A study of heavy metal contamination of roadside vegetation

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A STUDY OF HEAVY METAL CONTAMINATION OF ROADSIDE VEGETATION

by

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INTRODUCTION

General

The problem of lead pollution has attracted increasing attention in recent years. Many studies have linked the elevated levels of lead, found in air, soils, and vegetation close to busy main roads, with the emission of lead compounds from motor vehicles. Lead is the major heavy metal pollutant in the vicinity of roads. This metal is a natural component of the Earth's crust, in which it occurs at an average concentration of 16 p.p.m. It is an element with no known biological function and acts as a cumulative poison in the mammalian body.

Other heavy metals have received less attention as contaminants of the roadside environment. Evidence has been found which suggests that zinc and cadmium, from motor oils and tyres, may contaminate soils and plants near major highways (Lagerwerff and Specht, 1970). These elements occur together in nature; zinc is relatively abundant in the Earth's crust with an average concentration of 70 p.p.m. It is an essential mineral in both plant and animal metabolism. Cadmium has caused more concern because of its highly toxic nature, its long biological half life, and the ability of the mammalian body to concentrate this element (Jones, 1973). Cadmium is a fairly rare metal with an average concentration in the Earth's crust of only 0.5 p.p.m.

Much of the work, on heavy metal contamination of roadside vegetation, has been prompted by concern that crops grown close to busy roads might contain concentrations that are toxic to human and farm animal consumers. Effects upon roadside invertebrate fauna, and possible concentration along foodchains have been less studied. Elevated levels of lead have been reported in a variety of invertebrates sampled from sites near busy roads (Williamson and Evans, 1972; Giles et al., 1973; Price et al., 1974), but evidence suggests that invertebrate distributions and abundance are not affected by abnormally high concentrations of lead in soil and vegetation.

Generalisations cannot yet be made on the evidence for concentration along foodchains. Williamson and Evans (1972) found less lead in shrews from a roadside verge, than in their presumed invertebrate prey. However, Price et al. (1974) demonstrated biological concentration of lead from insect herbivore to carnivore trophic levels, in areas with high exhaust emission.

Variations have been reported in the heavy metal content of roadside plants between species, seasons, and parts of the plant (Motto et al., 1970; Haney et al., 1974; Rains, 1975). This might be expected to produce differences between the lead burdens of invertebrate herbivores and decomposers, and between species of herbivore with different food requirements. Some evidence in support of this is provided by Williamson and Evans (1972), who found higher levels of lead in millipedes and woodlice, which feed chiefly on decaying vegetation, than in other invertebrates from the same roadside site. However, lead estimates were not made for different components of the vegetation.

This study was carried out at a roadside site where heavy metal burden of the invertebrate fauna was already the subject of an extensive investigation (P. Williamson, personal communication). The work was undertaken in order to determine the lead, zinc and cadmium burden of a wide variety of plants which were potential components of invertebrate food chains, and to examine the distribution of lead within plants of selected species.

Origins of Lead Pollution.

It is generally accepted that lead has always been a component of both Man's environment and of Man himself, but levels have been elevated by many hundreds of years of industrial usage. The natural release of lead is minute by comparison with the quantity released by anthropogenic activity (Patterson, 1965).

The effects of changes in lead utilisation can be traced in natural materials. Murozumi et al (1969) found that the lead content of Greenland

snow was less than 0.001 $g.kg^{-1}$, in material dating from 800 A.D., rising to more than 0.2 $g.kg.^{-1}$ in recent years. Steep rises were detected after about 1750 and again after 1940, corresponding with the Industrial Revolution and increased use of leaded petrol respectively.

A similar historical lead gradient was detected in mosses from museum collections in Sweden (Ruhling and Tyler, 1968). The concentration more than doubled its previous level of 20 p.p.m., in mosses collected during the years 1875 - 1900. After 1950 there was a rapid increase to a level of 80 - 90 p.p.m. The authors suggested that the earlier rise reflected increased coal usage, whilst the second coincided with increased consumption of petrol. Lee and Tallis (1973) found a similar 19th century peak in the lead burden of mosses from Manchester museum, but were unable to detect a second increase in recent decades.

The most important single source of lead in the atmosphere is the combustion of leaded petrol. This view has been endorsed by official bodies both in Great Britain and the United States. The Department of the Environment (1974) reported that this source may account for 90% or more of airborne lead, whilst the National Academy of Science of America (1971) stated that 98% of traccable airborne lead is derived from petrol. Confirmatory evidence has recently been provided by the use of the isotopic ratio technique which enables the origin of environmental lead to be detected. By this means, Chow and Johnston (1965) showed that the dominant component of lead in Los Angeles air was derived from petrol. Ault et al., (1970) and Rabinowitz and Wetherill (1972) demonstrated that lead in soil and vegetation close to turnpikes had a similar isotopic ration to that in petrol.

The Nature of Exhaust Emission.

Most of the lead voided from exhausts is in the form of particulate lead chlorobromide (PbCl.Br). The combustion of lead alkyl anti-knock additives produces oxides initially, but these combine with halogen scavengers

to produce lead halides, ammonium complexes, and smaller amounts of other lead salts (Waldron and Stofen, 1974). Much of the airborne lead halide becomes converted into lead carbonates, oxides and oxycarbonate (Ter Haar, 1972). Very small amounts of organic lead are also present in exhaust emission; Hirschler and Gilbert (1964) found that 0.023% of lead burned by a single car was discharged in the organic form.

The size and nature of discharged lead particles determine their distribution; large particles (>10μm.) are deposited within a few hundred feet of the source, whilst small particles may be suspended for long periods and become more widely distributed. The nature of the vehicle and operating conditions influence both the amount of lead discharged and the size of aerosol particles (Hirschler and Gilbert, 1964; Ter Haar et al., 1972).

Contamination of roadside soils by lead, zinc and cadmium.

Background levels of lead in soils from different sites have been variously reported as 6-14 p.p.m. (Maclean et al., 1969), less than 50 p.p.m. (Motto et al., 1970), 25 p.p.m. (Davies and Holmes, 1972) and 10-20 g per gram dry weight (Smith, 1975). If 25 p.p.m. is accepted as a baseline, it is found that soil samples taken within a few meters of heavily travelled roads may range to more than 100 times baseline. Bevan et al. (1974) found concentrations between 2,600 and 3,000 p.p.m. in soil from the central reservation of the M.4 motorway.

Numerous investigators have studied the distribution of lead in soils at different distances from major roads (Ruhling and Tyler, 1968; Lagerwerff and Specht, 1970; Motto et al., 1971; Davies and Holmes, 1972; Haney et al., 1974; and Ward et al., 1974). Findings have generally been similar and may be summarised as follows.

1. Soils samples from sites up to 100 feet from major roads show lead concentrations above background. Lead content tends to increase with traffic volume and decrease with distance from roads.

2. Most of the lead is concentrated in the top 10 cms. of the soil; concentrations falling off rapidly with depth. This supports the view that the element is relatively immobile in the soil, but may have considerable significance for shallow rooted plants.

The distribution of zinc and cadmium was studied by Lagerwerff and Specht (1970); results were generally similar to those for lead. High concentrations of one metal did not necessarily entail high concentrations of the other or of lead. Cadmium levels ranged from 0.9 - 1.82 p.p.m. and zinc 32 - 85 p.p.m. in the top 5 cms. of soil, from sites at 8 meters from roads.

Chemical changes undergone by heavy metals in the soil are incompletely understood, but it has been speculated that reaction of lead with anions or with clay or organic matter complexes, converts this element into relatively insoluble forms (Smith, 1975).

The consequences of heavy metal contamination of soils are difficult to assess. It is possible that lead may be indefinitely recycled through biological systems (Ault et al., 1970). Some degree of protection may be conferred by the ability of plants to restrict their uptake of some elements present in excess (Purves, 1972), or to store them in structural materials (Peterson, 1971). Tyler (1972) drew attention to possible consequences of a different nature. He speculated that large quantities of heavy metal ions accumulated in litter, by ion exchange, could lead to blocking of negatively charged organic groups, increasing the resistance of litter to decomposition. It was suggested that this could result in less nutrient cycling and reduced productivity. This is contradicted by the work of Williamson and Evans (1972), who found that high concentrations of lead in soils and vegetation did not reduce the abundance of decomposers.

Contamination of Roadside Vegetation.

Many workers have studied the magnitude and distribution of the lead burden of roadside vegetation (Ruhling and Tyler, 1968; Motto et al., 1970; Page et al., 1971; Davies and Holmes, 1972; Lagerwerff et al., 1973;

Haney et al., 1974; and Graham and Kalman, 1974). Conclusions may be summarised as follows.

1. Wild and cultivated plants growing close to main roads have elevated lead levels; values as high as 950 p.p.m. (Graham and Kalman, 1974), have been reported in grass sampled within 20 meters of a highway. This compares with a possible background level in "natural" vegetation of less than 5 p.p.m. The lead burden decreases with distance from roads, especially in the aerial parts of plants; most of the lead is accumulated within 50 - 75 meters of the roadside, whilst between 100 - 150 meters concentrations fall to local background values.

2. Lead levels in vegetation increase with traffic volume, although the correlation is not always as clear as that shown by soils. The latter may be explained to some extent by the different nature of plant material examined at different sites.

3. The direction of the prevailing wind has a significant effect on the lead content of plants close to major roads; plants on the leeward side of roads generally contain more lead than similar plants on the windward side. The magnitude of the difference varies between sites but may exceed a factor of two (Page et al., 1971).

4. The lead burden is not evenly distributed throughout the plant; the aerial parts of plants contain more lead than underground organs. In several crop plants the distribution follows the order leaves>roots>stem; the magnitude of the difference between organs varies with species. In maize, the male inflorescence has been shown to contain more lead than any other part of the plant (Motto et al., 1970). The distribution of lead within wild species has been less studied.

Many factors influence the distribution of lead within a roadside plant, theseinclude the degree and duration of exposure, of different parts of the plant, to airborne exhaust particulates; the ability of the exposed surface to accumulate lead, and the extent of uptake and translocation of soil lead.

5. Reported lead levels show pronounced differences between species of plants but it is difficult to generalise, since few studies allow direct comparison of different species sampled from the same place, at the same time. Haney et al., (1974) compared one annual and one perennial species in each of three plant families and showed that Gramineae had significantly less lead than Fabaceae or Compositae. The study of crop plants suggests that rough, hairy plant surfaces accumulate more airborne lead per unit time than smooth surfaces (Page et al., 1971).

The zinc and cadmium contents of roadside plants has been less studied. Lagerwerff and Specht (1970) determined lead, zinc and cadmium in grasses adjacent to four busy roads. All three metals decreased with distance from roads, but metal concentration gradients with distance decreased in the order Pb, Cd, Zn. Zinc levels were 32 - 85 p.p.m. but cadmium only 0.49 - 0.95 p.p.m. in dry matter, at 8 meters from roads.

