of the lead pollution from vehicle exhausts

When petrol is burned in an internal combustion engine, most, but not all, of the lead alkyl is decomposed to inorganic lead. Ethylene dibromide and dichloride, also added to petrol, "scavenge" the lead from the engine in the form of volatile lead halides after combustion. The principal product is lead chloro-bromide,  $PbCl.Br$ , but there are in addition three complexes of this chemical with ammonium chloride, viz.  $\alpha NH_4Cl.2PbCl.Br$ ;  $\beta NH_4Cl.2PbCl.Br$ ; and  $2NH_4Cl.PbCl.Br$ . Traces of two other compounds, lead sulphate,  $PbSO_4$ , and a lead oxide-lead halide complex,  $PbO.PbCl.Br.H_2O$ , may also be found.

The relative proportions of these compounds vary according to the make and age of the engine and the temperature of the exhaust gases. Full throttle acceleration, when exhaust gas temperatures are higher, produces mainly lead chloro-bromide, whereas driving at lower speeds, e.g. in cities, favours the formation of ammonium-lead-halides. Two further variables that depend on the driving conditions are the proportion of input lead that is emitted in the exhaust at any time, and the size of the particles that are formed. Fast

driving and rapid acceleration may release up to 20 times the lead content of the petrol used, since lead that accumulated in exhaust system deposits during slower driving is also discharged, and the size of the particles is correspondingly larger.

The characteristics of exhaust emission under different driving conditions may be summarised as follows, the data taken from Hirschler and Gilbert (1964):

	% wt. of Pb compounds in exhaust		% lead input emitted	size of particles
	lead-chloro-brom.	amm.-lead-halides		
rapid acceleration	85-90%	10-15%	900-2,000 %	40% > 5 $\mu$
slower driving	approx. 50%	approx. 50%	30-55%	25% > 5 $\mu$

Hirschler and Gilbert (1964) also measured the ratio of inorganic to organic lead in the exhaust of one particular car. Using a series of filters and scrubbers to differentiate between the two forms, they collected 1,000 times more of the inorganic than the organic lead. Only 0.023% of the input lead was emitted in the organic form, considerably less than the proportion of unburnt hydrocarbon fuel. This is suggestive that the heat resulting from compression is alone responsible for the decomposition of a considerable proportion of the lead alkyls.

It is not known whether the car tested was typical; the proportion of organic lead in the urban atmosphere has been reported as being considerably higher, approx. 2% and up to 10% (Danielson, 1970). This may well be due to the more



rapid sedimentation of the larger of the inorganic particles.

Little is known about the length of life of the lead halide complexes once they are emitted. They are not very stable compounds and probably break down quickly to simpler lead chlorides, bromides and oxides. Once deposited on the soil, these may change to a variety of inorganic lead compounds, many of very low solubility.

#### Extent of lead pollution

The amount of lead that is mined and subsequently introduced into our relatively small urban environments each year has been calculated to be over 100 times greater than the amount leached naturally from soils and added to the oceans of the world (Chow and Patterson, 1962). Nine-tenths of the lead in the upper, mixed, zones of the open oceans of the Northern Hemisphere is likely to have had technological origins (Tatsumoto and Patterson, 1963), and the atmosphere of the Northern Hemisphere contains about 1,000 times the "natural" concentration of lead in air (Patterson, 1965).

A study of lead in annual strata of snow in Northern Greenland elegantly demonstrated the extent of global contamination and the relatively recent increase in airborne lead. Levels became detectable in snow deposited in 800 B.C. and rose only slowly until 1750 A.D. Around that time, with increasing industrialisation of many countries of the world, the rate increased, reaching 0.07 microgm/kg (parts per thousand million) by 1940. In the next 25 years it rose rapidly, increasing 300% to 0.22 microgm./kg in 1965. The lead content of the South Polar ice sheets, two low for detection before 1950, is now at 0.02 microgm./kg. (Murozumi, Chow and Patterson, 1969).

A similar "historical lead gradient" has been demonstrated in biological material by Ruhling and Tyler (1968), who have followed the rise in lead content of woodland mosses of Southern Sweden in museum specimens dating from 1860.

#### Toxicology of lead

The toxic effects of lead are very complex. There is no single harmful effect that is unique to lead (making identification of low level damage difficult) and the toxicology of organo-lead compounds is seemingly unrelated to the toxic effects of the lead ion. There exists the possibility of synergistic effects; levels of lead that would alone be harmless may be dangerous in the presence of other heavy metals or pesticides. Furthermore, vulnerability of an organism to damage is known to vary at different times of its life.

Inorganic lead has a wide range of effects on brain, peripheral nerves, blood, kidney and the vascular system. It is known to interfere with the production of red blood cells by affecting an enzyme, ALA-dehydratase, involved in haem synthesis (Hernberg and Nikkanen, 1970); it "follows" calcium to the skeleton, whence it is mobilised in pregnancy and in ageing (Hardy, 1965) and it can cause morphological changes in mitochondria and abnormalities in ribosomes (Westerman et al, 1965). The increased numbers of sterile marriages, and of pregnancies ending prematurely or in still birth, of lead workers in the 19th century demonstrate the effects of lead on the germ cells and its abortifacient action on the foetus.

There is now increasing awareness that the greatest risk of all may be to the mental development of the young. The analysis of British health statistics for the period 1964-68

by Bryce-Smith (1971) has shown that the greatest increase in admissions to mental hospitals and units occurred in the youngest age-groups, e.g. an increase in 100% for girls and 60% for boys in the 0-10 age group over the 4 year period. Bryce-Smith acknowledges that he has no proof of ~~causal~~<sup>causal</sup> relationship between the rise in mental disorder and the increase in urban lead pollution, but such a well-defined rise in illness predominantly in the younger age groups cannot be explained convincingly on purely procedural grounds (e.g. changes in admission policy or better diagnosis). He believes that it is highly suggestive that during the past few years some new causative influence, to which children are particularly vulnerable, has been increasingly at work. The dangers of lead poisoning in the young are greater since growing tissues are more susceptible to interference, homeostatic mechanisms are not so well developed, and growing bone is more labile. A course of chelation therapy has been used successfully in Switzerland to reduce blood levels of heavy metals in adults (report in The Guardian, May 19th, 1971) but the treatment must be used with caution and cannot reverse any damage already caused to the central nervous systems of children.

There are still those who believe that lead pollution is a negligible hazard to health. Kehoe (1961, 1964), working at the Kettering Laboratories, Cincinnati (a research institution primarily supported by the Lead Industries Association and Ethyl Corporation of America), maintains that the body adjusts to preserve a "lead balance" and that "the supposed chronic lead intoxication from environmental contamination is a myth not a fact." The experiments on which he bases his conclusions are unlikely to be repeated; they involved

increasing, by several times, the average daily intake of lead, by ingestion and inhalation, of human subjects with apparently no ill effects. The subjects were healthy male adults, neither the sex nor the age-group most susceptible, and he was noticing only the overt, clinical symptoms of lead poisoning. Kehoe is not, however, without supporters; Haley (1966) writes:

"Lead compounds in car exhaust may not be available, or if available not absorbed, or if absorbed not retained..... no contribution is made by them to the body burden of lead."

The toxic threshold for lead in the blood proposed by Kehoe, 0.8 ppm, is approximately double the levels which other workers believe are dangerous to exceed, e.g. 0.36 ppm (Moncrieff et al 1964) and 0.5 ppm (Egli et al 1957). Enzyme inhibition has been shown to occur even over the range 0.2-0.4 ppm (Hemberg and Nikkanen, 1970; Miller et al, 1970) i.e. at the levels now present among the general urban population. The conclusion by Bryce-Smith (1971) that "no other toxic chemical pollutant has accumulated in man to average levels so close to the threshold for overt clinical poisoning " seems unavoidable.

The effects of Organic lead have been poorly studied. It is known to be much more poisonous than inorganic lead, the toxic effects bearing a closer resemblance to organo-tin compounds than those of the (inorganic) lead ion. It is also absorbed readily through the skin and handled quite differently in the body. Cremer (1959) has shown tetra-ethyl lead to be toxicologically inert in in vitro biochemical systems, but in vivo becomes de-alkylated (in the liver of vertebrates) to much more toxic tri-ethyl lead compounds.

These produce their main effect on the brain.

Symptoms of organo-lead poisoning that have been described are given by Barnes and Magos (1968), they include (in man) irritability, emotional instability, insomnia, bad dreams and loss of appetite, all of which may easily pass unnoticed. In more severe cases there may be bizarre psychic disturbances that resemble alcoholic intoxication, schizophrenia or violent paranoia. Restlessness, tremors and excitability have also been produced in experimental animals.

In view of the difficulty in diagnosing organo-lead poisoning and of the insidious nature of the effects, the use of organo-lead compounds as pesticides can only be deplored by all those concerned with our health and with the environment. There are, however, indications that they may be used increasingly as such in the future. Willemsens (1964) writes: "The well known, and perhaps over-emphasised, toxicity for humans cannot be an excuse for their apparent neglect, since several other human toxic substances have been <sup>developed</sup> ~~described~~ in the fight against pests." (It should be noted that Willemsens works for the International Lead Zinc Research Organisation, established to investigate new uses and applications for lead and zinc in all areas of technology).

#### Lead in soil and vegetation

All soils contain significant amounts of lead. Their content varies according to the levels of lead in the parent material and various physical factors, such as the rate of weathering. Old Red Sandstone and Magnesian Limestone, for example, naturally produce soils with relatively high lead contents. The normal range would appear to be 10-200 ppm, but there is usually less than 50 ppm. Swaine (1955) has estimated the average for the earth's crust at 16 ppm.

Other workers that have studied natural lead levels in the soil are Jones and Hatch (1945); Prince (1957); Wright (1955) and Vinogradov (1959).

Soils with unnaturally high levels of lead may be found for some distance around lead mines (Donovan, Feeley and Canavan, 1969) and near industrial works using lead e.g. battery smelters (Marten and Hammond, 1966). There have also been a number of recent studies that have effectively demonstrated significant accumulations of lead in soils adjacent to highways, e.g. Warren and Delavault (1960); Cannon and Bowles (1962); Kloke and Riebartsch (1964); Cholak et al (1968); Ruhling and Tyler (1968); Motto et al (1970); Page and Ganje (1970). That the source of the lead contamination is that added to petrol has been confirmed by the isotopic studies of Chow (1970). There is close agreement between the findings of the different studies; their conclusions may be summarised as follows:-

1. The amount of "excess lead" in the soil is a function of traffic density and the time that there has been for accumulation to occur.
2. The highest accumulations are to be found within a few feet of the edge of the road, there being very little effect above 100 ft. away. Exact distribution will be affected by topography and the nature of the prevailing winds.
3. The surface inches of soil are the most highly contaminated. There, levels of lead may be 200-250 ppm in excess of those naturally present. Penetration into the soil is poor; below 2 ft. no elevation of lead levels in the soil have been recorded.

Such results are indicative of a rapid fall-out through

the air of the heavier, lead-containing, particles emitted from vehicle exhaust. On reaching the soil, highly insoluble compounds are formed that are not easily removed by leaching.

Concern that crops grown adjacent to well-used roads might be containing levels of lead that are dangerous to human health has initiated a number of studies on the accumulation of lead in vegetation. For example, the work of Cannon and Bowles (1962); Ruhling and Tyler (1968); Maclean et al (1969); Kleinman (1968); Ter Maar (1970); and Lagerwerff (1971).

Their results are complex and sometimes contradictory; it would seem that there are a number of complicating factors that make it difficult to draw generalisations. There are two sources of contamination, through the air, to the leaves, and through the soil, to the roots. Some contamination may only be superficial - washing the vegetation before analysis may remove half the lead from the aerial parts. Different plant groups accumulate heavy metals to a far greater extent than others; for example, bryophytes (depending to a far greater extent on precipitation and dust sedimentation as a source of their mineral supply) accumulate 5 to 10 times more than vascular plants on the same sampling site. Even among crop plants significant differences occur between different species and between different parts of the same plant. Furthermore, Mitchell and Reith (1966) have described a hundred fold increase in lead content between June and October with the same plant material, but without a concomitant change in either soil or air contamination. This finding renders uncertain all comparison of levels of lead in vegetation from different areas sampled at different dates.

The main conclusion to be drawn is that most of the contamination occurs within 75 ft. of the road, where crops

may contain 5-500 ppm lead. Levels of lead in the semi-natural vegetation of the roadside verge may exceed 1000 ppm. This compares to the 0.4-2.0 ppm lead found in plant material growing on uncontaminated sites. There is <sup>no</sup> legislation in Britain to control the amount of lead in foodstuffs, but 5 ppm is the recommended limit of the World Health Organisation. It would seem irresponsible for Ter Haar (1970) to conclude that there is no cause for concern: "Since leafy vegetables only form a fraction of the average U.S. diet, their high lead content is insignificant in comparison with the total lead ingested." The effects on vegetarians are disregarded.

Further investigation is needed to determine the exact nature of the relationship between lead in the soil, in the air and in the plant. There is reason to believe that plant uptake may be more closely related to soluble than to total lead; plants grown on soils of different lead concentrations take up very different amounts according to whether the lead is already in the soil or has been added as a soluble salt (Ruhling and Tyler, 1968). Brewer (1966) estimates that only 0.05-5 ppm of lead <sup>in the soil</sup> may be soluble, the amount being poorly correlated with total lead concentration. Keaton (1937) found a very high degree of fixation, i.e. conversion from soluble to insoluble forms: 3 days after adding approx. 3000 ppm. lead, as lead nitrate, only 17 ppm could be extracted in an acetate/acetic acid solution.

Nothing is known of the fate of organic lead in the atmosphere or what proportion of it there may be in the soil. If the figures given for the proportions of organic to inorganic lead in car exhaust and in the atmosphere are substantially correct, its dispersal may be more widespread than that of inorganic lead and the area contaminated correspondingly greater.



### AIMS OF STUDY

Since awareness of the problem of lead pollution has developed only recently, it is inevitable that there should be many aspects that have yet to be investigated. While it is understandable that the principal interest should be the possibility of danger to human life, this aspect would seem to have completely overshadowed study of the effects, if any, of lead pollution on all other species of animal life. Although there is other work now in progress, there have been, apparently, no published studies by ecologists on either the effects of lead on a natural fauna or whether there is any accumulation of lead in natural food chains. There are few toxicological studies of either inorganic or organic lead except on man and a limited number of experimental animals.

The present study was intended to help fill some of these gaps in our knowledge, within the time available. The aim was to study the soil and ground-living invertebrate fauna of roadside verges, living in conditions where lead accumulation is expected to be at its greatest, in order to see whether this might be affecting the mortality and distribution of the major groups. This could then be related to the levels of lead in soil, vegetation, invertebrate and vertebrate material.

The soil fauna of a disused lead mine was also to be studied, as well as the changes in soil fauna resulting from experimental treatment of a small area of semi-natural vegetation by soluble and insoluble lead salts.

## STUDY SITES

### 1. Weardale Lead Mines

The lead ores of the North Pennines have been worked for many hundreds of years, the period of most intensive mining being in the 18th century. Work largely ceased at the turn of the century due to the lowering of world prices by the large-scale importation of foreign ores. Some of the mines have since been re-opened to supply the need for fluorspar. (White, 1967)

Spoil heaps from disused lead mines were studied at Langdon Common (NY 849338) and at two sites near the village of Westgate (NY 903393 and NY 902393). All three sites had long ceased to have been worked. The spoil heaps had been colonised by a poor ground vegetation, chiefly Agrostis and Festuca spp., and were being grazed by sheep.

### 2. Roadside verges

Sites were sought that would give a reasonably uniform environment, i.e. without any major habitat discontinuities, for 100-150 ft. from the edge of a well used road. At that distance it was expected that the lead content of the soil should be near its natural level. Since only motorway verges are usually of this scale, it was necessary to sample from permanent pasture adjacent to the verge at two or the three sites. The three sites were:

- (1) South Road, Durham City (NZ 273413), west verge and adjoining field
- (2) A.1, at the Catterick By-Pass, North Riding of Yorks. (NZ 220007), west verge and adjoining fields
- (3) A.1(M) near Sherburn, Co. Durham (NZ 301423), west verge

Permission to work on the roadside verges of the City and County of Durham was obtained from Mr. H. Stone, the City Engineer, and from Mr. J. R. Tully, the County Engineer and Surveyor, respectively; and for those of the North Riding of Yorkshire from Lt.Col. G. A. Leech, County Surveyor, with the additional sanction of the Divisional Road Engineer of the Department of Environment.

The study on the motorway verge had to be discontinued when it was learnt that special dispensation for such work was required that the County Engineer was not, at that time, prepared to grant.

The verge at South Road is approximately 25 ft. wide where the study was made. Fencing and a recently planted hedge<sup>of</sup> hawthorn separates it from a field that extends towards the back of St. Mary's College for a further 200 ft. The field was cut for hay on 16th July, 1971, but until then supported a mixed and varied flora closely resembling that of the roadside verge; hogweed, Heracleum spondylium, was especially plentiful. See Plates 1 and 2.