Comparisons between reported heavy metal levels in vegetation are complicated, not only by differing abilities of species and organs to accumulate these metals, but also by seasonal changes. Guha and Mitchell (1966) examined changes in the composition of leaves from deciduous trees. Lead concentration fell during early Spring growth, due partly to a faster increase in dry matter than in lead uptake. Concentrations rose again from mid June (after growth had ceased), reaching a maximum in early October. Mitchell and Reith (1966) found similar changes in unpolluted pasture herbage from N.E. Scotland. Lead concentration remained low during active growth, but later rose steadily, even in the dormant plant, until late Winter or early Spring. It was suggested that the rise was caused by mobilisation of lead from the roots. Rains (1975) produced contradictory evidence, when lead content was measured in Avena fatua sods, maintained in a greenhouse, with filtered air supply. After a fall in concentration in the Spring, lead remained at a low level during the entire life cycle of the plants. However, field grown plants showed a steady rise from late June to a peak in late Winter. It was concluded that plant material which

has senesced can apparently continue to accumulate lead from the atmosphere. Tyler (1972) believed that the death, of aerial parts of plants, was accompanied by a reduction in the ability of roots to act as a barrier to translocation of heavy metals, but also that breakdown of epiderm and cuticle facilitated sorption by ion exchange.

Whatever the mechanism involved, the concentration of heavy metals, in plant litter and decaying organic matter, must be of considerable significance for the fauna which feed on these kinds of plant material.

Entry of heavy metals into roadside plants.

The relative contributions of soil uptake and aerial deposition to the heavy metal burden of contaminated plants is incompletely resolved, but it is clear that much of the foliar metal is present as a topical coating. Several studies have shown that some of the lead is removed by washing (Motto et al., 1970; Page et al., 1971; Lagerwerff et al., 1973; Ward et al., 1974). Reported values generally lie within the range 0 - 56% of the total lead content of leaves removed by this means. Large differences between species can to some extent be explained by differences in leaf surfaces, but comparisons are complicated by different washing regimes employed. Little (1973) obtained similar results for leaves contaminated with zinc, lead and cadmium from a smelter complex. Washing removed less cadmium and zinc than lead, but the distribution of each metal differed within leaves of any one species.

Arvik and Zimdahl (1974) showed, by means of cuticular penetration tests, that very little foliar uptake of lead occured on exposure to lead chlorobromide aerosols. Greenhouse culture experiments have shown that whilst lead is taken up by roots, little is translocated to aerial parts of plants (Motto et al., 1970; Lagerwerff et al., 1973; Rains, 1975). Ryegrass grown in culture solution with added lead, and in soils from 16 sites in England and Wales, demonstrated that roots provide a barrier restricting the upward translocation of lead (Jones et al., 1973 a, b). Alloway and Davies (1971) found little difference between the zinc content of the leaves and roots of radishes grown on mine waste contaminated soil, suggesting that zinc is more mobile within the plant.

METHODS

1. Study site.

The work was carried out on a 43 m. section of the central reservation of the A.1 road, 2.4 km south of Scotch Corner, North Yorkshire (N.Z. 202025). The location is shown in figure 1. The carriageways at this point are separated by a strip of land 25 m. wide which harbours a mixed flora of woodland, scrub and grassland species. Two rows of trees of mixed Craetagus oxycantha Linn., Fagus sylvatica Linn., and Fraxinus excelsior Linn., enclose a central glade, in which the vegetation largely comprises woodland understorey plants and grasses. The reservation is bordered on both sides by grass verges. Details of the site are illustrated in figure 2. The estimated traffic volume for this section of the road is 24,642 vehicles per day.

2. Collection of plant material.

With the exception of the transect (Section 2. 3.), all plant material was sampled from a central strip, 5m wide.

2. (i) General Survey of heavy metal concentrations in roadside vegetation.

Representative samples of leaves were collected, on 15th June 1976, from each of the twenty species listed in Table 1. These species possess a range of leaf types differing both in gross morphology and nature of leaf surfaces.

To reduce any variability in heavy metal burden due to age and position of plant material, leaves were collected only from plants growing in the central strip. The lowest two or three leaves from each plant were gathered, and a minimum of 20 were pooled to provide a single sample for analysis. In addition, senescent leaves were collected from the following four species, Rumex obtusifolius, Urtica dioica, Dactylis glomerata and Festuca rubra. Those from grasses, were taken from well bleached material at the base of clumps, and were the remains of the previous season's growth. Those from dicotyledons had grown during the current season, and generally were still attached to the stems.