Although this site was the most convenient of the three, especially for small mammal trapping, it was not ideal. The verge had been re-seeded after road improvements some three years ago and the field showed signs of earlier disturbance. A series of irregularities in its surface became obvious when the field was cut. The dumping of colliery waste was suspected from the former Elvet Colliery (less than a quarter of a mile to the north, on land now occupied by the Science Site of the University) but there is no indication of this on early 1 : 2500 scale Ordnance Survey sheets.

The County Engineer and Surveyor gave the following figures for traffic flow:

August, 1967                      5,200 vehicles per day

August, 1971              est. 5,500 vehicles per day

There was no record of application of herbicides.

The Catterick By-Pass of the A.1 was opened to traffic in 1959 and is a dual carriageway road with hard shoulder. The verge slopes, with a gradient of approx. 1 in 4, and is some 75 ft. wide. Again a low hawthorn hedge (4 ft.) and fencing separate it from the field that extends for a further 115 ft. See Plates 3 and 4.

In late spring the vegetation of the verge and field appeared to be similar in species composition and development. As the season progressed it became increasingly obvious that the soil of the verge was poorer and more porous, the micro-habitat drier and the vegetation coarser. The difference in the vegetation was not quantitatively surveyed. Superficial investigation indicated that those species common to both field and roadside verge (in mid-July) included Lolium perenne, Poa pratensis, Trifolium repens, Achillea millefolium, Heracleum spondylium, Plantago lanceolata, Senecio jacobea and Rumex crispus. Those found in the field, but not the verge, included Dactylis glomerata, Trisetum flavescens, Trifolium medium, Ranunculus acris, Medicago lupulina, Bellis perennis, Prunella vulgaris, Vicia sepium and V. cracca; and those on the verge, but not the field, Festuca rubra, Cynozurus cristatatus, Taraxacum officinale and Chrysanthemum eucanthemum. The field was not cut but was grazed by sheep from the end of July.

The County Surveyor estimated that the traffic flow was currently around 33,800 vehicles per day. No herbicides had been used in the past few years.

The A.1 Motorway is of much more recent construction.

The stretch as far as Sherburn was opened in April, 1969, and had linked up with the old A.1 at Chester-le-Street by September of that year. The verge at Sherburn is some 200 ft. wide and has a slope estimated at 1 in 6. The flora consisted of short grasses, presumably of the Ministry of Transport seed mixture (H.M.S.O., 1957), with few weed species.

No data <sup>are</sup> is yet available for the amount of traffic using this stretch of road, but it is likely to be in the order of 25,000-35,000 vehicles per day.

### 3. Experimental Plots

These were established, with the permission of the Assistant Surveyor to the University, on an area of disused allotments between South Road and St. Mary's College (NZ 274415). They are within 250 ft. of South Road. The vegetation predominantly consisted of coarse grasses, especially Arrhenatherum Elatius.

In addition to the sites mentioned above, soil sampling and small mammal trapping were also carried out in and adjacent to the Zoological Department Field Station, Hollinside Lane (NZ 274406).

Plate 1 South Road looking South, showing flora of verge  
and field

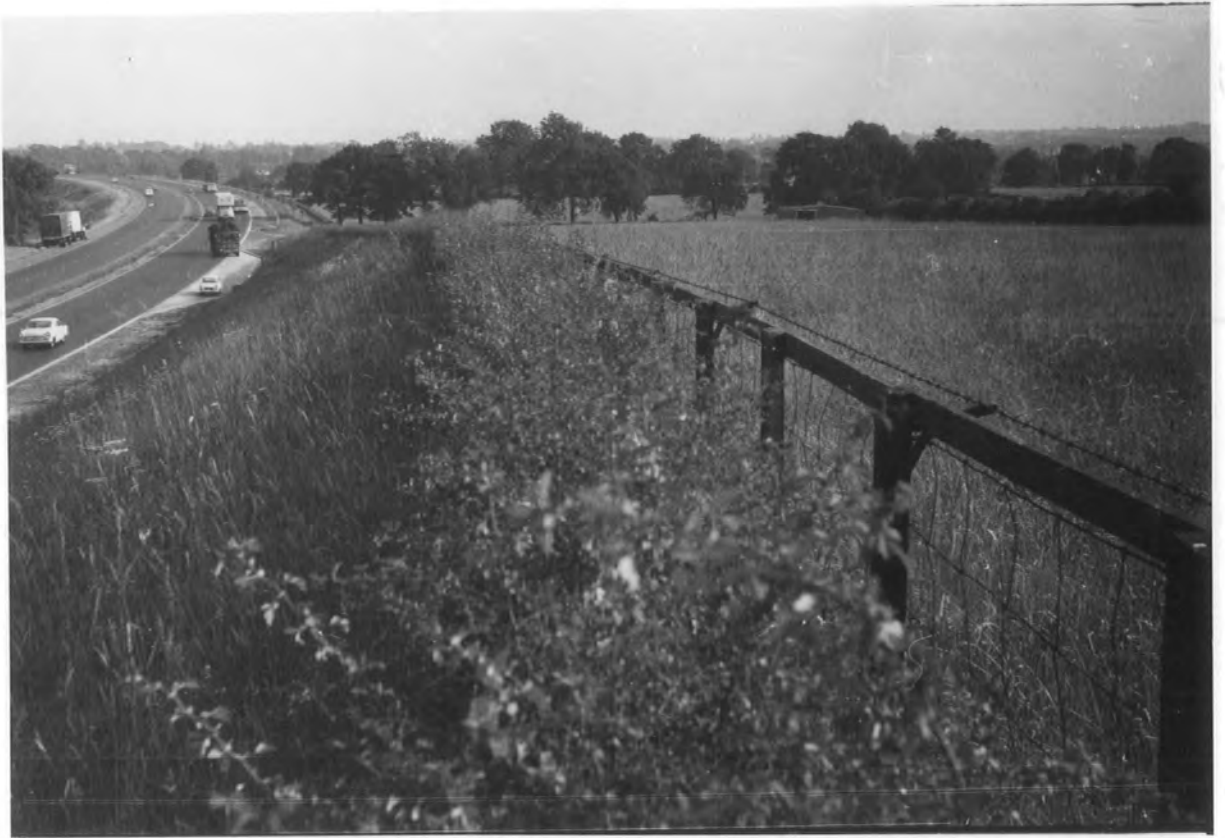
Plate 2 South Road looking North



Plate 3 : Catterick By-pass looking south, showing verge  
and field.

Plate 4 : Catterick By-pass looking north, showing verge.





## METHODS

### 1. Sampling techniques

#### (a) Invertebrates

The invertebrate fauna was sampled by extraction from soil cores and trapping with pitfalls. Neither method was wholly satisfactory; Macfadyen (1962) writes: "Any sampling scheme represents a balance in demands of accuracy and taxonomic range against resources in time and equipment..... The simultaneous sampling of all groups is quite impracticable." Extraction of soil cores was made with the core inverted in a Berlese-Tullgreen type funnel for 7 days. Since the method relies upon the animal's own mobility, only mobile stages can be obtained in this way. Those animals living in the soil water, e.g. enchytraeid worms, require a more specialised extractor device.

Soil cores, 11.5 cm. in diameter and to a depth of 8-10 cm., were taken from the spoil heaps of the lead mines, from the A.1(M) at Sherburn, and from the experimental plots. The voltage of the 60 w. lamps of the extractor funnels was increased from 120 v. to 240 v. by two increments of 60 v., and the animals were expelled into 70% alcohol solution. It was found that their sorting was extremely time consuming and, furthermore, insufficient bulk of material was collected to allow analyses of body lead content. When it was established that total numbers (of all species) of Collembola and Acarina were not greatly affected by the levels of lead in the soil, the remainder of the trapping was by the use of

pitfalls.

Pitfall trapping collected a greater quantity of material, primarily those animals moving over the surface of the ground. Since the size of the catch depends upon the behaviour and activity of the animals, it does not give an accurate measure of population size. The activity of beetles has been shown to be a function of several factors, including temperature and breeding condition (Briggs, 1960) and presence of pesticides e.g. D.D.T. (Dempster, 1968). The method is, however, widely used and can provide useful results if its limitations are realised. It is most important that the size of the catch should only be compared with another made at the same time and under approximately similar conditions.

Pitfall traps were used to sample the ground fauna of the three roadside verges and of the experimental plots. On the roadside sites, series of jars were sunk in lines running parallel to the edge of the road and at various distances away from it. The jars had a mouth diameter of 5.5-5.8 cm. A small quantity of 2% formalin was used to preserve the material and to prevent predation. They were left in position for 7-10 days before collection.

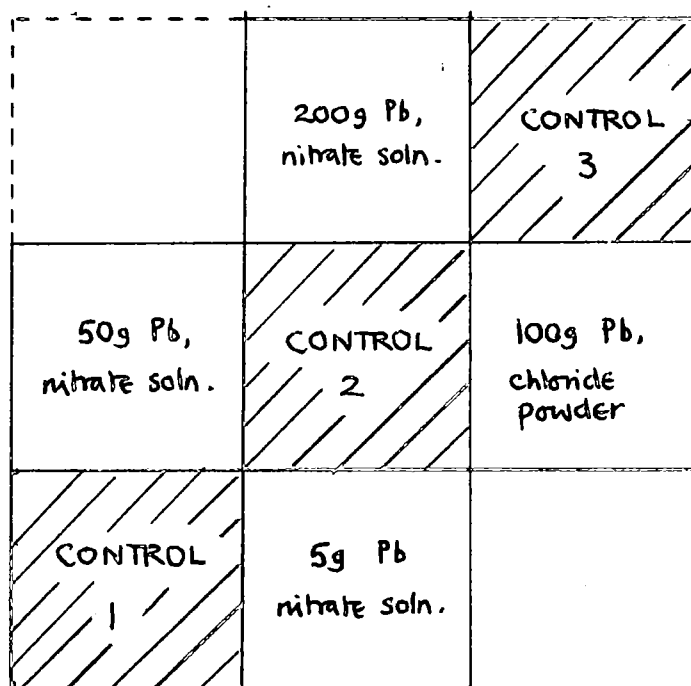
After sorting, the animals extracted from the soil cores and those caught in the pitfalls were kept in 70% alcohol until their analyses.

(b) Vertebrates

Small mammals were caught by the use of unbaited Longworth traps, visited daily. Those animals alive were killed with ether on cotton wool. The liver was removed and frozen until analysed. The remainder of the body was also kept frozen and the spleen and kidney were later removed.

## 2. The Experimental Plots

The area available was divided into 9 plots, each of an area of 90 sq.ft. (approx. 10 sq.m) Solutions of lead nitrate, containing 5 g., 50 g., and 200 g. of lead, were applied to three of the plots, and lead chloride powder, containing 100 g. of lead, to another. Of the remainder, three were sampled as controls and two were left unsampled. The layout of the plots was as shown below:



The nitrate solution was applied in 9 litres of water with a watering can. Four parallel crossings of the plot were made, walking backwards so as to avoid treading on areas that had just previously been sprayed. The three control plots received 9 l. of water in a similar manner. The 100 g. of lead, as lead chloride powder, was thoroughly mixed with several kg. of dry soil before application and the mixture scattered as a top dressing over the surface of the soil. Estimations were made of the extent to which the levels of lead in the surface soil might be raised, according to the depth of penetration, viz:

Amount applied	Increase in lead content of soil (ppm), assuming equal mixing to a depth of -			
	1 cm.	5 cm.	10 cm.	20 cm.
5 g.	50	10	5	2.5
50 g.	500	100	50	25
100 g.	1000	200	100	50
200 g.	2000	400	200	100

Light rain (c.0.2 inches) fell two days after the plots were sprayed. Four days after spraying, six pitfalls, arranged hexagonally, were set in each of the four experimental plots and the three controls. They were left in position for a further ten days. During this period two soil cores and two soil samples were taken off each plot.

Whenever the plots were visited, it was always in the same sequence, passing from the controls to the treated plots, but not vice versa, to avoid cross-contamination.

### 3. Analysis of lead

#### (a) Extraction

The lead content of the various materials was determined by Atomic Absorption Spectrophotometry. In order that they might be analysed they had first to be in an aqueous solution.

For soil there are a variety of methods used by different workers. No two are the same and some are extraordinarily complex e.g. Pawluk (1967); Mitchell (1964). Since a comparative value for soil lead content, rather than absolute accuracy, was required, the relatively unsophisticated mixture of nitric, hydrochloric and perchloric acids was used, as detailed below.

The soil samples were either taken directly from the site or else were sub-samples of soil-cores after extraction. In either case the sample (approx. 50-100 g.) was taken from

the top 2-5 cm., the surface vegetation having been removed and discarded. Soil taken directly from the site was oven dried at 110°C. until of constant weight (2-3 days). This was not necessary for soil that had been a week in the Berlese funnels.

Material from both sources was then loosely broken and a 5 g. sub-sample, accurately weighed, was taken from that that passed through a 0.2 mm. sieve. To this was added, in a 250 ml. conical flask, 5 mls. Perchloric Acid (60%)

10 mls. Hydrochloric Acid (36%)

and 20 mls. Nitric Acid (70%)

Analar Grades were used, Atomic Absorption Grades being unavailable. An electrically regulated sandbath was used to heat the mixture, gently at first, to prevent excessive frothing, and then more strongly, to drive off the acid fumes. This was continued for 3-4 hours when the bulk of the soil had been digested and the liquid was clear. De-ionized water was added to maintain the level of the liquid. At the end of the digestion, the mixture was filtered into graduated conical flask and made up to the mark with de-ionized water. Samples from the lead mines were made up to 250 ml. and all the others to 100 ml. They were then transferred to stoppered polythene bottles in which they were stored until analysed.

The few samples of vegetation that were taken were treated similarly to soil.

Samples of invertebrates (whole bodies) and vertebrate tissue were transferred to small pieces of aluminium foil and weighed before and after drying in an oven at 110°C. The material was then further transferred to 50 ml. flasks and the foil reweighed to obtain the wet and dry weights of the samples. Since much less than 5 g. of material was available,

correspondingly less acid was used. The mixture of acids was used on the first set of samples (invertebrates from South Road) but only Nitric on subsequent occasions. This was on the separate advice of Jefferies, of Monkswood, and Cameron, of Swansea (in lit.) who are both independently engaged in determining levels of heavy metals in biological material. It was found that 10 mls. of Nitric Acid completely dissolved 0.5 g. of tissue after less than half an hour of gentle heating. Digestion was continued for several more hours to reduce the acidity of the mixture, adding de-ionized water when necessary. The solution was then heated more strongly, until only 0.5 ml. remained, and was then made up to 5-10 mls. (accurately measured) with de-ionized water before filtering into the small sample tubes in which it was stored.

Using such a method Jefferies (pers. comm.) reports losses of less than 5% with samples of "spiked" liver tissue at 1, 5 and 10 ppm added lead.

The solution was still highly acid ( $\text{pH} \leq 1$ ) and concern was expressed that it might cause corrosion of the burner head. Further dilution would have reduced the levels of lead to values below which they could be measured for many of the samples. Instead an attempt was made at neutralising the solution for the first set of samples (invertebrates from South Road) by the use of Potassium Hydroxide pellets. The same number were also added to the acid blank controls. Unfortunately the amount required exceeded the solubility product of the potassium salts formed and a loose, white precipitate resulted. This was present when the volume of solution was made up to a known amount but was removed on subsequent filtration. The method was consequently abandoned for all further samples since there was the risk of losing

some of the lead in the precipitate and hence introducing a source of error of an unknown magnitude.

On all subsequent occasions the material was dissolved in the minimum of acid that was required (approx. 10 times its own weight) and the solution kept at 90°C. on the sand bath for at least 4 hours. Whenever levels of lead were high, the dilution factor was correspondingly increased. The solutions were only allowed to run through the spectrophotometer for the minimum time necessary to obtain a steady reading. De-ionized water was then run through for several minutes before reading the next sample.

Special attention was paid that all glassware should be spotless. Whenever biological material was being analysed, all the apparatus used had been previously washed in diluted Nitric Acid, rinsed in de-ionized water and dried. Soil contamination of the invertebrates could not be wholly avoided; two sources were recognised, viz:

1. That external to the animal; small soil particles adhering to the surface, especially if this was covered by hairs. Most, however, could be removed by rapid agitation in the 70% alcohol solution. That remaining was not thought to introduce a significant error unless the surface area to volume ratio was particularly large i.e. the smallest animals, or those with exceptionally long appendages e.g. Opiliones.
2. That internal to the animal, i.e. within the gut. The earthworms could be de-gutted but this was not practical for other groups. It remains to be investigated how much soil is accidentally ingested by soil living carnivores, herbivores, litter-feeders, etc., and whether this might significantly affect measurement of whole body heavy metal content.



(b) Analysis

Analyses were made on a Pye-Unicam SP 90 Atomic Absorption Spectrophotometer. An air/acetylene flame was used with a slit width of 0.1 mm. and wavelength of 283.3 ~~nm~~ nm. The wavelength 217 nm is the more readily absorbed but interference, especially by light scattering, is greater. The Pye-Unicam Handbook (1966) gives further details of the use of this apparatus.