Figure 1 Location of Study Site

Scale 1: 250,000

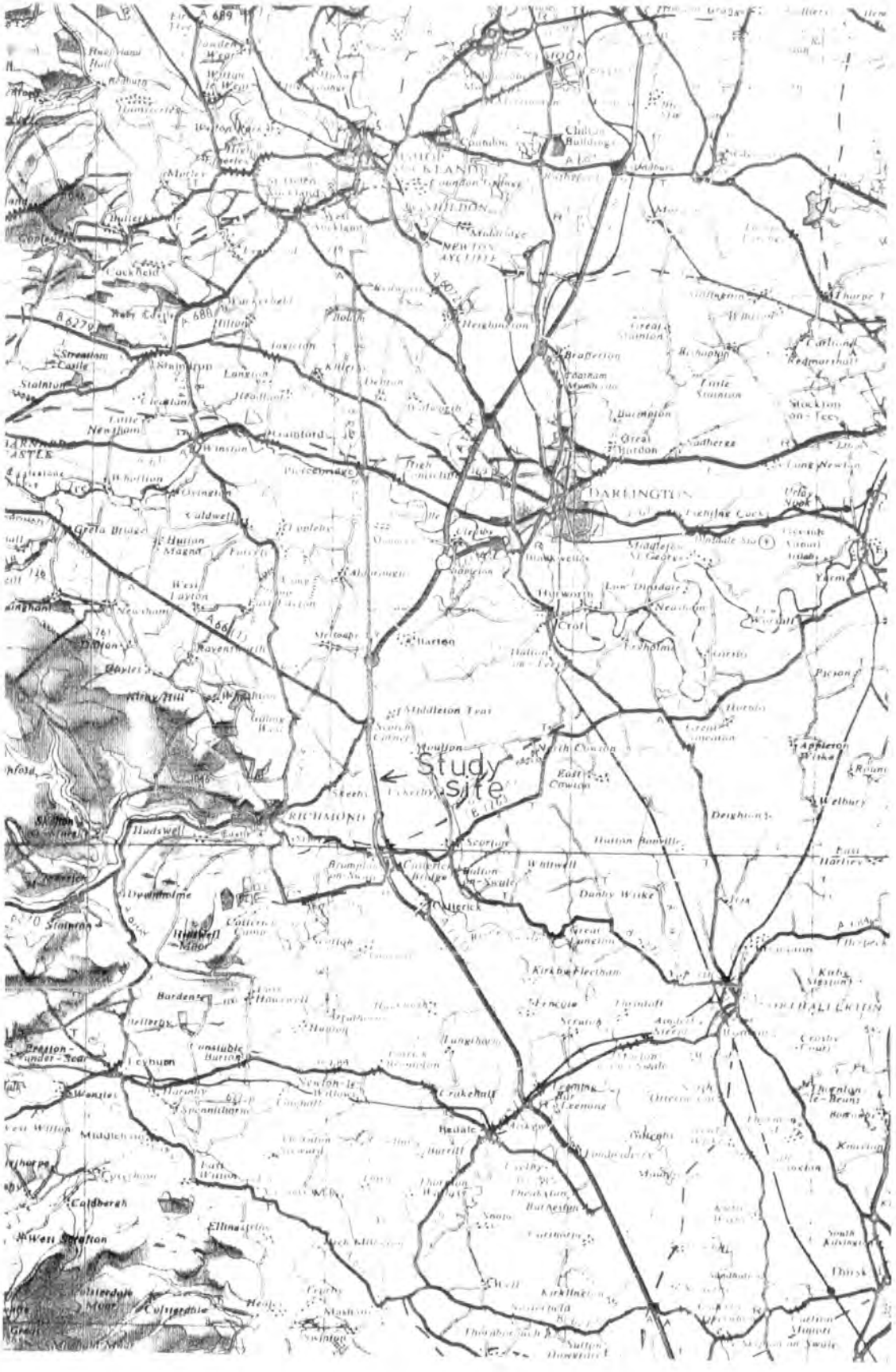


Figure 2 Sketch map of study area

Note : tree and shrub canopy not measured, included for illustration only

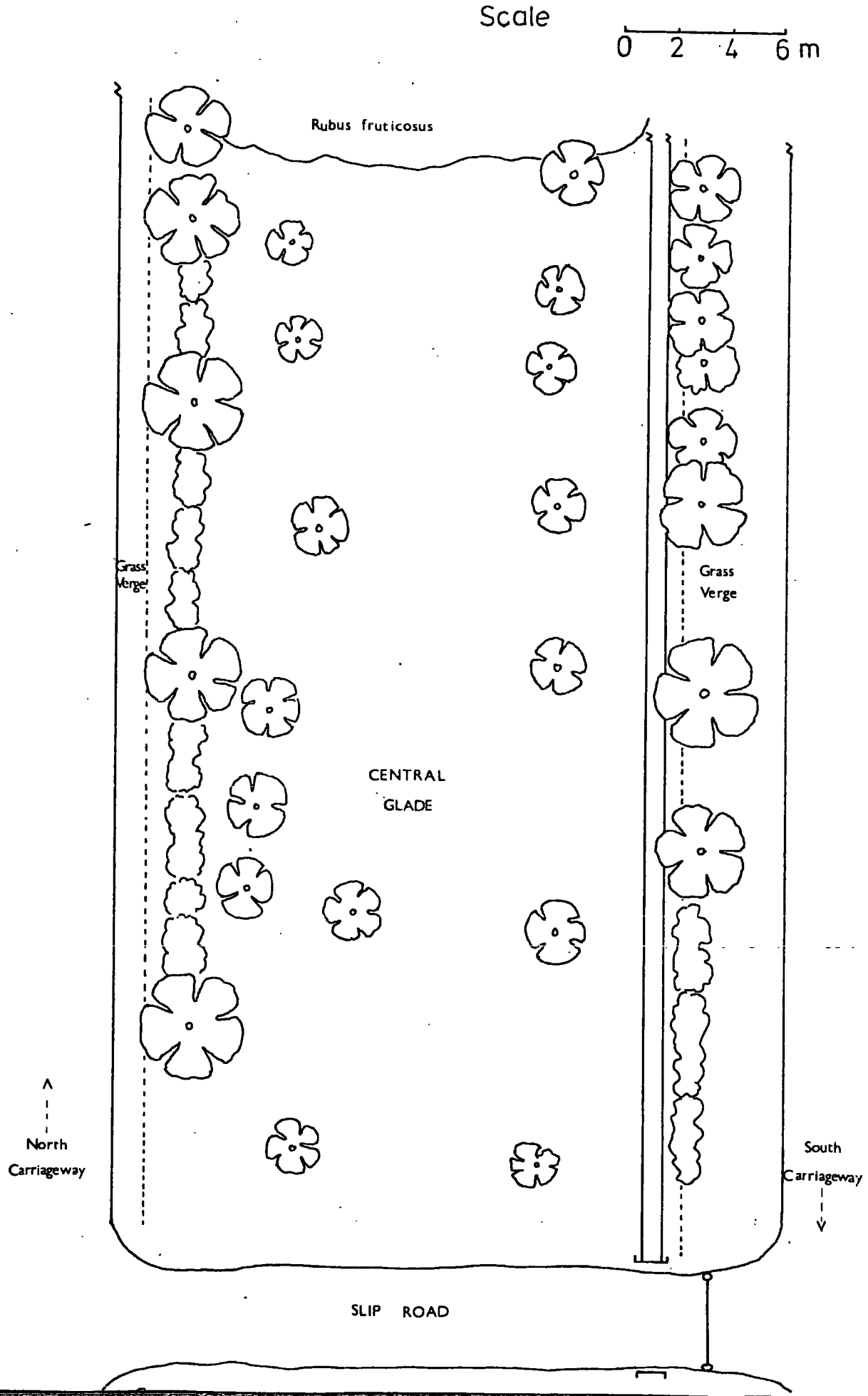


Plate 1 Study area viewed from northbound carriageway

Plate 2 Study area viewed from slip road

Plate 3 Part of the central glade

Table 1. Material collected for general survey.

Family	Species	Leaf type
Araliaceae	Hedera helix Linn.	Simple, glabrous
Compositae	Cirsium vulgare Linn.	Pinnatifid, hairy
Dioscoreaceae	Tamus communis Savi.	Entire, glabrous
Geraniaceae	Geranium robertianum Linn.	Deeply dissected, hairy
Gramineae	Arrhenatherum elatius Linn.	Entire
	Brachypodium sylvaticum Huds.	Entire, hairy
	Dactylis glomerata Linn.	Entire, ribbed
	Deschampsia caespitosa Linn.	Entire, ridged, rough
	Festuca rubra Linn.	Entire, folded
	Holcus lanatus Linn.	Entire, hairy
	Poa trivialis Linn.	Entire, scattered hairs
Labiatae	Glechoma hederacea Linn.	Entire, hairy
	Stachys sylvatica Linn.	Simple, scattered hairs
Papilionaceae	Vicia sepium Linn.	Compound, scattered hairs
Polygonaceae	Rumex obtusifolius Linn.	Entire, rough veins on lower surface with some hairs.
Rosaceae	Geum rivale Linn.	Compound, hairy
	Rubus fruticosus Linn.	Compound, hairy
Rubiaceae	Galium cruciata Linn.	Entire, hairy
Umbelliferae	Heracleum sphondylium Linn.	Compound, hairy
Urticaceae	Urtica dioica Linn.	Simple, scattered hairs

2. (ii) Distribution of lead within selected plant species.

A second collection was made in July 1976, to obtain material for an examination of the distribution of lead within the grass A. elatius, the labiate S. sylvatica and the nettle U. dioica. These species were selected because they were widely distributed throughout the site, and because the initial survey had shown that, of the dicotyledonous leaves examined, those of S. sylvatica and U. dioica contained the highest lead concentrations. Grasses, including A. elatius, contained comparatively little lead in growing leaves, but high concentrations in litter.

For each species a minimum of 10 complete plants were removed from each of three sites, of area about $4m^2$, within the central strip. The locations of the collecting sites for each species did not coincide because of variations in the plant distributions, but in each case, one site was situated at the northern end, one in the central region and one at the southern end of the study area. Plants from each site were divided into a number of fractions, detailed below, and material from ten or more plants pooled. Samples were analysed separately in order to detect differences in the lead burden between both parts of plants and parts of the study area.

The following parts of plants were separated (leaves are numbered from the tops) :-

(a) Arrhenatherum elatius. Flowering tillers were collected and treated as separate plants; in most cases tillers possessed six non-senescent leaves. The following fractions were removed : leaves 1 and 2, leaves 5 and 6, leaves 7 and 8 (if these were not senescent), attached recent senescent leaves, bleached senescent leaves from the base of clumps, inflorescences, and culms.

(b) Urtica dioica. The tips of most plants possessed approximately four small expanded leaves with short internodes, in addition to partially expanded leaves around the growing points. Plants also had up to 6 large expanded leaves (numbered 5 - 10 below). The following fractions were separated : tips, leaves 5 - 7, leaves 8 - 10 and stems. Senescent leaves

were also collected; some of these were still attached to stems, whilst others were trapped in surrounding vegetation and appeared to have become detached quite recently.

2. (iii) Transect across central reservation.

To determine whether lead levels in vegetation were correlated with distance from the carriageways and the direction of the prevailing wind, Urtica dioica was sampled across the central reservation from the northbound to the southbound carriageway. This species was selected because, in one area, it occured at frequent intervals across much of the study site. Stations were marked out at 2m intervals and material collected from the nearest clump of plants to each of these locations. In each case, the lowest two or three non-senescent leaves were removed from a minimum of ten plants, in order to provide sufficient material for analysis.

At the west (northbound) end of the transect U. dioica first occured at the 2m station, which was situated in a shallow ditch on the narrow grass verge. Plants did not occur at the 6m station or beyond the 16m station. The latter was close to the ditch at the east side of the site, within the area enclosed by trees, and lay about 8m from the southbound carriageway.

3. Washing of plant material.

A comparison of heavy metal levels, in washed and unwashed leaves, was carried out on ten of the species collected for the general survey of heavy metal levels. The material selected represented a range of leaf types and included some senescent material as follows :-

Non senescent leaves	Senescent leaves
Arrhenatherum elatius	Dactylis glomerata
Deschampsia caespitosa	Festuca rubra
Geum rivale	Urtica dioica
Hedera helix	
Rumex obtusifolius	
Rubus fruticosus	
Tamus communis	
Urtica dioica	

In each case, the collected leaves were divided into two portions, one of which was washed before analysis by immersion and agitation, for one minute, in 4 successive baths of distilled water. Leaves were then air dried overnight before being oven dried.

4. (i) Preparation for analysis.

Envelopes, strengthened with polythene tape, were used to hold plant material during drying, to avoid possible further contamination by heavy metals from trays. Material was dried in a vacuum oven at 40°C, for 72 hours. This regime was designed to minimise possible losses of lead; Von Sommer et al. (1971) have shown that drying in a forced draught oven, at temperatures as low as 35°C, may cause losses of up to 50% of the lead from fresh plant material.

All dried material was chopped finely with stainless steel scissors. Leaves of dicotyledons were then ground using an unglazed mortar and pestle; all parts of grasses and dicotyledonous stems were resistant to grinding by this method and analyses of these were carried out on the chopped material. Samples were stored over silica gel until required for analysis.

4. (ii) Analysis.

Heavy metal contents of the various materials were determined by Atomic Absorption Spectrophotometry. Suitable sample solutions were prepared by wet oxidation using nitric/perchloric acid digests, which were carried out in triplicate on each material. The method was based on that of Williamson (personal communication) which incorporates safety measures recommended by the Society for Analytical Chemistry (1959).

In most cases, 2 cm^3 of concentrated Nitric Acid (Aristar) were added to an accurately weighed sample of approximately 0.2 g in a 25 cm^3 conical flask. Grass samples were bulky and required 2.5 cm^3 of acid to achieve effective wetting of material. The cold acid was allowed to act for approximately 15 hours (overnight) by which time the danger of frothing had been eliminated. Small glass funnels, placed in the necks of the flasks, served as condensers.

Flasks were then warmed on a hotplate for approximately one hour, and simmered for approximately 15 minutes. The boiling rate was adjusted so that condensation washed down the insides of the flasks. The timing of the heating stages was varied so that almost all of the plant material was in solution at the end of boiling.

Digests were allowed to cool before adding 1 cm^3 of 60% Perchloric Acid (Foodstuffs Analysis Grade). Flasks were then reheated on the hotplate, the temperature being regulated to achieve a slow, even, boil without "spitting": in the case of grasses and some senescent materials, this required very careful adjustment. Simmering was continued until white fumes began to appear from digests; the glass funnels were then removed, digests allowed to evaporate to dryness, and heating continued until the evolution of white fumes had ceased.

After cooling, the residue was dissolved in 4 cm^3 of 3N Hydrochloric Acid (Aristar); in some cases slight warming was necessary to achieve solution. Digests were filtered through Whatman No. 1 filter papers into screw-topped polythene bottles in which they were stored, under refrigeration, until analyses could be completed.

Reagent blanks were included and received identical treatment to the samples.

Analyses were carried out on a Pye Unicam SP90 Atomic Absorption Spectrophotometer equipped with hollow cathode lamps. An air/acetylene flame was used in each case, and operating parameters were as follows :-

	Wavelength	Slit Width	Air	Acetylene
Lead	283.3 nm	0.15 mm	4.5 1/min	0.7 1/min
Zinc	213.9	0.15	4.5	0.7
Cadmium	228.8	0.15	4.5	0.7

Fresh standards were prepared from master stock solutions for each series of analyses; these were made up as follows -

(a) Lead : The stock solution of 1000 p.p.m. Pb consisted of 0.9152 g of $Pb(CH_3COO)_2 3H_2O$ dissolved in deionised water, acidified with $5cm^3$ of concentrated Nitric Acid, and made up to 500 cm^3. Appropriate working standards within the range 0.5 - 12 p.p.m. were prepared by successive dilution.

(b) Zinc : The stock solution of 1000 p.p.m. Zn consisted of 2.1994 g of $ZnSO_4 \cdot 7H_2O$ dissolved in deionised water, acidified with 5 cm^3 of concentrated Nitric Acid, and made up to 500 cm^3. Working standards were prepared within the range of 0.5 - 7 p.p.m.

(c) Cadmium : The stock solution of 500 p.p.m. Cd was prepared by dissolving 0.5706 g of $3CdSO_4 \cdot 8H_2O$ in deionised water, acidifying with 5 cm^3 of concentrated Nitric Acid, and making up to 500 cm^3. Working standards were prepared within the range 0.1 - 0.8 p.p.m.

A standard curve was prepared for each batch of analyses and this was checked by respraying the standards after every twenty to thirty samples. Typical standard curves are shown in figures 3 to 5. Reagent blanks were sprayed and the results for samples adjusted accordingly. Blanks did not contain detectable levels of lead and the results for zinc and cadmium were equivalent to 0.1 and 0.03 p.p.m. in solution respectively.