A master stock solution, at 5,000 ppm lead, was prepared by dissolving 3.995 g. of lead nitrate in 200 ml. de-ionized water and making up to 500 ml. From this, by successive dilutions, was prepared the standards used, at 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 20 and 24 ppm. lead. A new standard curve, at least for the range 1-16 ppm, was prepared for each series of analyses. A typical standard curve is shown (Fig.1).

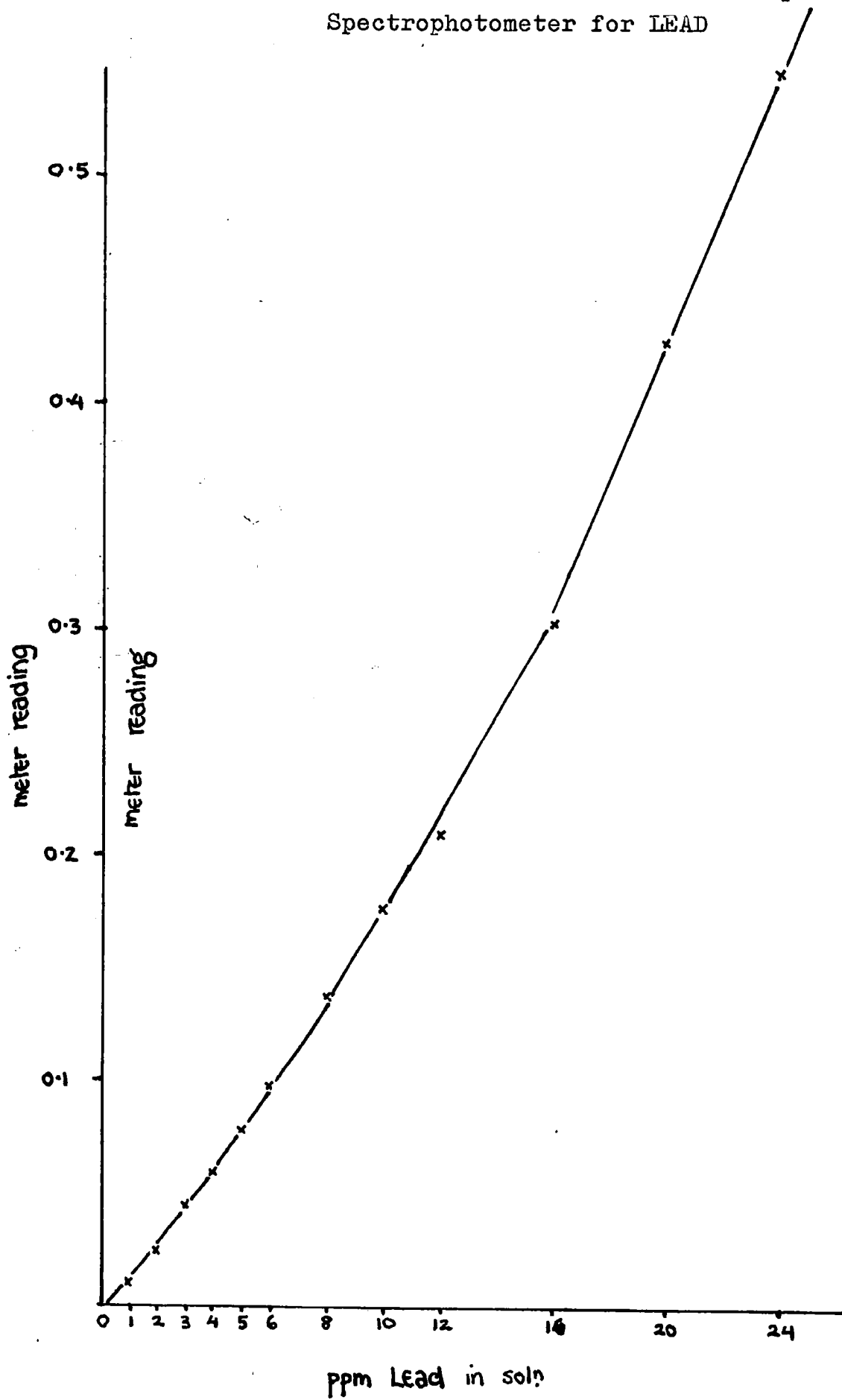
A pair of acid blanks were included with each series of samples in order that contamination ~~of~~ by the acid ~~and~~ during the extraction procedure might be allowed for. When potassium hydroxide was used to neutralise the samples, 1 ppm lead was found in the blanks. Otherwise they contained less than 0.3 ppm, near to the limit of detection of the machine. N.B. The theoretical sensitivity is "that concentration of element in aqueous solution that gives actual absorbance of 1%" (i.e. meter reading on the 0 to 100 scale reduced from 100 to 99). It is a function of conditions within the flame itself and does not depend on either the monochromator or the light sensitivity of the detector. For the machine used the sensitivity is given as 0.7 ppm (for lead). This should be distinguished from the limit of detection - that concentration that gives a signal equal to full noise amplitude. It was found that levels of lead in solution that were less

than 0.2-0.3 ppm could not be reliably measured. If the material had been diluted 10-20 times in preparing the solution, this is equivalent to levels of lead in the original material of around 5 ppm dry weight.

Chemical interference effects are said to give very little trouble when working at concentrations 20-200 times the limit of detection, but become increasingly important when "working near the limit." The reliability of results of less than 10 ppm dry weight is consequently not very great.

Fig. 1 :

Typical Standard Curve for SP90 Atomic Absorption Spectrophotometer for LEAD



## RESULTS

### 1. Weardale lead mines

Six soil cores were taken from the overgrown spoil heaps of Langdon Common and two from adjacent rough pasture. Their fauna was extracted and the amount of lead in the soil analysed. The results were inconclusive; they showed wide variations in the numbers of soil invertebrates apparently uncorrelated to the lead content of the soil.

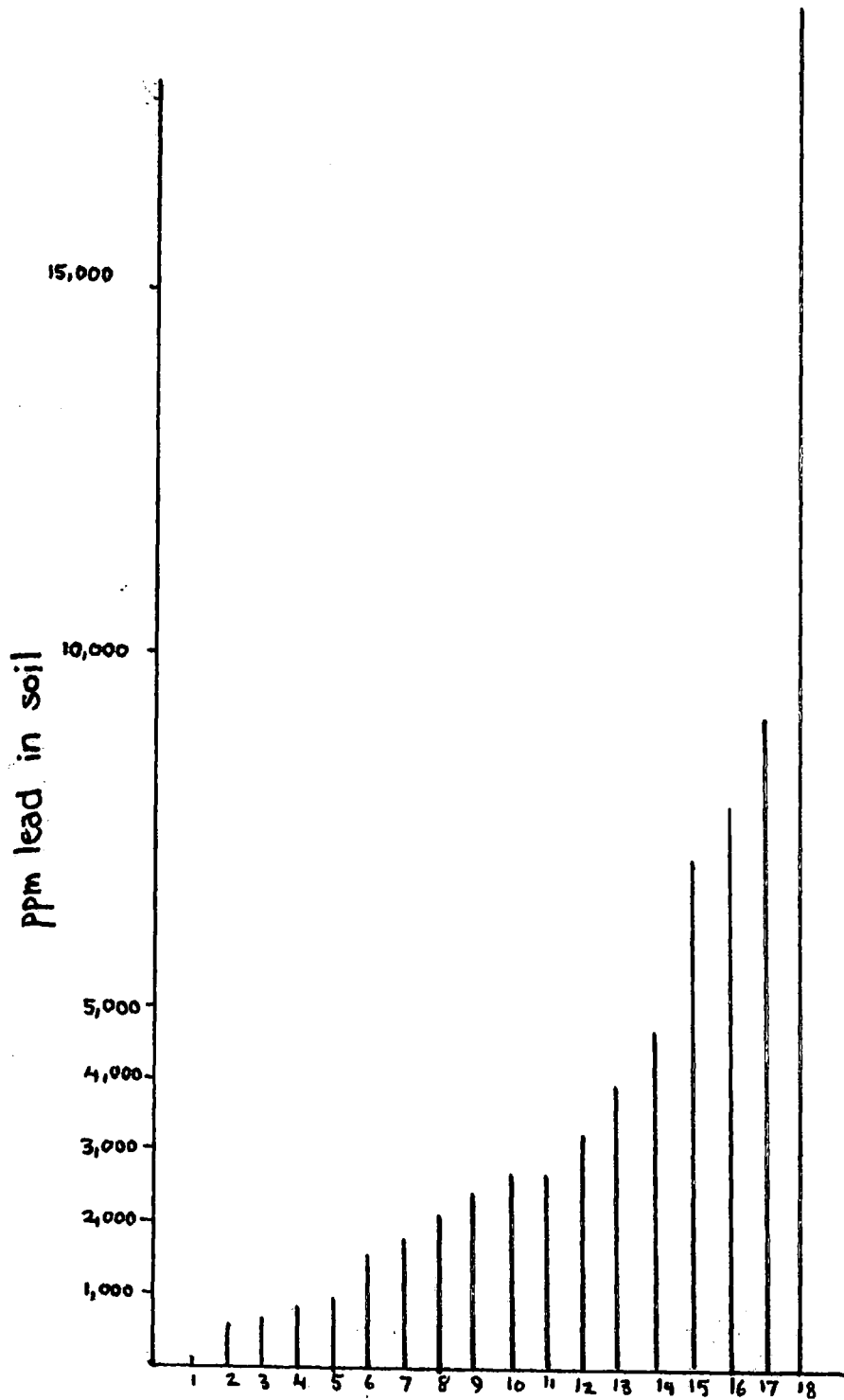
Consequently a further 18 cores were taken from the two sites at Westgate. The conditions at the two sites were considered sufficiently similar to allow the 18 samples to be re-arranged to present one continuous series of increasing lead content, from less than 200 ppm to nearly 2,000 ppm Pb (Fig.2). The results of the extractions are displayed in Table 1 and graphically in Figs. 3 and 4. Correlations between the number of individuals of each group and the lead content of the soil were examined using Kendall's rank correlation coefficient,  $T_B$ . Only the mites (*Acarina*) showed a significant correlation, ( $0.05 > p > 0.02$ ), but this was for an increase in numbers with increase in lead. It is highly improbable that any biological significance can be attached to this finding.

The concordance of the samples (agreement between the groups over what constitutes a favourable or unfavourable habitat) is highly significant,  $p \leq 0.001$ .

The levels of lead in plant and animal material were several orders of magnitude less than those found in the soil.

Two samples of mixed (unwashed) above-ground vegetation gave levels of 22 and 24 ppm lead when analysed, and a single sample of coleopteran material 13 ppm (dry wt.). Insufficient material of other groups was collected to permit analyses.

Fig. 2 : Lead Content of Westgate Soil Samples



Samples, re-arranged in order of increasing lead content

KEY:

sample	ppm lead	sample	ppm lead	sample	ppm lead
1	165	7	1,800	13	3,850
2	655	8	2,100	14	4,500
3	680	9	2,550	15	7,100
4	800	10	2,625	16	7,750
5	900	11	2,700	17	9,000
6	1,700	12	3,050	18	19,000

Table 1 : Westgate

Numbers of individuals extracted from each of 18 soil cores

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Acarina	340	236	239	167	248	221	232	385	250	457	113	284	352	312	416	402	277	374
Collembola	159	392	324	252	187	147	280	334	227	180	102	275	265	152	62	243	358	268
Coleoptera	86	1	2	2	1	1	4	2	13	8	5	2	19	1	4	3	3	2
Coleoptera larvae	6	13	2	1	5	13	15	1	16	4	2	3	20	2	4	9	11	7
Diptera I.	-	7	3	1	1	2	3	3	5	1	5	-	-	2	-	8	4	3
Hemiptera	16	3	11	1	5	5	13	1	13	5	-	6	5	19	20	6	7	19
Thysanoptera	2	5	2	3	2	3	5	3	2	2	-	1	16	1	-	2	5	8
Chilopoda	1	-	4	-	6	1	12	-	-	3	3	7	7	2	2	-	23	1
Araneae	-	2	-	-	2	-	-	-	1	1	7	-	-	-	2	-	-	-
Oligochaetae	1	1	2	1	-	2	-	-	-	-	6	-	-	-	-	2	-	-

The samples are arranged in ascending order of lead content; the key on

fig. 2 gives the amount of lead that each contained.

Fig 3 ; Soil Invertebrates extracted from Westgate  
Soil Cores

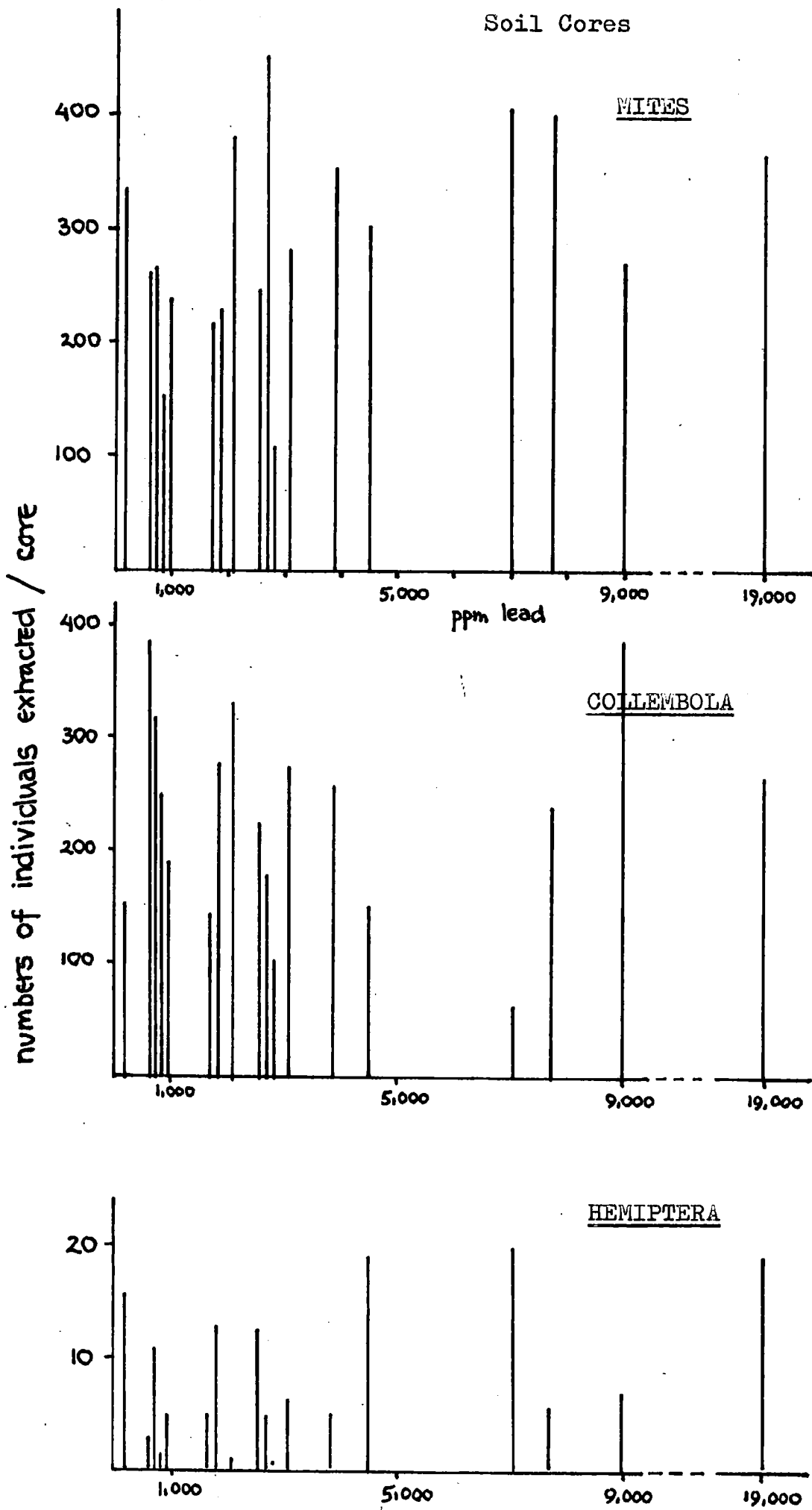
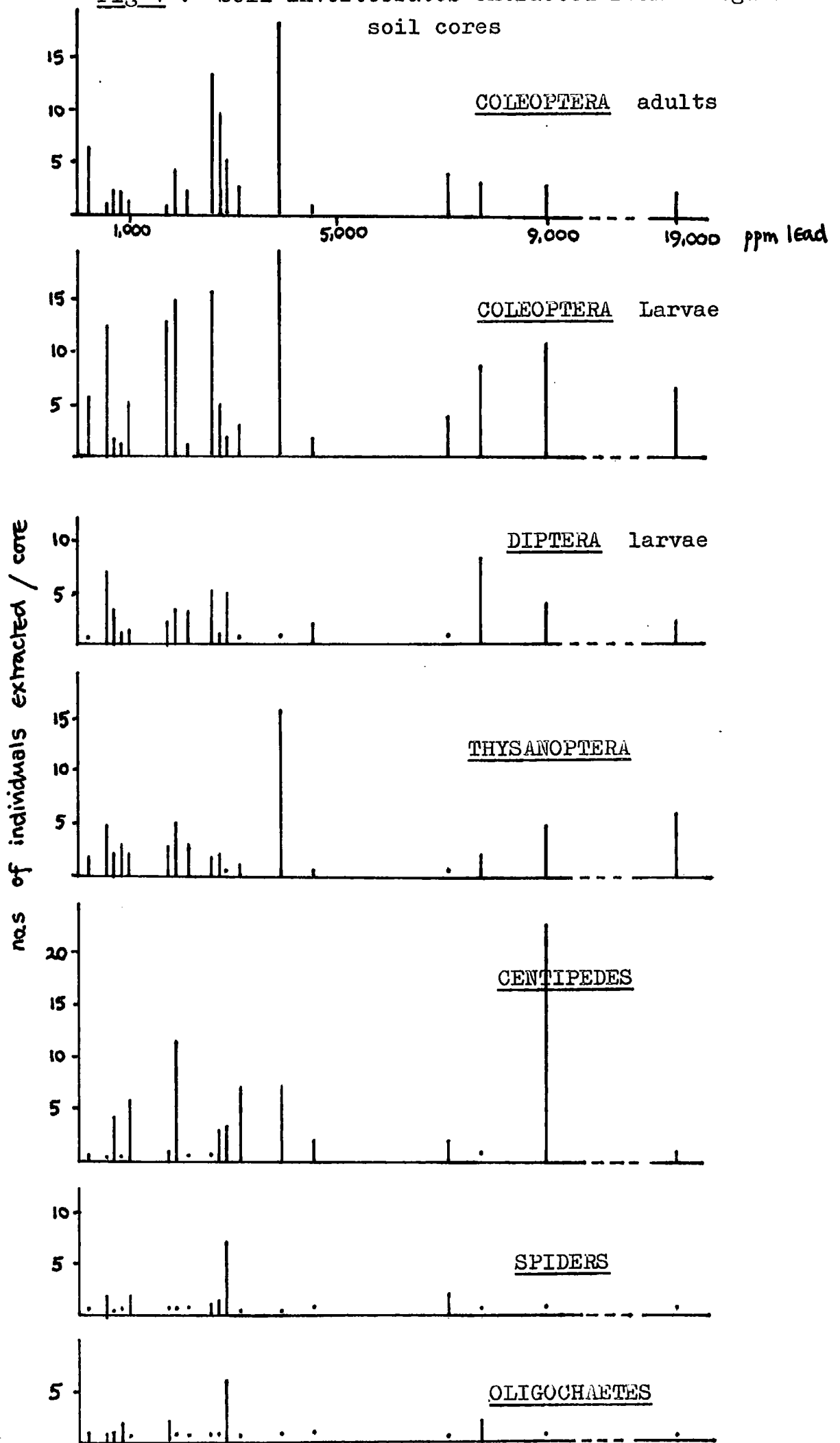




Fig 4 : Soil Invertebrates extracted from Westgate soil cores



## 2. The A.1(M) at Sherburn

Soil samples were analysed from soil cores taken at four stations, A-D, 15, 30, 60 and 115 ft. from the edge of the hard shoulder. A progressive decline in lead content of the soil was found.

ppm. lead in soil

Position	A	B	C	D
distance, from road, ft.	15	30	60	115
soil lead ppm.	109.7	102.7	85.7	84.3
± standard error	6.5	3.2	2.3	3.5

The "Students t-test" was used to test for the significance of these differences. Although there was no significant difference between either A and B or C and D, the differences between B and C ( $p = 0.01-0.02$ ) and A and D ( $p = 0.02-0.05$ ) were both significant.

Six pitfalls were set at each of the stations A, B and D. The results of the soil core extractions are presented in Table 2 and of the analysis of the pitfall catches in Table 3.

Since the t-test is valid only for normal or near-normal distributions, it would be <sup>inappropriate</sup> ~~inapplicable~~ to use it to test for the significance of differences in animal numbers from different samples. There are two reasons why a departure from normality might be expected. Firstly, the distribution of each species in the soil is more likely to be aggregated than random (as demonstrated by the work of Cole, 1946, and Hairston, 1959), and, secondly, when only low numbers are present in the sample the distribution is necessarily truncated. The simplest alternative, the chi-squared test, may be used on the totals of the original data but not on

any quantities derived from them.

If the null hypothesis is that there is no difference between the sampling stations across the Motorway verge then there is reason to reject the null hypothesis for many of the animal groups. For some, this is an effect of large numbers that the taking of more samples might even out. In other cases, especially the surface living groups and the vegetation feeders (e.g. Thysanoptera, Hemiptera, Aphids, Lepidopterous larvae), there is evidence of a real decline in numbers, not the nearer they are to the Motorway but the further they are away from it. There is no group for which the combined totals of stations A and B (high lead content) are significantly less than those of C and D (natural lead content). For the pitfalls the data from station C are lacking; nevertheless in no case are the combined totals for A and B significantly less than twice those of D. The toxic effects of lead, if any, are lost under the influence of other more important factors determining the distribution of the fauna of the verge.

It is possible that drift of chemical sprays used on an adjacent crop of cereal, less than 20 ft. from station D, are responsible for this effect. This could not be proved or disproved without further sampling, which, without special permission, would constitute a breach of Motorway regulations. Consequently the investigations at this site had to be prematurely discontinued.

Table 2: A.1(M) at Sherburn

Total no. individuals extracted from three soil cores  
at each of four stations

	A	B	C	D	$\frac{A + B}{C + D}$
Acarina	152	146	208	137	0.86 N.S.
Collembola	307	277	375	99	1.23
Thysanoptera	354	295	270	101	1.75
Staphylinidae	12	2	2	3	2.80
Other Coleoptera	2	1	2	1	(1.00)
Coleoptera larvae	11	41	12	7	2.74
Diptera larvae	2	5	9	5	0.50 N.S.
Aphids	202	120	27	4	11.10
Other Hemiptera	25	41	9	7	4.76
Araneae	11	17	15	4	1.47
Chilopoda	3	3	1	-	(6.00)
Diplopoda	1	1	1	-	(2.00)
Oligochaetes	6	1	3	5	0.88 N.S.
Opiliones	1	1	-	1	(2.00)

Table 3: A.1(M) at Sherburn

Total no. individuals caught  
in six pitfall traps at each of three stations

	A	B	D	$\frac{A+B}{2D}$	
Staphylinidae	68	33	26	1.94	
Carabidae	43	16	17	1.74	
Other Coleoptera	2	6	6	0.67	N.S.
Coleoptera larvae	1	2	1	(1.50)	
Diptera larvae	0	2	2	(0.50)	N.S.
Lepidoptera l.	15	9	0	(inf.)	
Aphids	73	25	4	12.25	
Other Hemiptera	6	8	3	2.67	
Lycosidae	12	12	4	3.00	
Other Araneae	131	84	49	2.19	
Chilopoda	0	1	0	(inf.)	
Diplopoda	11	5	9	0.89	N.S.
Oligochaetes	1	3	2	(1.00)	
Opiliones	24	23	7	3.34	

Small samples bracketed. Significance of decreased numbers at stations with increased lead tested;

N.S. = not significant.

### 3. Cattorick By-Pass

Soil lead was analysed from the central reservation and from six other positions at various distances along a 300 ft. transect from the edge of the road. Samples of mixed (unwashed) above-ground vegetation were collected from five positions along the sampling transect. The results unequivocally show a decline in lead content with increasing distance from the road, closely resembling the pattern of that found by other workers.

ppm lead in soil and vegetation  
(see also Figs. 5 and 6)

Position	Central reserva- tion	Verge			D	Field	
		A	B	C		E	F
distance from road, ft.	-	9	33	65	85	165	300
soil lead, ppm.	203	111.5	102	70.5	70.0	69.0	70.5
± st. error	7.0	6.5	8.0	1.5	2.0	1.6	0.5
vegetation lead, ppm	-	135.0	32.5		18.0	10.5	8.0
± st. error	-	23.0	5.5		3.0	1.5	1.0

Using the t-test, significant differences are to be found between the levels of lead in the soil of the central reservation and station A ( $p = 0.001-0.01$ ), and between those of station B and C ( $p = 0.02-0.05$ ). There is no evidence for any accumulation of lead in the soil at station C i.e. the levels are down to background across the distance of the verge itself.

Pitfalls were set at the five stations designated A-E; the first three occupying positions of near-verge, mid-verge and far-verge, and the latter two at 10 ft. and 90 ft. into the field. Initially five pitfalls were set at each station, in a line each 6 ft. away from the next. When these were collected they were replaced by eight jars in each row.

The data from the two occasions are combined and presented as totals for 13 jars (Table 4 and Figs.7-9).

In examining the results it is important to bear in mind that the existence of a correlation between the levels of lead in the soil and the numbers of a particular group does not prove that the two are causally related. The effect of other factors has first to be examined and assessed, or, preferably, their variation eliminated in an experimental situation.

The results indicate that the change from verge to field represents a discontinuity of far greater importance to nearly all animal groups than the effects, if any, of increased lead in the soil. In passing from verge to field there are abrupt increases in the catches of ground beetles (Carabidae) and most groups of spiders (the Lycobidae and Thomisidae are exceptional in this respect), but equally abrupt decreases in the catches of ants (Formicidae), woodlice (Isopoda) and harvest-men (Opiliones). To examine the effects of lead more critically, one has to compare the totals caught at the three stations of the verge. Only the ants (sp. Myrmica ruginodis) show a consistent decrease in numbers when approaching the edge of the road. Statistically the result may be highly significant, ( $p < 0.001$ ), but this is not especially meaningful when dealing with such highly aggregated, colonial, insects.

There is every reason to support that the siting of their nests is governed primarily by such factors as soil texture, cover and microhabitat, and that in these respects station C is preferable to B, and B to A. The slope of the verge would itself be sufficient to provide the necessary variation in conditions across its width. There are almost no ants to be found in the field.

There are other groups that show a decrease from C to B, i.e. across the region of greatest increase in the concentrations of lead in soil and vegetation. These include the ground beetles, beetle larvae, woodlice and millipedes (Diplopoda), but none of the differences are significant when compared with the  $\chi^2$ -test. There are, however, significant increases between B and A for the Hemiptera ( $p < 0.001$ ) and the millipedes ( $p = 0.01-0.02$ ).

Table 4: Catterick

Total numbers of individuals caught in 13 pitfalls at each of 5 stations

	A	B	C	D	E
Staphylinidae	120	125	114	111	97
Carabidae	48	46	55	163	147
Other Coleoptera	60	79	45	74	51
Coleoptera larvae	31	26	39	60	41
Diptera larvae	2	4	-	-	-
Lepidoptera larvae	3	-	2	-	2
Symphyta larvae	3	4	1	11	23
Formicidae	39	81	300+	1	1
Other Hymenoptera	3	5	5	3	5
Hemiptera	64	27	11	32	18
Lycosidae	53	42	29	14	8
Thomisidae	13	17	11	8	2
Other Araneae	47	45	42	205	159
Opiliones	173	206	119	51	29
Isopoda	165	155	188	9	3
Diplopoda	58	34	39	13	27
Chilopoda	5	4	3	2	1
Gastropoda	1	2	-	2	2
Orthoptera	-	1	4	-	-
Oligochaetae	2	1	1	2	3



Fig.5 : Lead Content of Soil at Catterick

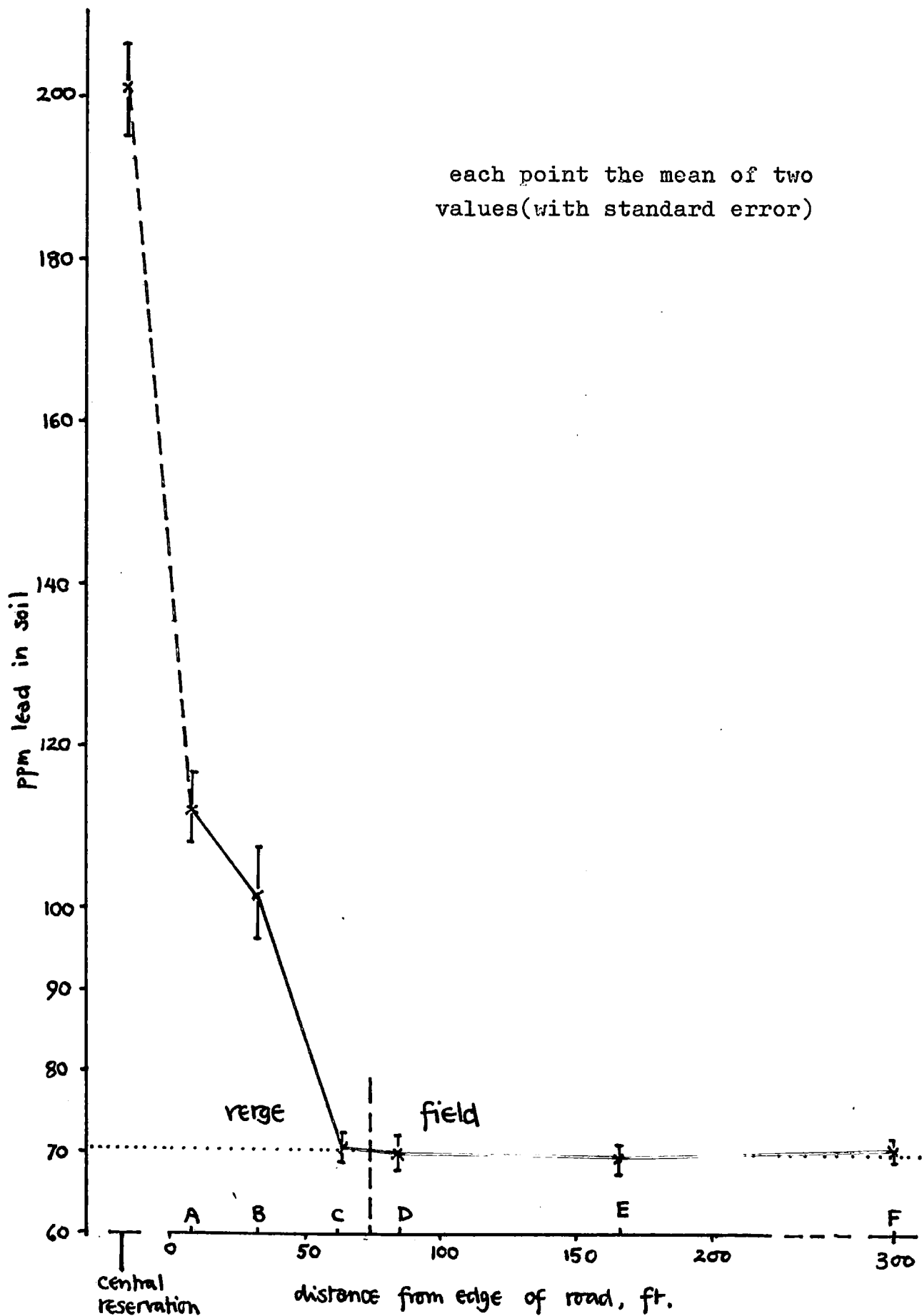


Fig. 6 : Lead Content of Vegetation at Catterick

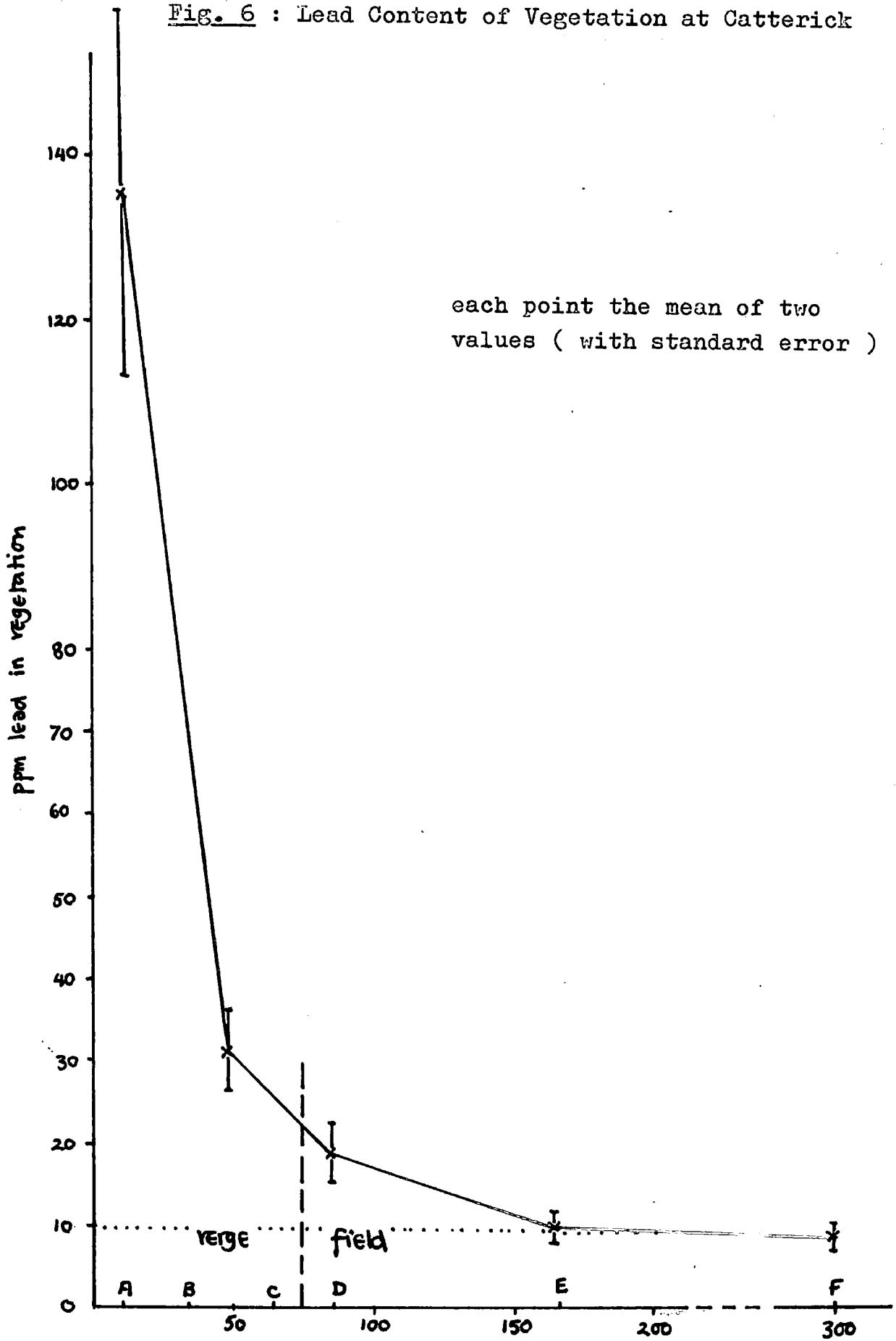


Fig. 7 : Invertebrates caught in Pitfall Traps

( Catterick )