In order to reduce heavy metal contamination from external sources, glassware was washed first in 10% Hydrochloric Acid, followed by four washes in distilled water with a final rinse in deionised water. All solutions were made up in deionised water.

4. (iii) Correction for background absorbence.

Atomic Absorption Spectrophotometry is specific for a given element but the response of a particular element may be influenced by chemical interference from other components of the sample solution (Cooke, 1970). De Vries et al. (1975) have drawn attention to the problem of absorption caused by other components of plant ash solution, when determining cadmium and lead by Atomic Absorption Spectrophotometry.

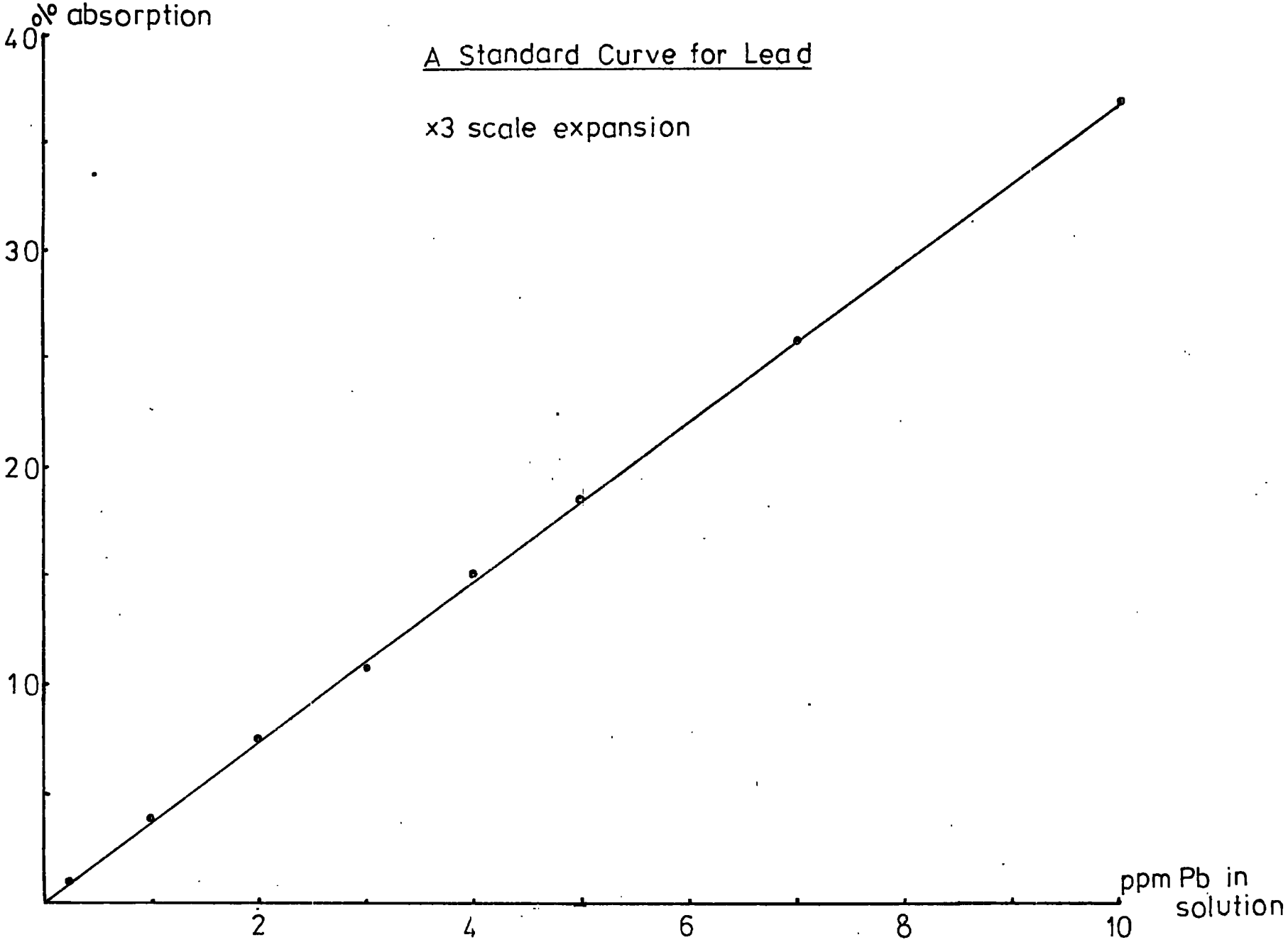

% absorption
40
30
20
10
A Standard Curve for Lead
x3 scale expansion
2
4
6
8
10
ppm Pb in solution

Figure 3

Figure 4 Standard Curve for Zinc

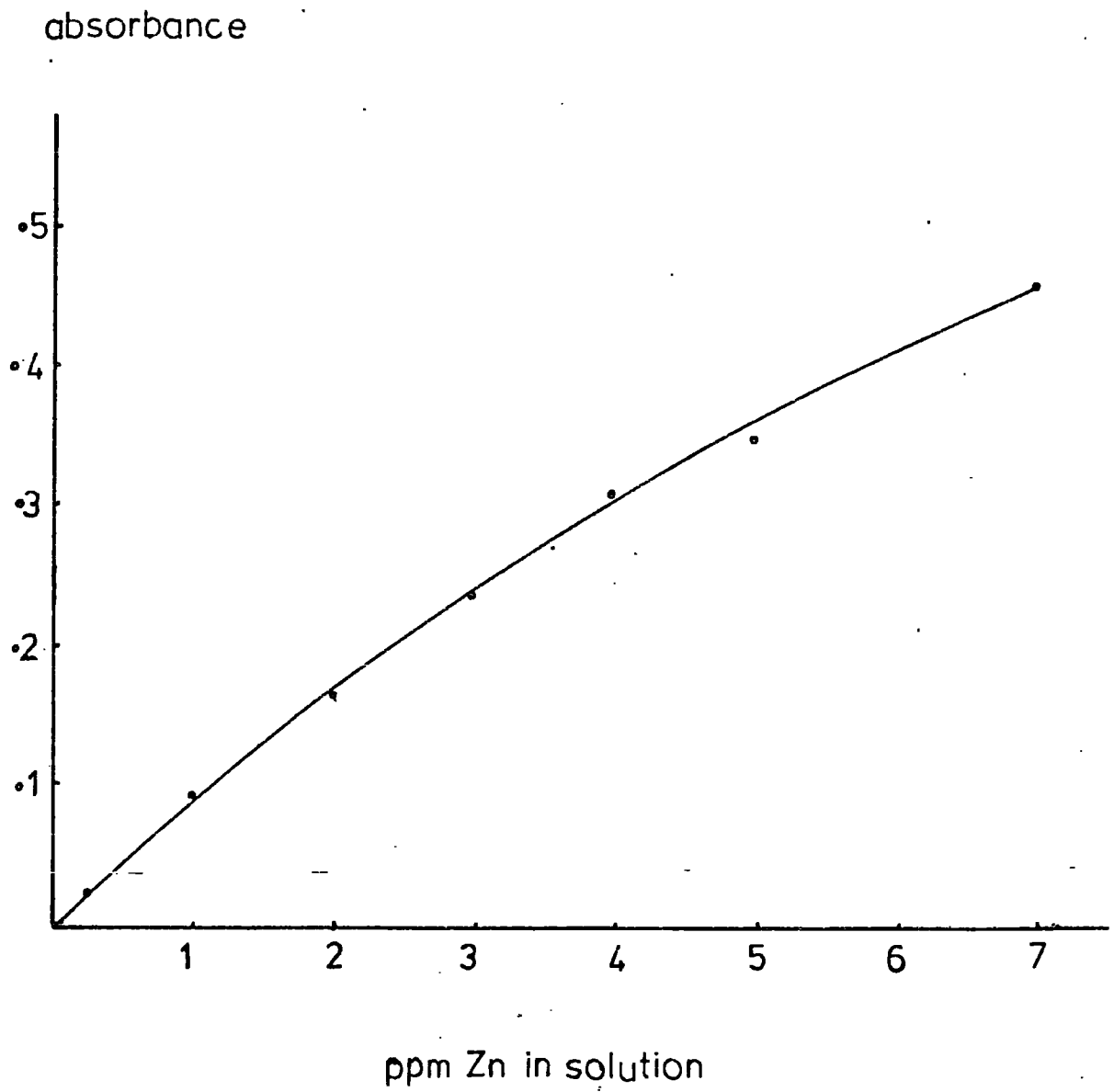
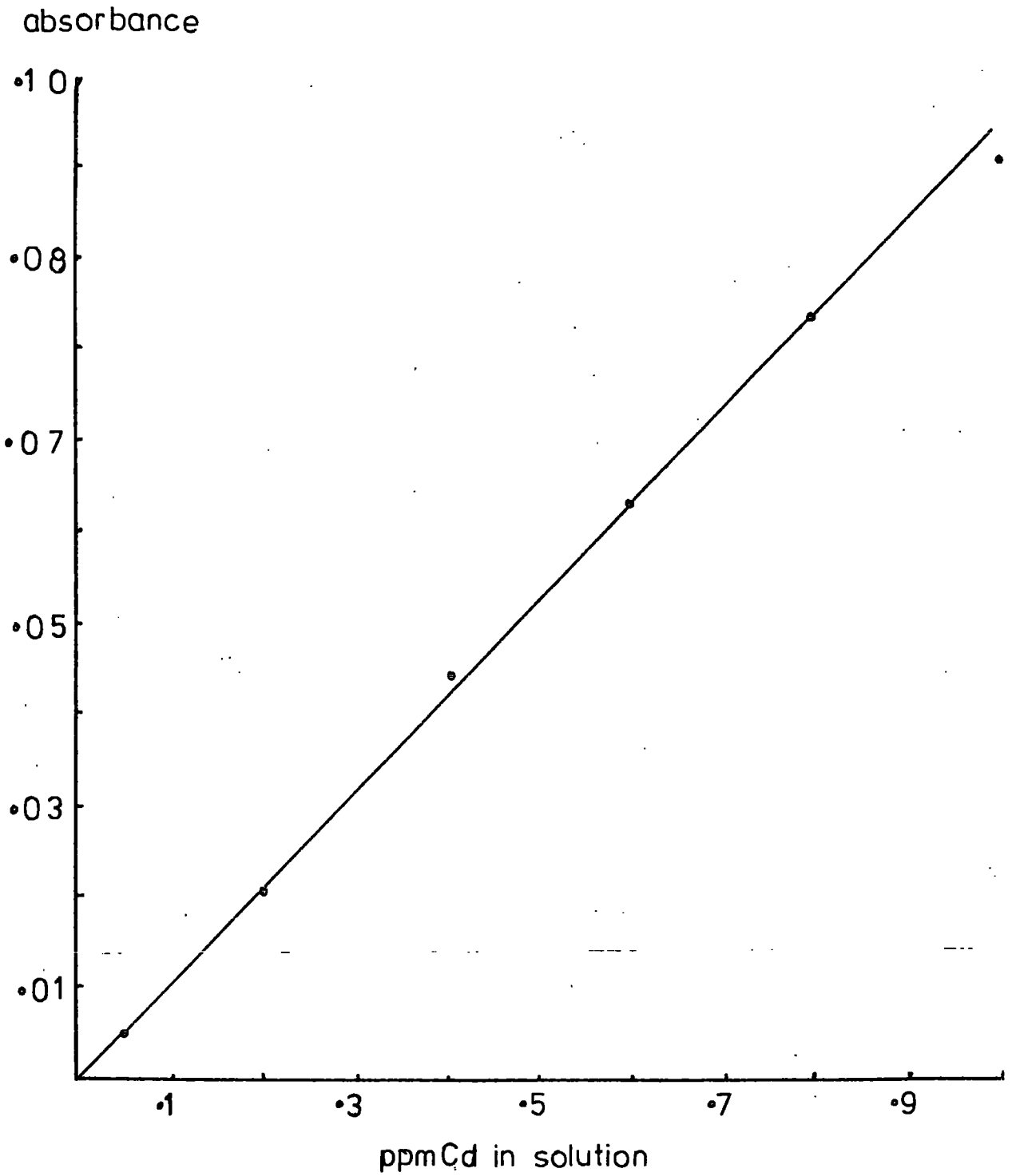


Figure 5 A Standard Curve for Cadmium



Absorption readings may be corrected by the use of a hydrogen continuum lamp, but this was not available during my study. An attempt was made to determine correction factors by a method of Williamson (personal communication), which has been used effectively in analyses of invertebrates for heavy metals. Using methodology modified from De Vries et al. (1975), complexed heavy metals are extracted from suitable digests using an organic solvent. The aqueous remainder of the digest is subjected to Atomic absorption Spectrophotometry to give a value for background interference.

Determinations were carried out on ten types of plant material, which were considered to be representative of the range of materials analysed in the study. These were, U. dioica leaves, U. dioica senescent leaves, S. sylvatica leaves, S. sylvatica senescent leaves, A. elatius leaves, A. elatius senescent leaves, D. glomerata leaves, D. glomerata senescent leaves, C. vulgare leaves, and G. robertianum leaves. Four accurately weighed samples of each plant material were taken, the sample sizes being approximately 0.1, 0.2, 0.3 and 0.4 g respectively. These were subjected to the usual acid digestion procedure, but after evaporating to dryness, were dissolved in 2.5 cm^3 of 3N Hydrochloric Acid and the resultant solutions transferred to 5cm^3 volumetric flasks. One drop of Thymol Blue indicator was added to each flask and the pH adjusted to 2.8 (pink to yellow colour change) by addition of concentrated (880) Ammonia. Final adjustment was achieved using 3N Ammonia and 3N Hydrochloric acid. Solutions were made up to volume with deionised water before transferring to test tubes.

Heavy metals were extracted using a 1% w/v solution of Diethylammonium Diethyldithiocarbamate (DDDC) in Methyl Isobutyl Ketone (MIBK): the solution was freshly prepared for each series of extractions. 3 cm^3 of DDDC/MIBK solution were added to each digest solution and the mixture agitated for 1 minute. Tubes were then left for 1 - 2 minutes to allow the organic layer to rise to the surface, it was then removed by means of a bulb pipette. The remaining solutions were filtered through Whatman No. 1 papers,

and stored in polythene bottles until analyses could be completed.

The absorption of solutions was measured on the Atomic Absorption Spectrophotometer at the resonance wavelengths for lead, zinc and cadmium. When aspirating these solutions, a white deposit accumulated in the burner head slit leading to quite rapid deterioration of the flame. As a result of the need to respray many of the samples, insufficient solution was available, and absorption could not be measured at all three wavelengths in every case.

The results were inconsistent, particularly in the case of zinc and cadmium, and correction factors could not be determined for these two elements. Results are displayed in Appendix 1. "Apparent" correction graphs for lead are displayed in figures 6 and 7 but great reliance should not be placed on the correction factors determined for this element. Results for lead are presented throughout the study in an uncorrected form; an indication of possible corrected values is shown in Table 2 and Appendix 2.

Cooke (1970) suggests an alternative method for the determination of background correction values by measuring at a wavelength, emitted by the hollow cathode lamp adjacent to the resonance wavelength for the element, but at which the element itself does not absorb. Suitable lines are not available for zinc or cadmium, but an attempt was made to determine a correction value for lead in this manner. Scanning the spectrum emitted by the lead lamp showed that the nearest non-absorbing line, of suitable intensity, occured at 348 nm, this is far removed from the resonance wavelength for lead at 283.3nm. The extent of "scatter" due to background interference is wavelength dependant and decreases significantly at wavelengths greater than 300 nm. Absorption at the 348 nm line cannot therefore be considered to provide an accurate indication of interference. Values measured at this wavelength were equivalent to 0.1 - 0.4 p.p.m. in solution.

Figure6 Apparent Correction Factors for Lead

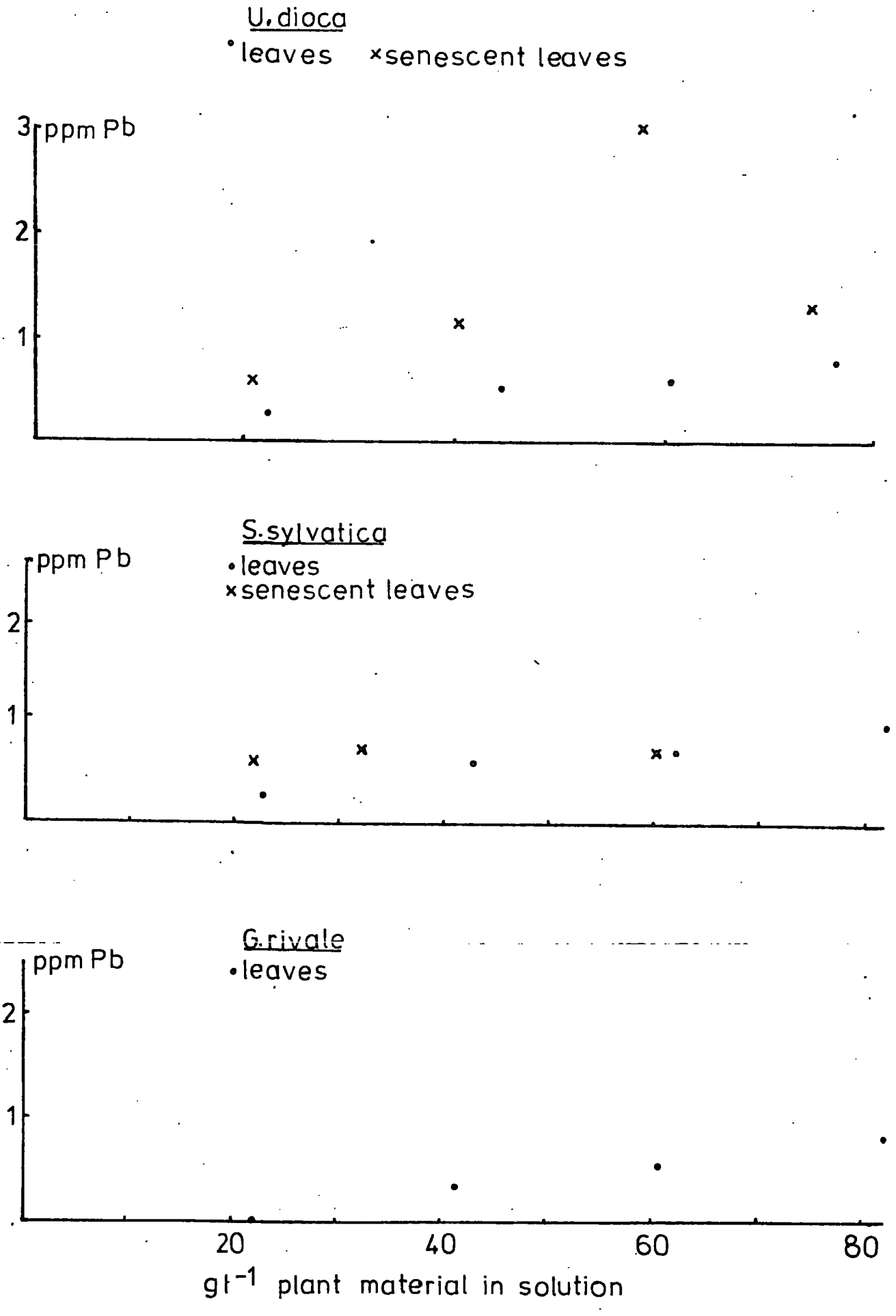
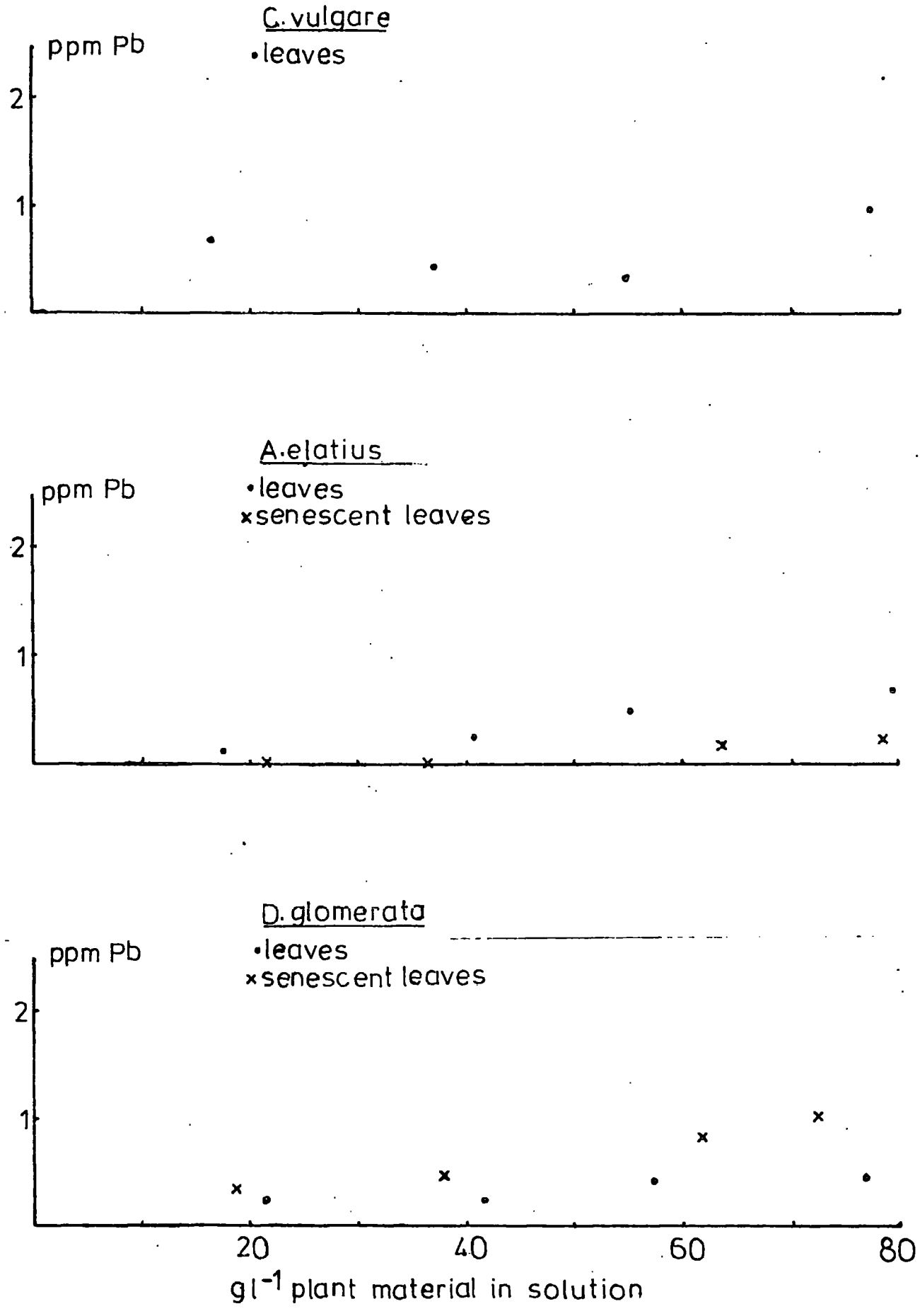