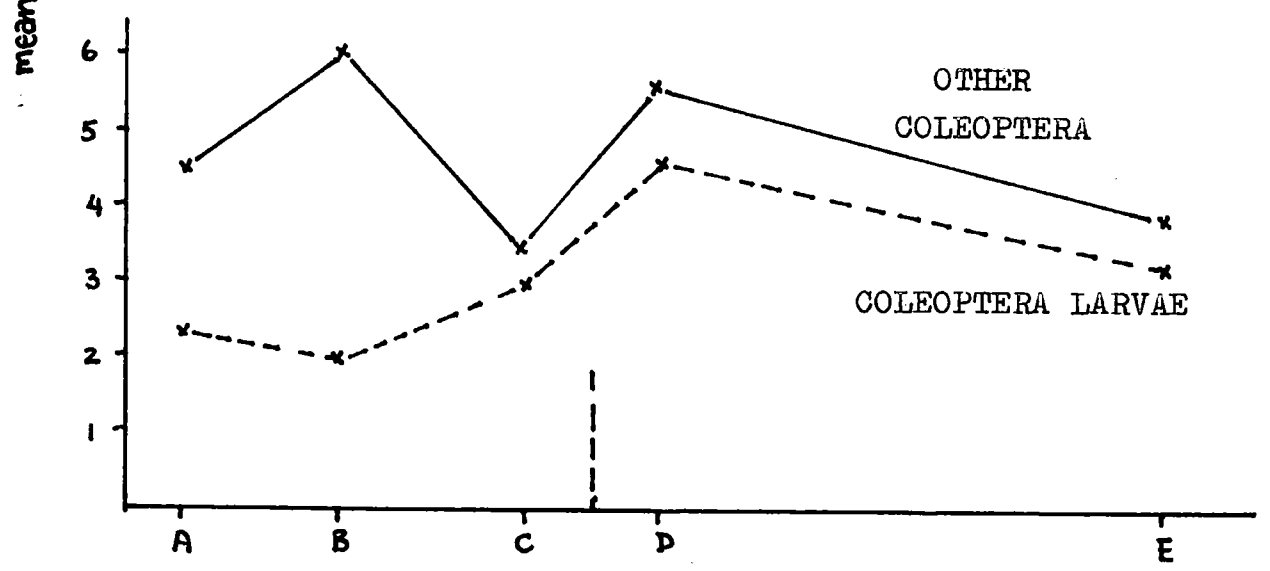
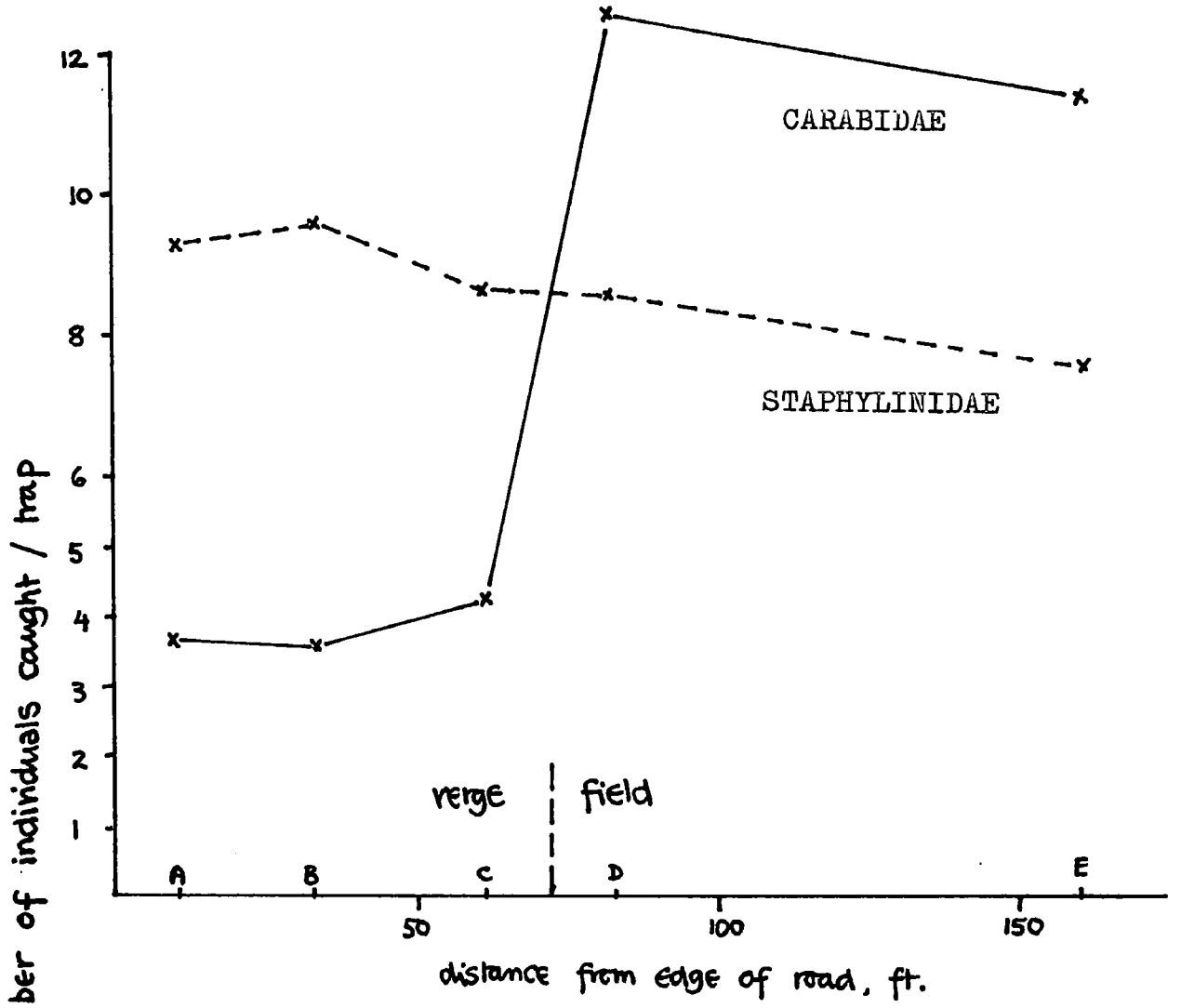


Fig. 8 : Invertebrates caught in Pitfall Traps

( Catterick )

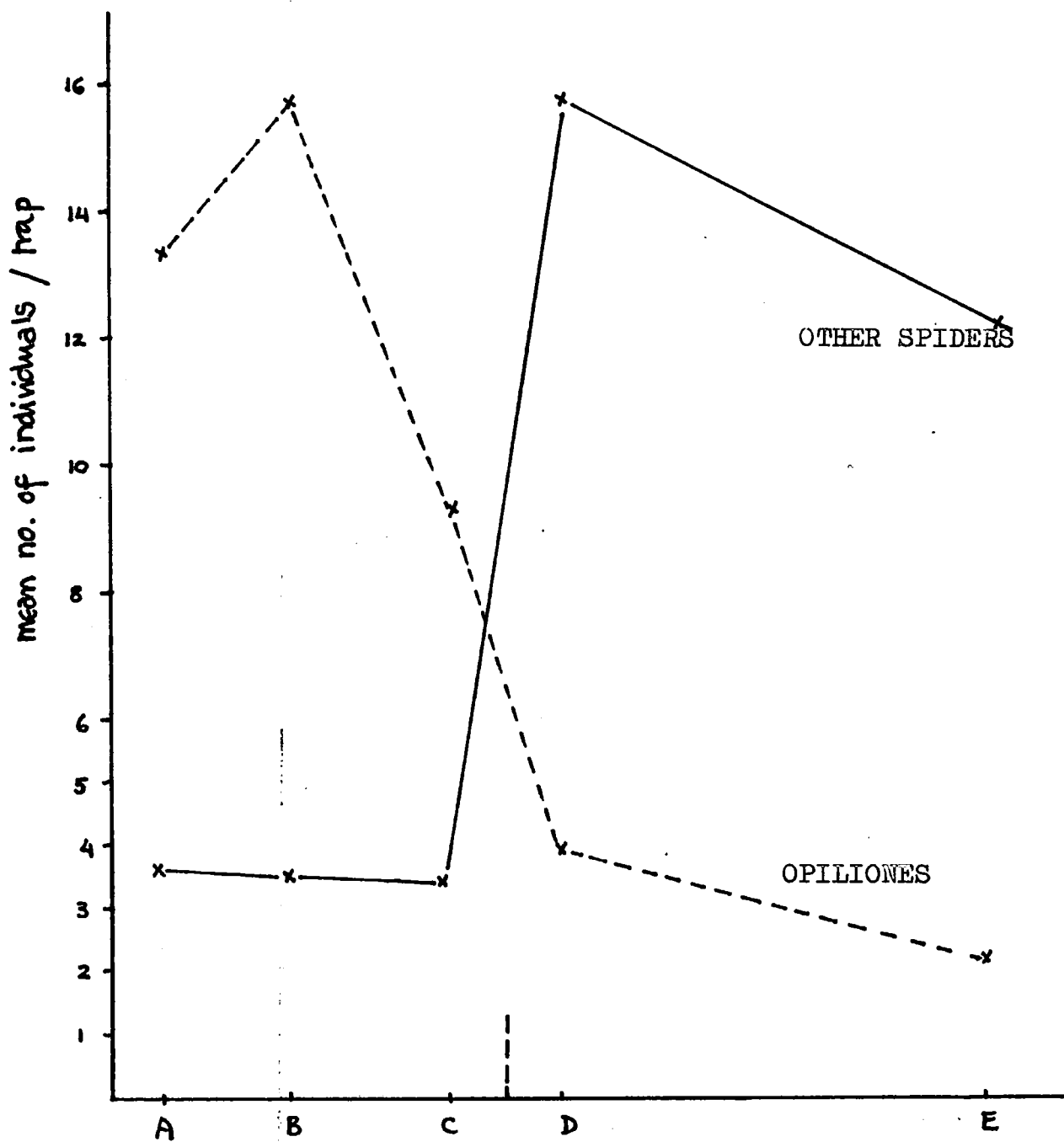
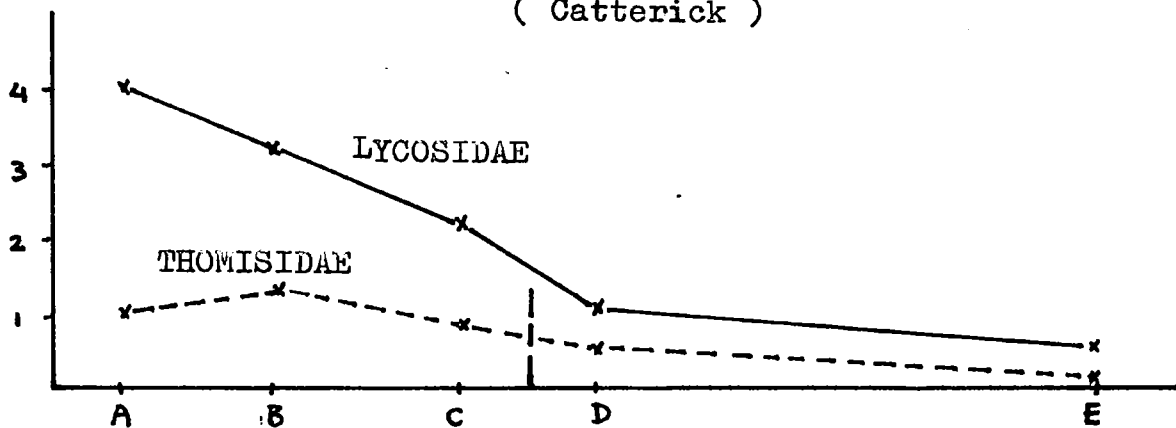
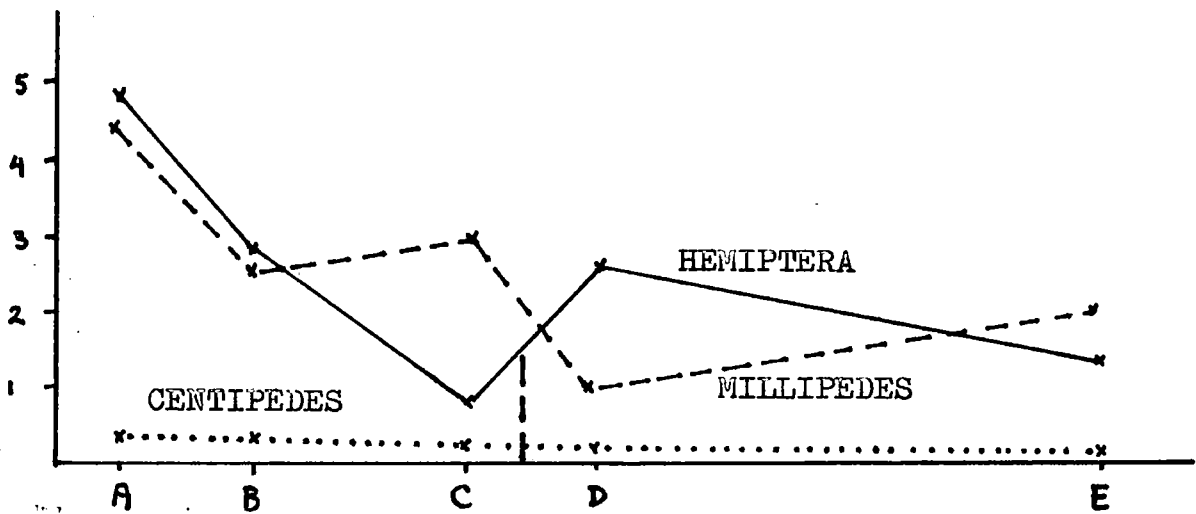
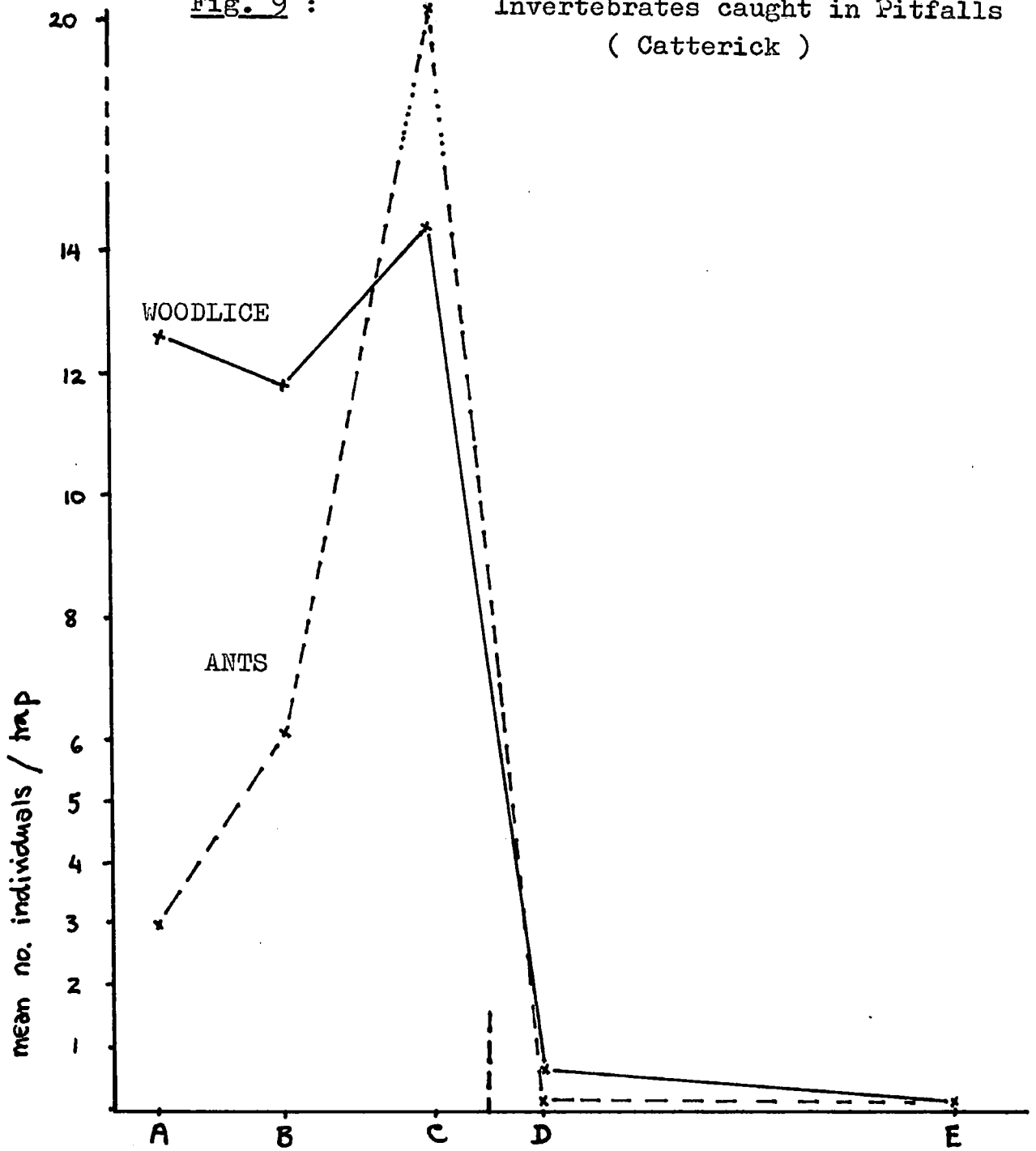