Figure 7 Apparent Correction Factors for Lead



RESULTS

5. General survey of heavy metal concentrations in roadside vegetation.

5. (i) Heavy metal levels in leaves.

Lead, zinc and cadmium were determined in the leaves of 20 plant species; results are presented in Tables 2 to 4. In every case the concentration of lead found was greater than the maximum natural background level of 5 p.p.m. proposed by Smith (1975).

Variation between species was considerable; mean concentrations ranged from 12.33 p.p.m. in the dry matter of *D. caespitosa* to 81.81 p.p.m. in *S. sylvatica*. Grasses contained less lead than other plants sampled; application of the "Students t-test" showed this to be a significant different (grasses 20.89 ± 2.30 (7 samples); dicotyledons 39.80 ± 4.55 p.p.m. (13 samples); $t = 3.71$, $p < .002$).

Although leaves of the different species of dicotyledons varied in morphology, it was not possible to correlate variations in their lead contents with most morphological differences. Three species (*H. helix*, *T. communis*, and *R. obtusifolius*) possessed glabrous or almost glabrous leaves and, as expected, these had relatively low lead concentrations, 28.36, 29.41 and 21.99 p.p.m. respectively. However, the lead content of the leaves of hairy species ranged between the relatively low level of 20.38 p.p.m. in dry matter of *G. rivale* to the high level of 81.71 p.p.m. found in *S. sylvatica*.

Zinc concentrations in the different species ranged from 15.51 to 105.13 p.p.m. in dry matter. A comparison with background levels is difficult because the natural zinc content of plants varies widely; Hewitt et al. (1975) suggest 15 - 75 p.p.m. in dry matter as a range for the "normal" zinc content of leaves. However the levels found in the present study were comparable with those reported by Lagerwerff and Specht (1970), for plants growing near busy main roads. The zinc content of grasses was less than that of other plants sampled and this was shown to be a significant difference by application of the "Student's t-test" (grasses 25.43 ± 2.84

p.p.m. (7 samples); dicotyledons 49.69 ± 5.90 p.p.m. (13 samples); $t = 3.71$. $p < .002$).

No evidence was found of correlation between leaf morphology and zinc concentrations; levels in species of dicotyledons with hairy leaves ranged from the relatively low value of 25.59 p.p.m. found in R. fruticosus to the high value of 105.13 p.p.m. in G. hederacea. Some glabrous leaves contained relatively high zinc levels (H. helix 61.01 and T. communis 47.17 p.p.m. in dry matter), whilst those of R. obtusifolius contained a relatively low concentration (29.02 p.p.m.).

Those species sampled, which contained high lead concentrations, did not necessarily have high zinc concentrations; the relationship between concentrations of the two metals is shown in figure 8. Correlation between lead and zinc levels was examined using Kendall's rank coefficient: no significant correlation was found ($T_B = 0.41$; $p > 0.1$).

Great reliance cannot be placed in the results of cadmium determinations, since replicate analyses often showed considerable variation. The range of values found was low and the effect of background interference is uncertain. None of the grasses examined contained levels of cadmium which were detectable by the method employed, whilst values for other plants ranged from 0 (below detection level) to 1.58 p.p.m. in dry matter. Most of the values were within the range reported for natural vegetation (Inskip, 1973) of 0.1 - 1.0 p.p.m. in dry matter: only C. vulgare (1.58 p.p.m.) exceeded this by any large amount.

5. (ii) Comparison of heavy metal contents of living and senescent leaves.

The heavy metal contents of living and senescent leaves were compared in the following plant species, U. dioica, R. obtusifolius, D. glomerata and F. rubra. Results are detailed in Table 5. Levels of lead and zinc were significantly greater in senescent than in living leaves of all four species. The differences between concentrations in these two kinds of plant material were greater for lead than for zinc, and, for both elements, greater in grasses than in the two species of forbs examined. Senescent leaves of

the two grasses contained particularly high concentrations of lead, representing approximately 9 - 10 fold increases over concentrations found in living leaves.

The cadmium analyses provide an indication of increased concentrations in the senescent leaves of R. obtusifolius, but this may not be real in view of the overall variability in the results of cadmium determinations.

5. (iii) Comparison of heavy metal concentrations in unwashed and washed leaves

Heavy metal concentrations were compared in unwashed and washed leaves of nine plant species and in senescent leaves of three species; results of analyses are shown in Table 6. The differences were examined using "Student's t-tests" and levels of probability for significant differences are shown in Table 7. Washing did not affect the concentrations of lead or zinc significantly in leaves of the four species C. vulgare, R. obtusifolius, A. elatius and D. caespitosa, or in senescent leaves of D. glomerata. In addition, washing did not lower the concentrations of zinc significantly in living or senescent leaves of U. dioica. No evidence was found that "apparent" cadmium levels were significantly affected by the washing process.

In the remaining species (senescent and non-senescent leaves), the percentages of heavy metals removed by washing were within the ranges 15 - 57 and 10 - 32 for lead and zinc respectively. Details for each species are listed in Table 8. The considerable variation in losses which occured between species could not be related to differences in leaf morphology.

With one exception (F. rubra, senescent leaves), washing removed a greater percentage of lead than zinc. To discover whether lead and zinc "deposition" was influenced by similar factors, correlation between percentages of the two elements removed from species was examined by Kendall's rank coefficient T_B. A significant correlation was not found ($T_B = -0.523$; $p > .05$).

Analysis of leaves of H. helix produced an anomalous result in the case of zinc, suggesting that the concentration of this element was increased

significantly in leaves when they were washed. It seems probable that contamination occured during some part of the washing process or preparation of material for analysis, since all values for washed leaves were higher than all values for unwashed. The distilled water used contained insignificant amounts of heavy metals, but contamination could have occured whilst leaves were being air-dried.

6. Distribution of lead within plants of selected species.

Lead concentrations were determined in different fractions from plants of three species, sampled from each of three sites within the study area. Sites were numbered as follows : 1a, 2a, 3a for U. dioica, 1b, 2b, 3b for S. sylvatica, and 1c, 2c, and 3c for A. elatius. Sites for the three species were in the same section of the study area, but did not coincide, and were separated by several meters in some instances. Sites 1a - 1c were in the southern part, 2a - 2c in the central section, and 3a - 3c in the northern part of the study area. Results are detailed in Appendix 2 and are shown graphically in figures 9 - 11.

6. (i) Distribution of lead within plants.

(a) Urtica dioica : Stems contained the lowest lead concentrations; mean values did not exceed 11 p.p.m. in dry matter. In the remaining fractions of plants, lower (older) parts contained more lead than upper (younger) parts. The magnitude of concentrations descended in the order senescent leaves > leaves 8 - 10 > leaves 5 - 7 > tips > stems. At all sites the lead content of the lowest group of living leaves (8 - 10) was approximately 1.5 times, and that of senescent leaves almost twice, that of the upper group of large, expanded leaves (leaves 5 - 7).

(b) Stachys sylvatica : The distribution of lead concentrations within plants followed a similar trend to that in the previous species. Levels decreased usually in the order senescent leaves > leaves 3 or 4 - 5 > leaves 1 - 2 > inflorescence > stems. The relationship between leaves 3 and leaves 4 - 5 differed between sites, and at site 2b, inflorescences contained as much lead as the lowest living leaves.

The magnitude of the difference in concentrations, between upper and lower living leaves, was similar to that found in U. dioica, but senescent leaves contained between 2 and 5 times as much lead as the upper leaves. A high level of lead (more than 250 p.p.m. in dry matter) was found in senescent leaves from site 2b.

(c) Arrhenatherum elatius : The distribution of lead concentrations showed a similar trend to those found in the previous species, and decreased in the order old senescent leaves > recent senescent leaves > living leaves > inflorescences > culms. The difference between concentrations found in upper and lower living leaves was less marked than that in the previous species, except at one site, where plants possessed an additional pair of leaves (leaves 7 - 8). An exception to the general trend occured at site 3c where inflorescences contained as much lead as the lower, living leaves.

6. (ii) Differences between sites.

For each species, lead concentrations were compared between each pair of sites. Differences were examined by a series of "Student's t-tests" for each of the parts of the plants analysed. Results are displayed in Table 9 and appropriate values of "t" are displayed in Appendix 3. In most cases the lead contents of parts of plants differed significantly between sites. Stems were a consistent exception, but the lead content of these was low and replicate analyses variable. This may be associated with difficulties which were experienced in obtaining representative sub-samples of chopped stem material.

Less instances of significant differences were found in the lead contents of equivalent parts of U. dioica, between sites, than in the other species examined.

A general comparison of plant contamination by lead, amongst three sites for each species, shows that all fractions of U. dioica plants were most contaminated at site 3a, less at site 2a and least at site 1a. S. sylvatica generally contained least lead at site 3b, whilst relative levels of lead in material from sites 1b and 2b were not consistent for the

different fractions of plants analysed. Relative levels of lead in A. elatius, from three sites, varied considerably for the different fractions of plants analysed.