Fig. 9 :

Invertebrates caught in Pitfalls  
( Catterick )



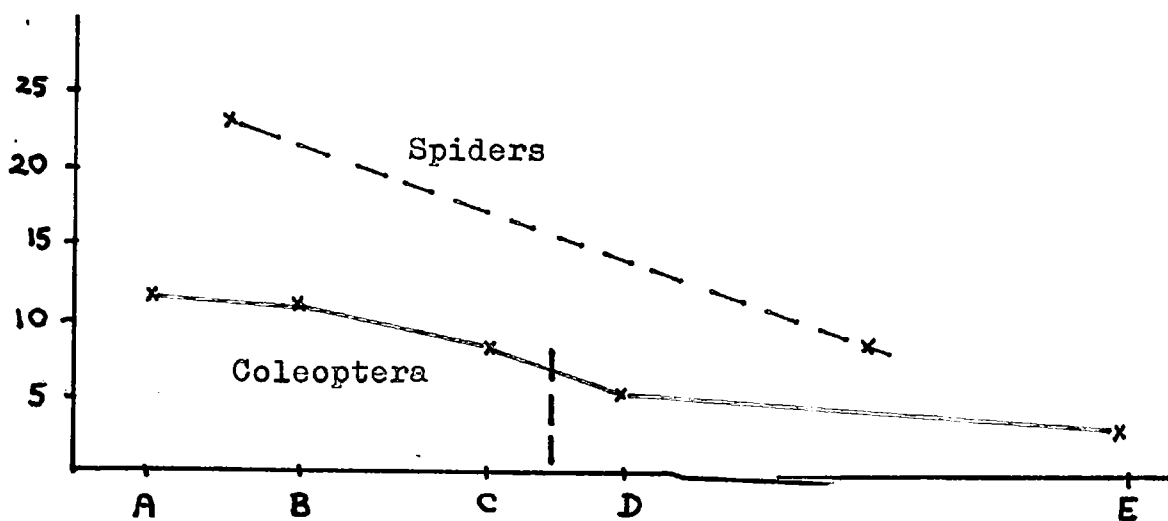
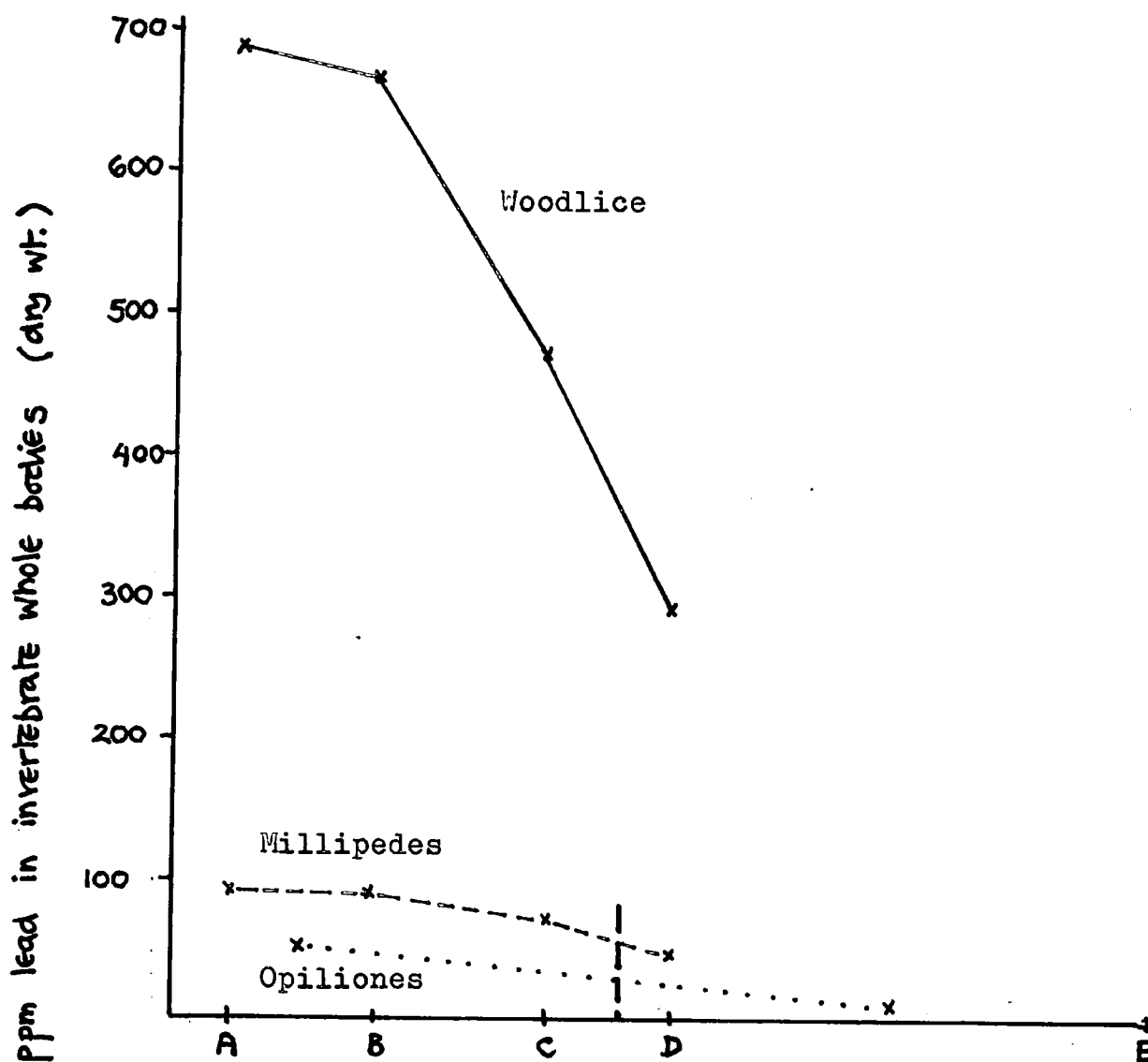
Sufficient invertebrate material was collected for analysis of lead content of a number of groups. Coleoptera were analysed from each of the five collecting stations and millipedes and woodlice for the four nearest to the road. For other groups the material from two collecting stations was combined to provide sufficient for analysis i.e. 0.5-1.0g dry wt. An estimate of the standard error is given for the Coleoptera whenever it was possible to prepare duplicate samples for analysis from the same collecting station. The results are summarised below and graphically in Fig.10.

ppm. lead in invertebrate whole bodies (dry wt.)

	A	B	C	D	E
Beetles: Coleoptera	11.5	11.0	7.75	4.7	5.0
± St. error	-	1.0	0.75	0.2	-
Millipedes: Diplopoda	82	79.5	68.6	42	-
Woodlice: Isopoda	682	665	467	288	-
Harvest-men: Opiliones	45		-	12	
Spiders: Araneae	23		-	11	

In all the groups there is a progressive decline in lead content reflecting the gradient in the soil and vegetation. Levels of lead in animals collected from the verge are 2-3 times greater than those in animals from the field. Contamination by soil could not account for all of this difference; the levels of lead in the soil at stations A and B are only 140-160% greater than those of the field. There are remarkable differences between different groups; the levels found in the woodlice and millipedes being extraordinarily high. They would not seem to be affected by their body burden of lead - both these animals are at their most plentiful within a few feet of the road. Almost all the woodlice were of the species Philoscia muscorum.

Fig. 10 : Lead Content of Invertebrate Whole Bodies  
( dry weight ) at Catterick



Calcium interference was suspected, since the flame was highly coloured by this element. However, solutions of calcium of up to 0.1 M failed to produce any interference effects greater than the equivalent of 1 - 2 ppm lead when tested on the atomic absorption spectrophotometer. It must be concluded that the levels of lead in these animals are genuinely of the order of magnitude observed.



#### 4. South Road

At this site the recent disturbance to the verge, caused by road improvements and re-seeding, have markedly affected the lead profile of the soil. Unlike the other sites studied, soil samples taken a few feet apart had widely different levels of lead and the standard deviation and standard error of the mean are correspondingly higher. It was suspected that even at distances greater than 100 ft. from the road the levels of lead were still unnaturally high. Soils taken from the Field Station were analysed for comparison. The maximum values for lead content of the soil were found 36 ft. away from the edge of the road, on the far side of the hedge and into the field.

ppm. lead in soil:

(See also Fig.11)

Position	Verge		Field		Field Station Hollinside Lane
	A	B	C	D	
Distance from road, ft.	10	22	36	125	$\frac{1}{2}$ mile from road
soil lead, ppm	131	146	166	119	87.5
$\pm$ st. error	10.0	36.6	20.0	11.5	8.9

The four soil samples analysed from each station were not sufficient to show any significant differences between their means. There are, however, significant differences between the lead contents of the soils from South Road and those from the Field Station, (o.g.  $p = 0.001-0.01$  when soil from C and the Field Station are compared).

When the results of the soil analyses were known, the first series of pitfall traps had already been set. It was

decided nevertheless to continue the collection of invertebrate fauna, since, in the absence of a well defined lead profile, it might be possible to identify other factors affecting distribution.

The lead content of the vegetation was not analysed. Had it been, it might have been possible to say whether the plants were receiving their contamination principally from the soil or the air.

The results from two consecutive series of 10 pitfalls at each station are combined to give a total for 20 traps (see Table 5 and Figs. 12 and 13). The differences between numbers caught in the verge and in the field are not so great as at Catterick. Numbers of ants, woodlice and millipedes once again decrease in passing from verge to field, with an increase in the numbers of weevils (Curculionidae). Station B is particularly interesting since it shows a minimum in numbers of many groups (e.g. Opiliones, Lycosids, Staphylinids and Carabids) but a maximum in numbers of ants and woodlice. This station is situated at the top of the bankside of the verge where the soil is at its driest.

If lead in the air - or any other produce emitted in vehicle exhaust - were affecting distributions, then a decline in numbers at those stations nearest to the road might result, regardless of the distribution of lead in the soil. Only the isopods show a significant decrease between Stations B and A, ( $p < 0.001$ ), but this is much more likely to be caused by a change in quality of the microhabitat.

If, on the other hand, it is lead in the soil that is more important, then a minimum of numbers would be expected at C but a recovery by Station D. Only the Opiliones increase across the field, but not by a statistically significant amount.

Table 5: South RoadTotal numbers of individuals caught in 20  
pitfalls at each of 4 Stations

	A	B	C	D
Staphylinidae	133	74	144	111
Carabidae	28	11	19	19
Elateridae	7	9	4	4
Curculionidae	235	237	321	323
Other Coleoptera	25	14	20	9
Coleoptera larvae	35	47	66	65
Diptera larvae	96	75	91	67
Lepidoptera larvae	1	6	4	7
Symphyta larvae	2	4	3	3
Formicidae	c.2,500	c.3,000	440	59
Other Hymenoptera	-	5	3	3
Hemiptera	63	55	66	40
Lycosidae	96	75	91	67
Other Araneae	231	143	147	156
Opiliones	121	96	102	136
Isopoda	57	112	45	2
Diplopoda	120	48	61	27
Chilopoda	16	6	2	1
Gastropoda	5	6	12	5
Orthoptera	2	-	1	-
Dermaptera	1	4	-	-
Oligochaetae	6	-	5	3

Fig. 11 : Lead Content of Soil at South Road

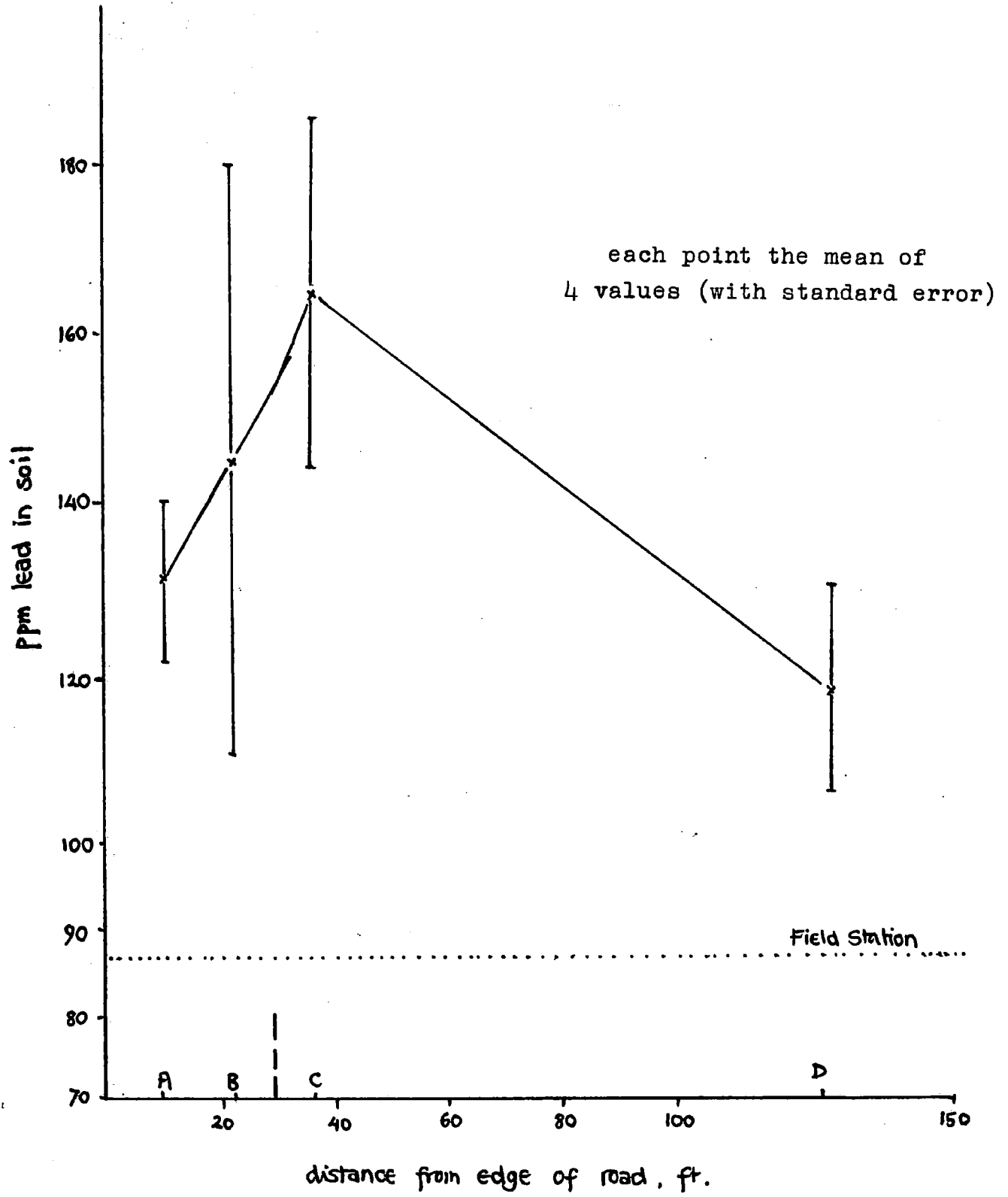


Fig. 12 : Invertebrates caught in Pitfalls  
( South Road )

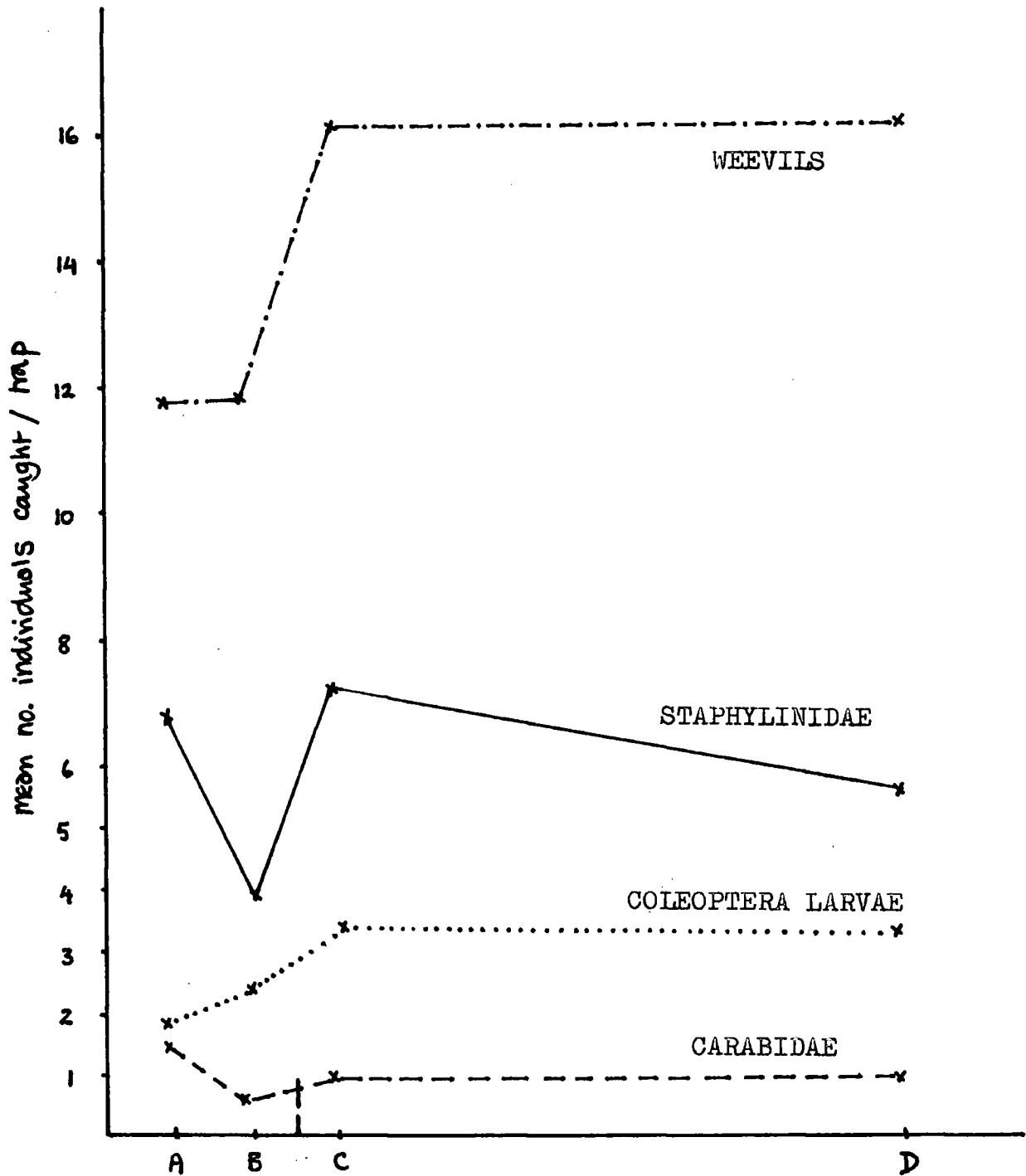


Fig. 13 : Invertebrates caught in Pitfalls  
( South Road )

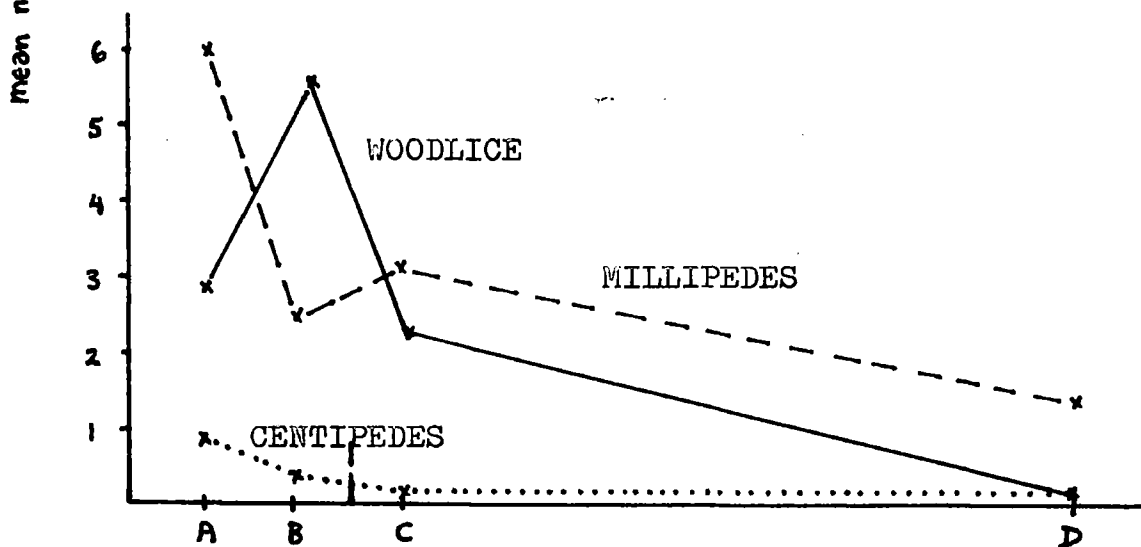
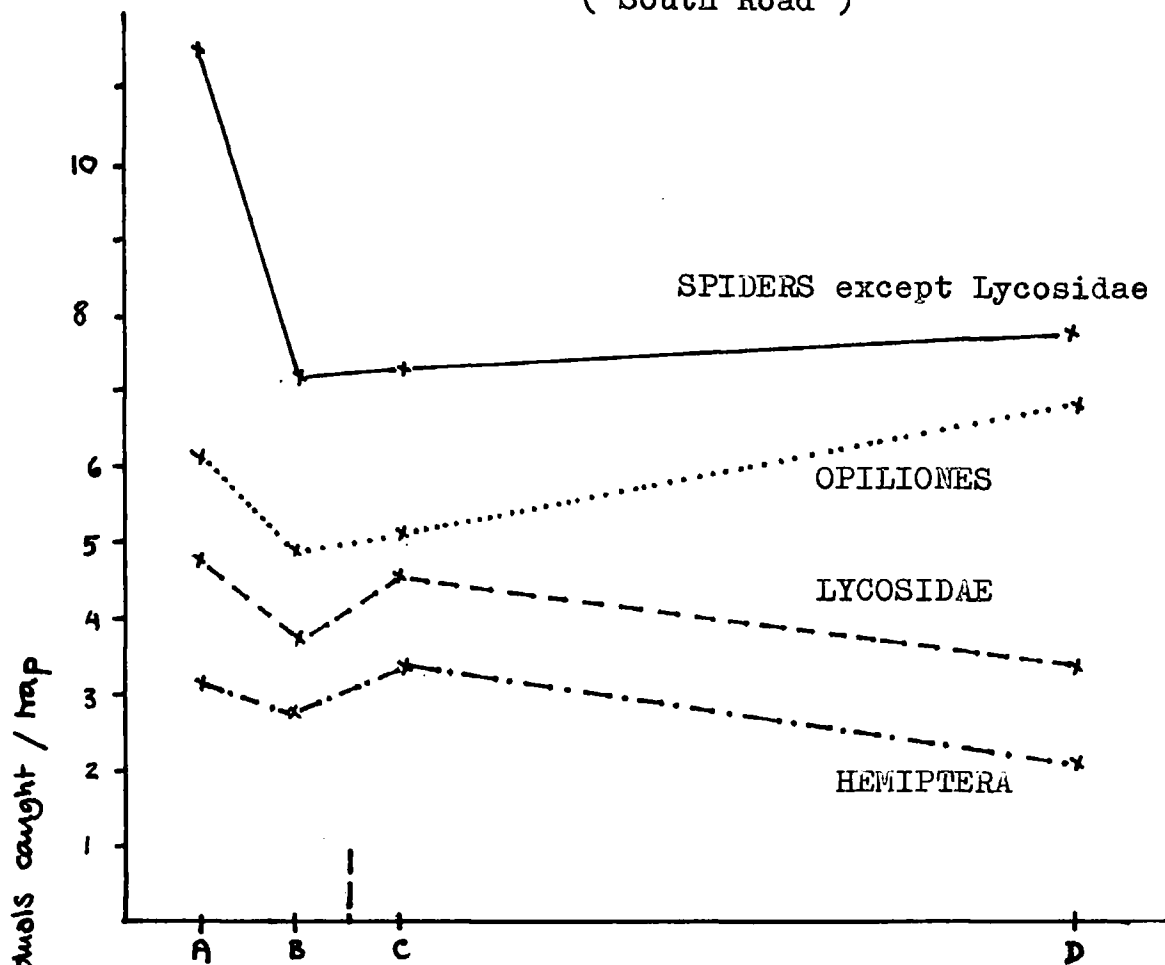
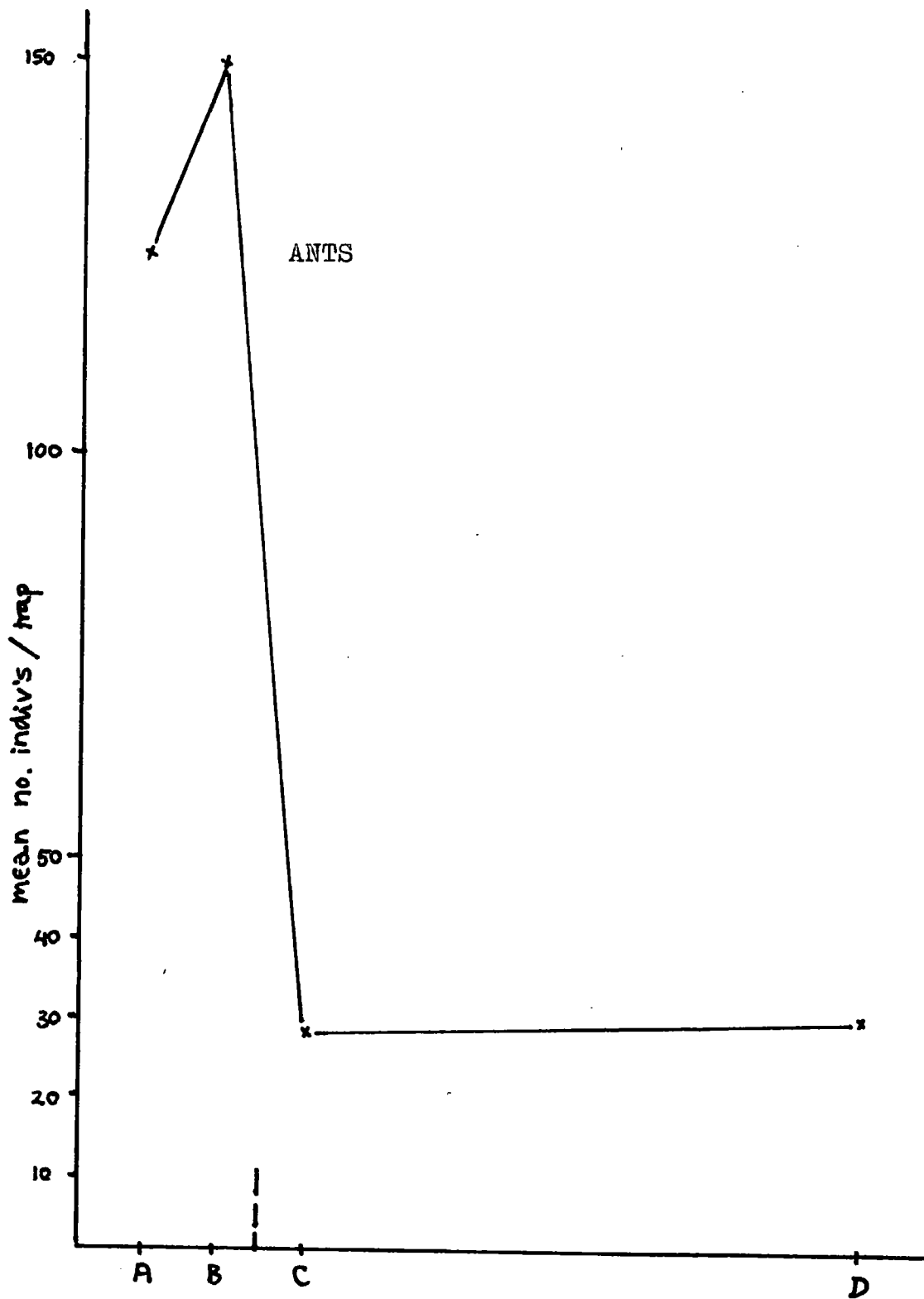


FIG. 14 : Invertebrates caught in Pitfalls  
( South Road )



The results of the analyses of invertebrate material are given below. Material from Stations A and B were pooled to provide sufficient for analysis of duplicate samples and an estimation of the standard error.

ppm. lead in invertebrate whole bodies (dry wt.)

		A + B	C	D
Beetles:	Coleoptera	16.0 ± 3.4	16.5	21.0
Spiders:	Aranæ	17.5 ± 1.8	22.0	25.8
Harvest-men:	Opiliones	30.5	40.0	30.0
Woodlice:	Isopoda	380.0	280.0	-
Millipedes:	Diplopoda	43.0 ± 9.7	40.5	47.5
Earthworms:	Oligochaetes*	18.1	-	-
Ants:	Formicidae	14.5 ± 3.5	-	-

\* Earthworms de-gutted before analysis.

Further sampling would be required before it could be known whether the maximum lead levels in invertebrates were at the verge, just into the field or at the back of the field. The data suggests that different groups behave differently in this respect, but the standard errors, where estimated, are high. The relative amounts of lead to be found in different groups agrees well with the work at Catterick. The woodlice again contain exceptionally high levels and the millipedes, opiliones and spiders are in the same order as before. Woodlice were also collected (of mixed species) from soil and litter in Great High Wood. Their analysis showed them to contain 145 ( $\pm 7.5$ ) ppm lead; an abnormally high amount for biological material in the absence of gross contamination.

Longworth traps were set at various positions on the verge, at the back of the field (approx. 200 ft. from the



edge of the road), and at various sites at the Field Station and in Great High Wood. The most commonly caught species were the bank vole (Clethrionomys glareolus); the short tailed vole (Microtus agrestis); the wood mouse (Apodemus sylvaticus), and the common shrew (Sorex araneus). Shrews caught in the pitfalls were also analysed. The results of the analyses are given below, with standard errors when duplicate samples could be prepared.

ppm. lead in vertebrate tissue (dry wt.)

	<u>South Road, verge</u>	<u>South Road, back of field</u>	<u>Field Station and Great High Wood</u>
<b>LIVER:</b>			
<u>Sorex</u>	14 ± 3.3	11	-
<u>Clethrionomys</u>	13.5 ± 2.6	10	7.5
<u>Microtus</u>	10.5	5	5
<u>Apodemus</u>	12	9.5	9?
<b>KIDNEY:</b>			
<u>Sorex</u>	27	17.5	-
<u>Clethrionomys</u>	13	5	5
<u>Microtus</u>	9.5	5	9.0
<u>Apodemus</u>	-	6.5	5.0
<b>SPLEEN:</b>			
<u>Sorex</u>	5	5	-
<u>Clethrionomys</u>	-	5	-
<u>Microtus</u>	8	-	5
<u>Apodemus</u>	-	-	8?

The results are highly suggestive of higher levels of lead in the tissues of animals living nearer the road. There would seem to be more lead in the kidney than the liver, and least of all in the spleen. More samples would make the pictures clearer, but continual trapping at one site would catch increasing numbers of 'immigrants' and less of the native population. The different diets would not seem to be greatly affected the amounts in body tissues, although the insectivorous shrew would seem to have slightly higher levels of lead, at least in the kidney.