7. Comparison of lead contents in plants sampled on two occasions.

Lead concentrations were compared in three species of plants which were sampled in both June and July. These were U. dioica, S. sylvatica and A. elatius; materials common to both samples were the lower living leaves of all three species and senescent leaves of U. dioica only. The data used was that collected for the general survey and for the study of lead distribution within plants (Sections 5 and, 6 (i)). Sampling methods used on the two occasions were not identical and so data from the three sample sites for the July collection were pooled, and taken to be representative of the area as a whole.

The application of "Student's t-tests" showed that the lead concentrations were significantly greater in material sampled in the July collection for U. dioica leaves ($t = 6.49$; $p < .001$), A. elatius leaves ($t = 2.46$; $p < .05$), and for senescent leaves of U. dioica ($t = 4.41$; $p < .002$). Lead concentrations in S. sylvatica leaves did not differ significantly between the two collections ($t = .87$; $p > 0.1$).

8. Lead concentrations in vegetation from a transect across the site.

Lead concentrations were determined in the lowest, living leaves of U. dioica plants collected along a transect across the central reservation. Results are presented in Table 10 and graphed in figure 12.

The highest lead concentration was found at the 2m station which was the only site outside the tree-enclosed area, and also the nearest site to the road. Differences in concentrations, within the area enclosed by trees, cannot be closely related to either distance from carriageways or prevailing winds. The sample taken cannot be considered representative of the area as a whole, since it comprised one fraction of plants, of a single species, collected along one transect. It is possible that much of the variation could have been caused by local differences in conditions at sample sites.

The lowest concentration was found at the 10m station, which was also approximately 14m from the southbound carriageway. The 16m station was the nearest to the southbound carriageway, being approximately 8m from this part of the road. The application of a "Student's t-test" to a comparison between the 8 and 16m stations, showed that lead concentrations in the vegetation sampled were not significantly different ($t = 0.28$; $p > 0.1$).

Table 2. Lead concentrations in leaves of twenty species (p.p.m. in dry matter)

Species	Mean lead concentration	Standard error	Apparent corrected value
Hedera helix	28.36	1.17	22.2 - 19.1
Cirsium vulgare	42.53	2.61	35.6 - 42.3
Tamus communis	29.41	4.79	16.5 - 23.2
Geranium robertianum	41.28	1.68	28.4 - 35.1
Arrhenatherum elatius	24.99	2.66	16.8 - 18.1
Brachypodium sylvaticum	23.17	1.39	14.9 - 16.3
Dactylis glomerata	18.39	1.06	10.2 - 11.5
Deschampsia caespitosa	12.33	1.12	4.1 - 5.4
Festuca rubra	14.27	1.44	6.0 - 7.4
Holcus lanatus	24.04	1.45	15.8 - 17.1
Poa trivialis	29.04	1.71	20.8 - 22.1
Glechoma hederacea	47.02	0.46	34.1 - 40.8
Stachys sylvatica	81.71	3.79	68.8 - 75.5
Vicia sepium	33.69	0.49	20.8 - 27.5
Rumex obtusifolius	21.99	2.05	9.1 - 15.8
Geum rivale	20.38	0.52	7.5 - 14.2
Rubus fruticosus	29.78	2.44	16.9 - 23.6
Galium cruciata	40.42	2.44	27.5 - 34.2
Heracleum sphondylium	42.63	1.90	29.7 - 36.4
Urtica dioica	58.20	0.88	45.3 - 52.0

Note : "Apparent corrected values" were derived by applying possible maximum and minimum correction factors for background interference (see figures 6 and 7).

Table 3. Zinc concentrations in leaves of twenty species (p.p.m. in dry matter)

Species	Mean zinc concentration	Standard error
Hedera helix	61.01	1.20
Cirsium vulgare	74.04	6.09
Tamus communis	47.17	0.66
Geranium robertianum	33.06	1.07
Arrhenatherum elatius	15.51	0.79
Brachypodium sylvaticum	23.67	1.67
Dactylis glomerata	18.41	0.22
Deschampsia caespitosa	30.90	1.85
Festuca rubra	25.04	0
Holcus lanatus	26.47	0.58
Poa trivialis	37.99	3.68
Glechoma hederacea	105.13	0.71
Stachys sylvatica	38.76	0.70
Vicia sepium	43.58	*
Rumex obtusifolius	29.02	0.50
Geum rivale	35.63	1.00
Rubus fruticosus	25.59	0.80
Galium cruciata	34.56	4.29
Heracleum sphondylium	69.44	0.71
Urtica dioica	42.20	2.43

* two analyses only carried out

Table 4. Cadmium concentrations in the leaves of twenty species (p.p.m. in dry matter)

Species	Mean cadmiun concentration	Standard error
Hedera helix	0.80	0.17
Cirsium vulgare	1.58	0.21
Tamus communis	0.60	0.08
Geranium robertianum	0.58	0.14
Arrhenatherum elatius	0	-
Brachypodium sylvaticum	0	-
Dactylis glomerata	0	-
Deschampsia caespitosa	0	-
Festuca rubra	0	-
Holcus lanatus	0	-
Poa trivialis	0	-
Glechoma hederacea	0.48	0.04
Stachys sylvatica	0.39	0.07
Vicia sepium	0.10	0.10
Rumex obtusifolius	0.24	0.07
Geum rivale	0.34	0.05
Rubus fruticosus	0	-
Galium cruciata	0.75	0.09
Heracleum sphondylium	0.20	0.15
Urtica dioica	1.03	0.31

Table 5. Comparison of heavy metal contents of senescent and non-senescent leaves.

Mean values with 95% confidence limits (uncorrected).

Non-senescent

p.p.m. in dry matter

	Lead		Zinc		Cadmium	
U. dioica	58.2	56.44 - 59.96	42.2	37.40 - 47.00	1.03	0.41 - 1.65
R. obtusifolius	21.99	17.88 - 26.09	29.02	28.02 - 30.02	0.24	0.10 - 0.38
D. glomerata	18.39	16.27 - 20.51	18.41	17.97 - 18.85	0	-
F. rubra	14.27	12.83 - 15.71	25.04	25.04	0	-

Senescent

	Lead		Zinc		Cadmium	
U. dioica	93.36	83.85 - 96.53	50.40	48.2 - 52.60	1.13	0.97 - 1.29
R. obtusifolius	59.29	55.71 - 62.87	37.41	36.61 - 38.21	0.90	0.79 - 1.01
D. glomerata	199.3	188.02 - 210.58	57.68	53.68 - 61.68	0.33	0.10 - 0.58
F. rubra	139.04	132.44 - 145.64	60.36	55.48 - 65.24	0	--

Results of Student's t-tests for comparisons of metal contents in senescent and non-senescent leaves

	Lead		Zinc		Cadmium	
	t	p	t	p	t	p
U. dioica	11.58	< .001	3.07	< .05	0.32	> .1
R. obtusifolius	13.71	< .001	7.99	< .002	0.09	> .1
D. glomerata	22.50	< .001	91.20	< .001	-	-
F. rubra	37.46	< .001	14.48	< .001	-	-

Figure 8 Correlation between lead and zinc concentrations in leaves of 20 species

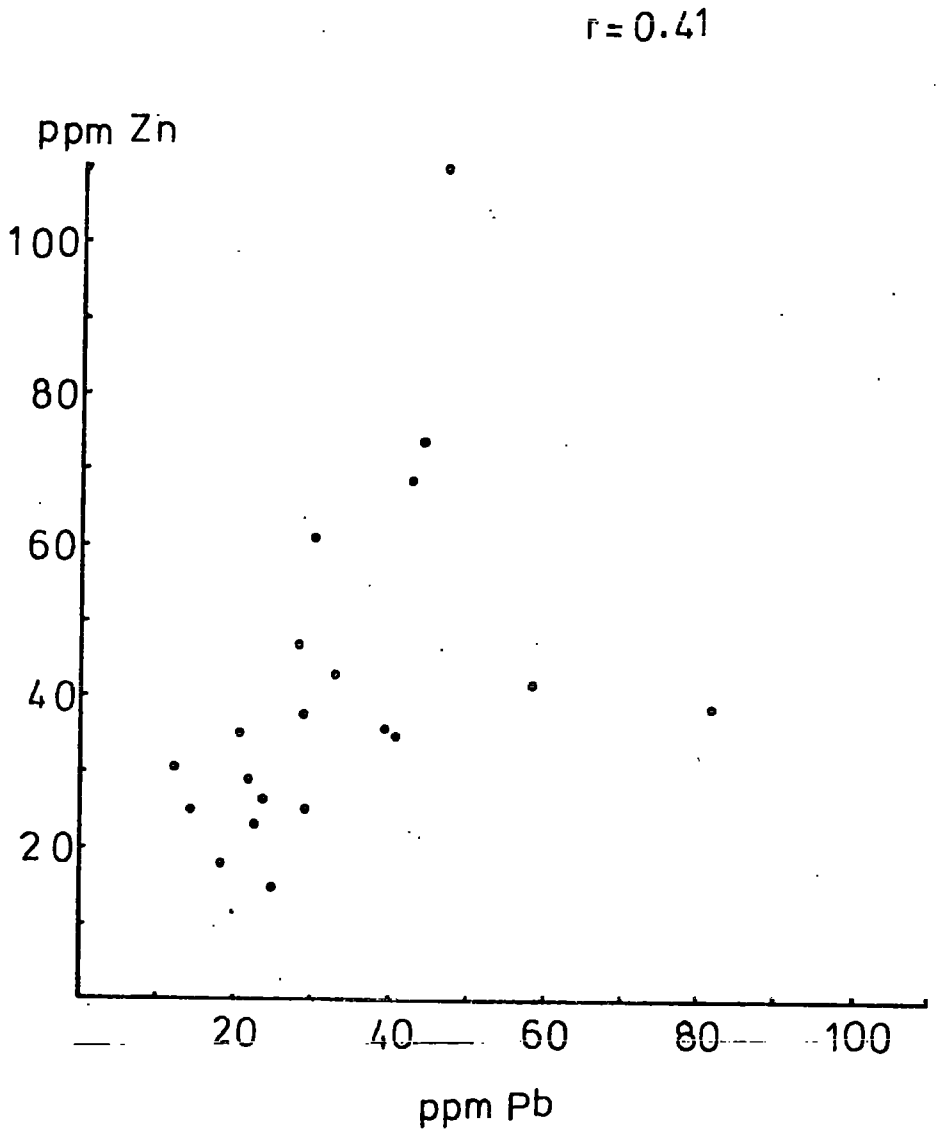


Table 6. Heavy metal content of unwashed and washed leaves.