### 5. The Experimental Plots

The analyses of the soil samples showed that the application of lead compounds had raised the levels of lead in the soil by the order of magnitude estimated, assuming penetration and equal mixing to a depth of 5-10 cm. There were quite considerable differences between the pair of samples from each plot, but this was to be expected with the method of application used. The lead levels found in the soil of the control plots were surprisingly high. It is not known what levels have been found previously for urban garden soil or what the sources of the contamination might have been. The transfer of such amounts of lead from the experimental plots to the controls is unlikely, considering the method of sampling and the precautions taken to avoid such cross-contamination.

		ppm lead in soil				
Plot No.	Controls	Pb(NO <sub>3</sub> ) <sub>2</sub>			PbCl <sub>2</sub>	
	1-3	4	5	6	7	
lead applied	-	5 g.	50 g.	200 g.	100 g.	
soil lead, ppm	177	192	293	399	292	
± st. error	9.7	18.0	12.0	23.0	28.0	
increase in soil lead	-	15	126	222	115	

significance of difference between controls and 4: N.S.

" " " " " " 5: p = 0.001 - 0.01

" " " " " " 6: p = 0.001 - 0.01

" " " " " " 7: p = 0.01 - 0.02

The results of the extraction of soil cores and the sorting of the pitfall catches are given in Tables 6 and 7 respectively.

Table 6: Experimental PlotsTotals for 6 pitfalls from each of 7 plots

	Controls			Pb(NO <sub>3</sub> ) <sub>2</sub>			PbCl <sub>2</sub>
	1	2	3	4	5	6	7
Staphylinidae	27	28	19	18	16	13	13
Carabidae	9	3	7	7	8	19	7
Curculionidae	20	24	33	42	20	11	46
Other Coleoptera	2	5	6	2	2	4	2
Coleoptera larvae	-	2	6	3	2	2	-
Diptera larvae	-	-	1	4	-	-	1
Lepidoptera larvae	-	-	1	-	-	1	1
Aphids	15	6	9	8	25	17	6
Other Hemiptera	11	8	12	10	5	9	6
Lycosidae	2	-	-	1	1	-	-
Other Araneae	9	6	16	12	10	16	11
Opiliones	11	20	31	23	7	19	33
Diplopoda	3	1	6	2	1	10	7
Chilopoda	-	-	-	-	-	-	1
Gastropoda	1	1	1	-	1	-	-
Isopoda	9	11	25	15	8	19	26
Oligochaetes	1	-	-	1	-	-	-

Table 7: Totals for 2 soil cores from each of 7 plots

	1	2	3	4	5	6	7
Acarina	230	150	178	149	132	188	168
Collembola	136	74	168	96	62	95	123
Thysanoptera	30	14	16	15	22	19	17
Diplura	2	1	1	1	2	3	-
Staphylinidae	-	5	1	5	3	2	3
Coleopteran larvae	6	1	3	8	2	3	4
Dipteran larvae	4	-	4	-	1	-	3
Hemiptera	11	7	4	7	6	3	4
Araneae	16	7	7	5	7	3	5
Diplopoda	-	-	4	1	-	4	-
Chilopoda	5	3	6	2	3	2	6
Isopoda	4	7	9	7	3	5	2
Oligochaeta	2	2	6	3	4	1	-

There is no evidence from the results that during the time between application of the lead and taking the samples there occurred any significant change in either the soil or ground living fauna. Most of the values of the totals of plots 4-7 fall within the range of values of the controls and none are significantly different from them. The numbers of some groups would appear to decline across plots 4-6 but the numbers of others rise or are indifferent. The taking of further samples would be desirable but was not possible in the time available.

## DISCUSSION

No evidence has been found that artificially high levels of lead in the soil have any effect on the invertebrate fauna of the Weardale lead mines, of either of the three roadside verges, or of the Experimental Plots.

At Weardale the lead content of the soil, which may be many thousands of ppm (up to 2% by weight), might be determined by relatively few particles of lead ore. Consequently there might be a poor correlation between the amounts of insoluble and soluble lead. It remains to be investigated whether the toxic effects of soluble lead, or other aspects of soil quality (e.g. amount of organic matter; suitability for plant growth), are the principal cause for the observed variations in the numbers of soil invertebrates.

The levels of lead in above-ground vegetation and the single sample of coleopteran material analysed were not especially high. The vegetation was not washed before analysis in order to give a more accurate estimate of the amount of lead in the food of herbivores. The amounts of lead in beetles are much the same when they are living in soil containing several thousands ppm lead, as at Weardale, or around a hundred, as at Catterick or South Road. Soil contamination of the sample would not seem to be an important factor in determining the results of these analyses.

Sheep being grazed at Weardale were at liberty to wander over the spoil heaps, although there are several reported cases of poisoning of domestic animals at similar sites, e.g. Donovan et al (1969). The risk would seem greater if

the mine is being worked when fallout of dust could add considerably to the lead content of the vegetation. Even if the sheep at Weardale are not themselves being poisoned, their carcasses might well represent a hazard to human health. The desirability of grazing such habitats deserves thorough investigation.

The results of the studies on roadside verges are complicated by a wide variety of other factors. Whereas the lead found in the spoil-heap from a mine is entirely in the form of inorganic compounds, there is the possibility that small quantities of organic lead might also be present by the roadside. Other pollutants might also be present.

At the Motorway, significant accumulations of lead had occurred within the two years that it had been opened. However, these were not especially high in comparison to the natural lead levels of the soil. There was no evidence that the lead content of the soil was affecting the distribution of any invertebrate group, but the interpretation of the data was made more difficult by interference effects, believed to be caused by the drift of pesticide sprays.

Disturbances to the verge at South Road had greatly affected the amounts of lead to be found in the surface soil. Other sources of lead contamination were also suspected, since the levels of lead in the soil 100 ft. from the road was considerably higher than those found at the Field Station, Hollinside Lane. In the absence of a well-defined lead profile, there was no evidence that gaseous constituents of vehicle exhaust, e.g. carbon monoxide, unburnt hydrocarbons or oxides of nitrogen, were significantly affecting the fauna of the verge. The traffic flow at this site was, however, considerably less than that of either the Motorway

or the A.1 at Catterick.

The results obtained from Catterick are considered to be the most important. Over the distance of the verge, the lead content of the vegetation fell from 135 ppm to less than 35 ppm, and that of the soil from 111 ppm to 70 ppm. Yet the majority of the constituents of the fauna of the verge remained indifferent to these changes. Differences in micro-habitat of the near-verge and far-verge were sufficient to account for the changes in distribution that were found. Other heavy metals besides lead have been found in road-side soils, although not in such concentrations. Lagerwerff (1967) found cadmium, nickel and zinc were also accumulating at such sites; cadmium, a product of the attrition of car tyres and the burning of motor oils, reaching levels of 0.56 ppm.

The purpose of the experimental plots was to isolate the effects of lead and to determine whether some groups were more susceptible than others. During the period of time for which they were studied no significant changes in the soil or ground living fauna were found. The plots were relatively small and a re-invasion of the larger arthropods e.g. Carabids, might have occurred between the time of spraying and sampling. It is, however, unlikely that the smaller and slower moving of the invertebrates could have fully recovered their numbers. One must suppose that the application of lead had yet to have its effect or else the animals were relatively insensitive to it.

It is not known why so many invertebrate groups should seem to be so insensitive to such a toxic poison. It may be that they have become adapted to the wide range of levels of lead found in natural soils; alternatively, the effects of lead on invertebrates may not be so critical as they are to

the more highly developed vertebrates.

The analyses of lead in vegetation, invertebrate and vertebrate tissue did not support the view that there might be progressive increases in concentrations along a food chain. The greatest differences between the levels of lead in invertebrates did not occur as a result of different feeding habits, i.e. whether carnivore, herbivore or detritus feeder, but were associated with differences in composition of the exoskeleton.

The greatest amounts of lead were found in woodlice and millipedes, both with cuticles strengthened by the deposition of calcium salts, principally calcium carbonate, (50%) and calcium phosphate (5-10%), (Richards, 1951). In woodlice, the ratio of calcium to lead is closely similar to that found in the plant material on which they feed. It can be calculated that they concentrate calcium by some 20-25 times, i.e. from the 1% or less of the leaf litter on which they feed to the 20% or so of their bodies. If they concentrate lead similarly, and their food contains 30 ppm lead (as it might well do on the verge at Catterick), then the levels of lead in their bodies might well reach 600-750 ppm (as they do on the verge at Catterick).

Millipedes feed on similar material but do not have such high levels as lead in their bodies as woodlice; the biochemical mechanisms responsible for the formation of their exoskeletons must therefore be capable of some degree of discrimination between the two inorganic ions.

The principal predators of woodlice on roadside verges are not known with any certainty. Shrews almost certainly take isopods in their diet; it is possible that Clethrionomys



and Apodemus might also occasionally. Paris and Sikora (1967) used radioactive tracer methods to show that lycosid spiders would take Armidillidium vulgare, at least when young and unable to roll into a ball.

No previous mention of abnormally high levels of heavy metals in either the isopods or the millipedes has been found in the literature.

The analyses of tissues of small mammals gives values similar to those reported by Jefferies (in lit.). He reports finding 13-16 ppm (dry wt.) in the whole bodies of Apodemus, Clethrionomys and Microtus living near the roadside, and about 3 ppm for those from farmland sites.

It is not known what levels of lead a small mammal might have to contain before symptoms of poisoning are apparent. Sub-lethal effects might be present at almost any concentration, e.g. increased susceptibility to stress. In the field the mortality rate would naturally be high and any unoccupied territories would be taken over by wandering animals.

SUMMARY

1. The amount of lead released into the environment as a consequence of man's industrial and technological processes has risen dramatically within the last 25 years.
2. Tetraethyl lead, used in petrol as an anti-knock, is the principal source of lead in the air and soil of urban environments.
3. Lead is highly toxic, especially in its organic form. At the levels now present it represents a subtle but serious hazard to human health.
4. Studies made at the spoil heaps of lead mines and experimental treatment of a semi-natural habitat have shown the invertebrate soil fauna to be apparently unaffected by high levels of inorganic lead in the soil.
5. The presence of accumulations of lead in soil and vegetation adjacent to well-used roads was confirmed. No evidence was found that such contamination might affect the distribution of ground living invertebrates.
6. Levels of lead were measured in a variety of invertebrates and in the liver, kidney and spleen of four species of small mammals. The highest amounts were most frequently found in those animals living nearest to the road. Woodlice and millipedes were found to contain excep-

tionally high levels of lead, presumably associated with calcium in their exoskeletons. The evidence for progressive accumulation through a food chain is inconclusive.

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APPENDIX

A.1(M) at Sherburn

- (i) Soil Core data
- (ii) Pitfall Trapping data

A.1(M) at Sherburn

(i) Soil core extraction data :

	A			B			C			D		
	1	2	3	1	2	3	1	2	3	1	2	3
Acarina	38	55	59	35	64	47	82	69	57	53	48	36
Collembola	145	67	95	125	85	67	65	185	125	29	56	14
Thysanoptera	89	60	205	120	107	68	52	128	90	33	41	27
Staphylinidae	4	5	3	2	-	-	1	-	1	1	1	-
other Coleoptera	-	-	2	-	-	1	2	-	-	-	-	1
Coleoptera l.	3	1	7	16	21	4	3	7	2	1	3	3
Diptera l.	-	1	1	2	2	1	5	2	2	1	-	4
Aphids	56	54	92	52	39	29	7	10	10	2	-	2
other Hemiptera	7	10	11	11	21	10	1	5	3	-	2	5
Araneae	2	8	1	8	3	6	4	6	5	1	1	2
Chilopoda	-	1	2	1	-	2	1	-	-	-	-	-
Diplopoda	-	-	1	-	1	-	1	-	-	-	-	-
Oligochaetes	4	1	1	-	-	1	-	1	2	2	3	-
Opiliones	1	-	-	-	1	-	-	-	-	-	-	1

(ii) No's caught per Pitfall trap :

	A						B						D					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Staphylinidae	7	14	13	17	8	9	4	2	7	11	4	3	4	4	10	6	2	-
Carabidae	3	9	4	9	10	8	2	3	4	2	1	4	-	1	6	8	-	2
other Coleoptera	-	-	2	-	-	-	2	-	1	1	1	1	1	-	-	2	1	2
Coleoptera l.	1	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-
Diptera l.	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	1	1
Lepidoptera l.	3	1	4	3	4	-	1	2	-	-	2	4	-	-	-	-	-	-
Aphids	18	21	9	9	10	6	6	5	3	1	6	4	-	3	-	-	-	1
Other Hemiptera	-	3	2	1	-	-	2	1	1	1	1	2	1	-	-	1	-	1
Lycosidae	1	2	2	3	-	4	-	1	4	6	1	-	1	-	1	1	-	1
Other Araneae	21	14	31	25	16	4	8	16	15	5	18	22	16	8	9	7	2	7
Chilopoda	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Diplopoda	-	2	3	-	2	4	2	-	1	1	-	1	1	3	3	2	-	-
Oligochaetes	-	-	1	-	-	-	-	-	-	3	-	-	-	-	1	1	-	-
Opiliones	5	3	6	7	-	3	-	1	3	3	3	13	-	1	5	1	-	-

Catterick

Pitfall Traps : Series 1

Catterick : Series # 1

No's caught per Pitfall Trap

	A					B					C					D					E				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Staphylinidae	9	2	2	17	20	3	4	7	8	4	5	7	10	14	5	9	12	7	8	9	6	11	13	11	9
Carabidae	2	3	5	1	2	2	1	-	-	1	2	2	-	-	1	9	4	2	6	7	9	11	21	24	21
Other Coleoptera	2	4	-	1	1	2	1	1	-	-	2	2	1	3	3	-	4	5	1	2	6	-	1	1	-
Coleoptera l.	-	-	-	1	-	-	-	1	-	-	-	-	2	-	-	-	1	2	-	3	1	4	-	1	2
Diptera l.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lepidoptera l.	-	1	-	-	1	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	1	-	1	-	-
Symphyta l.	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	1	-	1	-	-	-	-	-	-
Formicidae	1	2	2	-	1	-	3	3	4	3	3	3	1	4	2	-	-	-	-	-	-	-	-	-	1
Other Hymenoptera	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemiptera	16	7	6	7	16	1	3	3	1	3	3	1	1	-	-	4	2	4	2	5	2	-	3	3	-
Lycosidae	9	4	9	8	12	4	6	-	14	4	3	2	15	4	2	3	1	1	2	1	-	1	-	-	1
Thomisidae	2	2	1	2	3	3	2	-	4	2	2	-	1	3	2	4	2	2	1	-	-	-	-	-	2
Other Araneae	5	1	3	6	4	2	2	7	2	5	1	1	3	9	1	35	10	15	14	18	15	16	9	13	2
Opiliones	7	4	10	7	6	2	6	1	1	2	6	1	5	8	-	-	5	-	-	1	-	-	1	-	-
Isopoda	8	24	17	56	31	17	48	21	18	15	9	49	38	56	10	1	4	-	-	-	-	-	-	2	1
Diplopoda	10	4	1	6	11	4	5	6	2	2	2	11	4	11	5	-	1	-	2	1	5	2	-	-	-
Chilopoda	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Gastropoda	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Orthoptera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oligochaetae	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-

Catterick

Pitfall Traps : Series 2



South Road

Pitfall Traps : Series 1





South Road

Pitfall Traps : Series 2



Experimental Plots

Soil Core extraction data

Experimental Plots

No's extracted per Soil Core

	Controls						Pb(NO <sub>3</sub> ) <sub>2</sub>						PbCl <sub>2</sub>	
	1		2		3		5g		50g		200g		100g	
	I	II	i	II	I	II	I	II	I	II	i	II	i	II
Acarina	103	127	65	85	89	90	87	62	72	60	65	123	83	85
Collembola	57	79	36	38	90	78	57	39	40	22	56	39	45	78
Thysanoptera	12	18	12	2	6	10	4	11	13	9	9	10	9	8
Diplura	2	-	-	1	1	-	1	-	2	-	-	3	-	-
Staphylinidae	-	-	2	3	-	1	2	3	1	2	1	1	3	-
Coleoptera l.	4	2	-	1	1	2	4	4	-	2	-	3	4	-
Diptera l.	4	-	-	-	2	2	-	-	-	1	-	-	3	-
Hemiptera	6	5	2	5	-	4	5	2	2	4	2	1	-	4
Araneae	6	10	3	4	5	2	2	3	3	4	2	1	3	2
Diplopoda	-	-	-	-	3	1	-	1	-	-	-	4	-	-
Chilopoda	4	1	-	3	-	6	2	-	3	-	-	2	3	3
Isopoda	2	2	3	4	2	7	6	1	2	1	2	3	1	-
Oligochaetae	2	-	1	1	4	2	3	-	1	3	1	-	-	-

Experimental Plots

Pitfall Traps