	Unwashed						Washed					
	Lead		Zinc		Cadmium		Lead		Zinc		Cadmium	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
H. helix	28.36	1.17	61.01	1.20	0.8	0.17	18.39	1.23	75.28	1.91	0.81	0.11
C. vulgare	42.53	2.61	74.04	6.09	1.58	0.21	36.54	1.82	85.48	4.60	1.28	0.09
T. communis	29.41	4.79	47.17	0.66	0.60	0.08	15.91	0.45	41.90	0.53	0.20	x
R. obtusifolius	21.99	2.05	29.02	0.50	0.24	0.07	22.58	3.04	29.11	0.50	0.07	0.03
R. fruticosus	29.78	2.44	25.59	0.80	0	-	12.71	1.06	20.27	0.61	0.13	0.13
G. rivale	20.38	0.52	35.63	1.00	0.34	0.05	12.78	0.14	31.93	0.68	0.25	x
U. dioica	58.20	0.88	42.20	2.43	1.03	0.31	34.04	0.58	35.35	2.02	0.95	0.06
A. elatius	24.99	2.66	15.51	0.79	0	-	17.32	2.03	14.77	0	0	-
D. caespitosa	12.33	1.12	30.90	1.85	0	-	13.93	0.72	25.51	1.59	0	-
Senescent												
U. dioica	96.36	3.17	50.40	1.10	1.13	0.08	54.32	4.78	42.59	2.63	1.37	0.11
D. glomerata	199.30	5.64	57.68	2.00	0.33	0.12	208.77	18.45	58.70	3.54	0.06	0.03
F. Rubra	139.04	3.30	60.36	2.44	0	-	177.97	1.42	40.57	1.06	0	-

x two analyses only

Table 7. Probability levels for significant differences in heavy metal contents of unwashed and washed foliage.

	Lead		Zinc		Cadmium	
	P	"t"	P	"t"	P	"t"
H. helix	<.01	5.87	<.01	6.31	NS	0.296
C. vulgare	NS	1.88	NS	1.49	NS	1.31
T. communis	<.05	2.80	<.01	6.22	x	x
R. obtusifolius	NS	0.16	NS	0.13	NS	2.24
R. fruticosus	<.01	5.22	<.01	5.32	NS	1.0
G. rivale	<.001	14.11	<.05	3.06	x	x
U. dioica	<.001	22.92	NS	2.17	NS	0.25
A. elatius	NS	2.29	NS	0.94	-	-
D. caespitosa	NS	1.20	NS	2.21	-	-
Senescent						
U. dioica	<.002	7.33	NS	2.73	NS	1.76
D. glomerata	NS	0.49	NS	0.25	NS	2.18
F. rubra	<.01	5.85	<.002	7.43	-	-

NS = not significant

x = insufficient replicate analyses carried out

- = metal not detected in either material

Table 8. Percentages of heavy metals removed by washing.

	Lead	Zinc	Cadmium
H. helix	35	+23	(+1)
C. vulgare	(14)	(+15)	(19)
T. communis	46	11	x
R. obtusifolius	(+3)	(0)	(71)
R. fruticosus	57	21	(+13)
G. rivale	37	10	x
U. dioica	42	(16)	(8)
A. elatius	(31)	(5)	-
D. caespitosa	(+13)	(17)	-
Senescent			
U. dioica	44	(16)	(+21)
D. glomerata	(+5)	(+2)	(82)
F. rubra	15	33	-

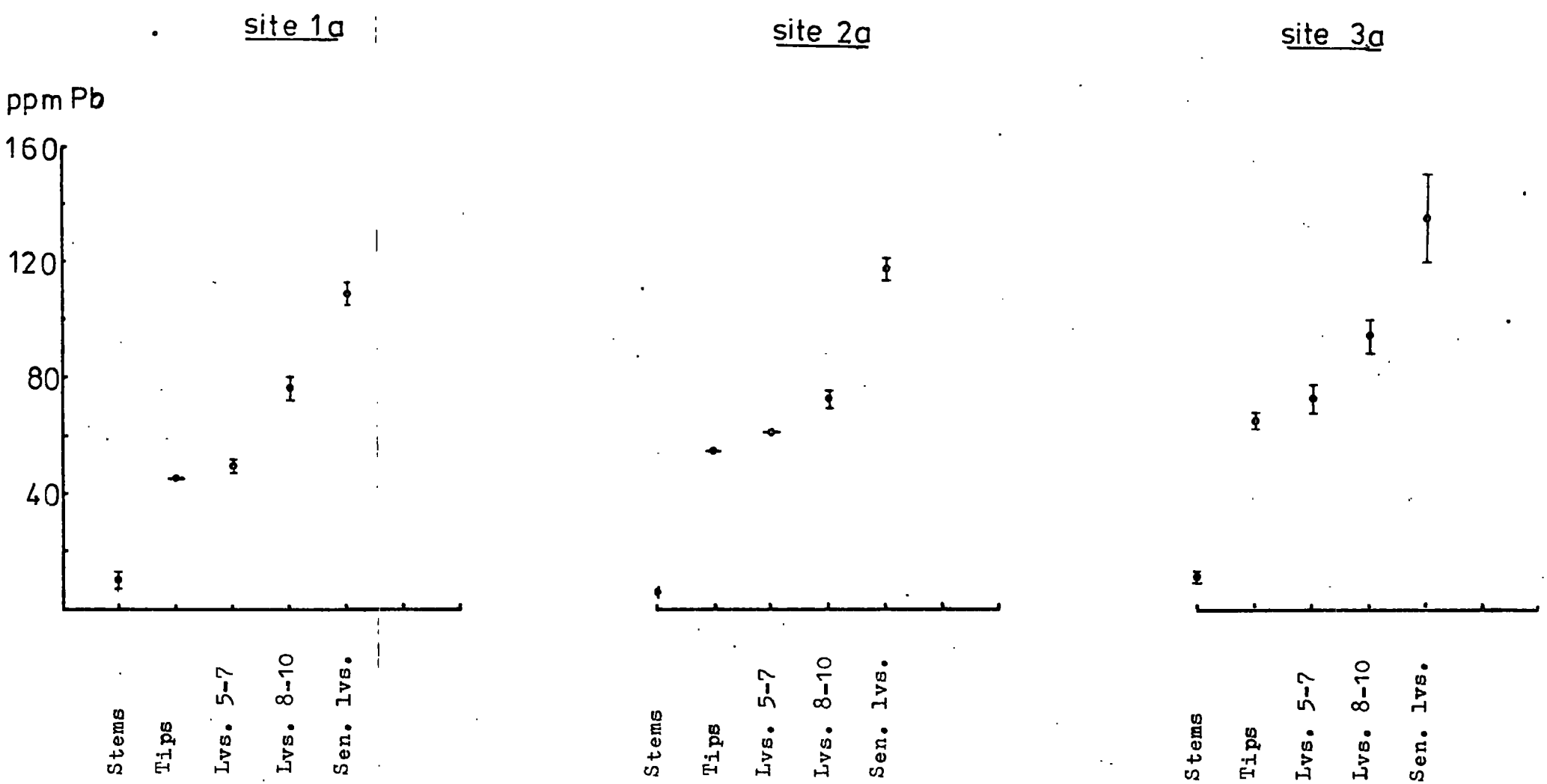
\+ = apparent increase

( ) = difference not significant

\- = metal not detected in either material

x = insufficient replicate analyses carried out

Figure9 Distribution of lead within plants - U.dioica mean values+2 standard errors



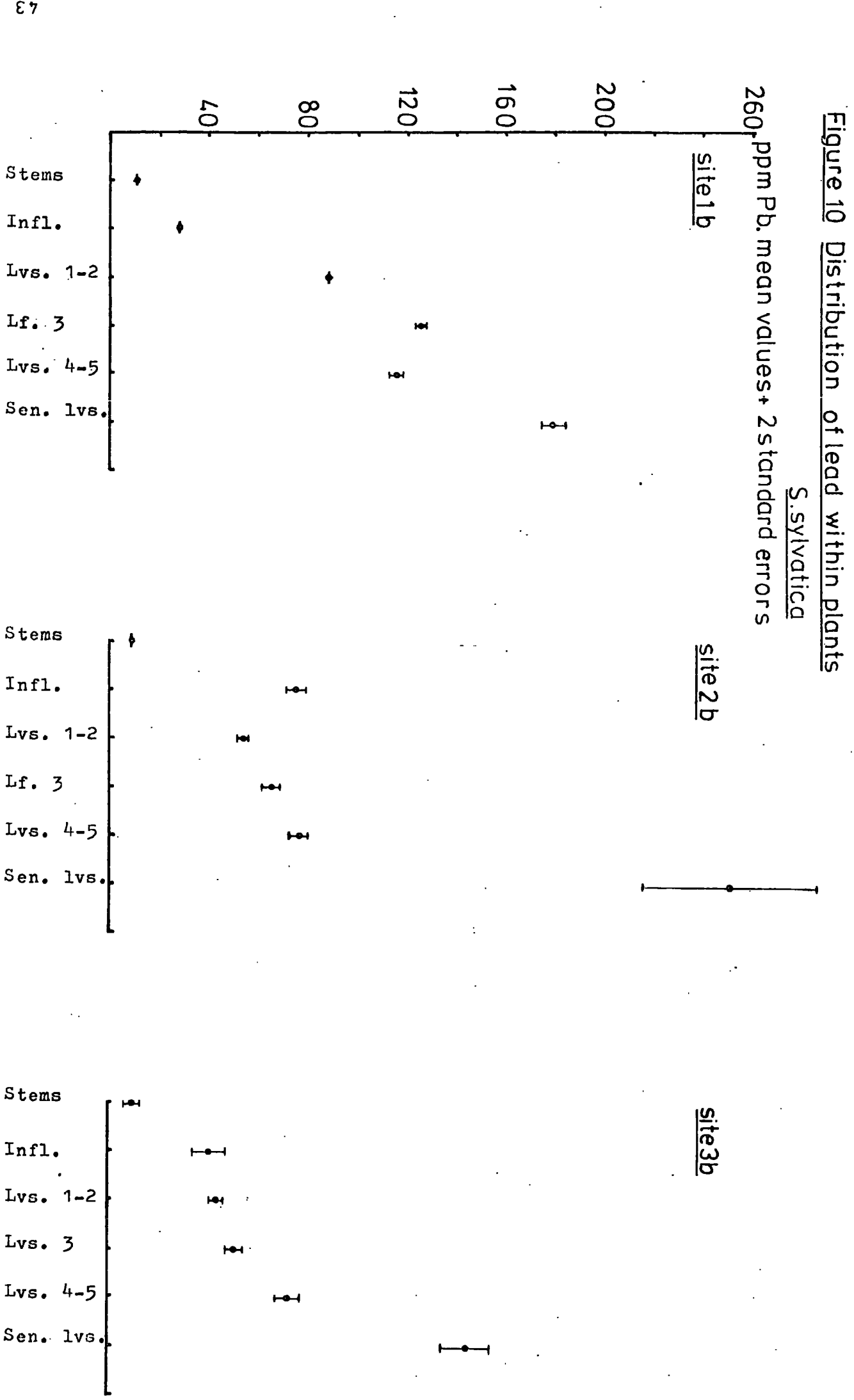


Figure 10 Distribution of lead within plants

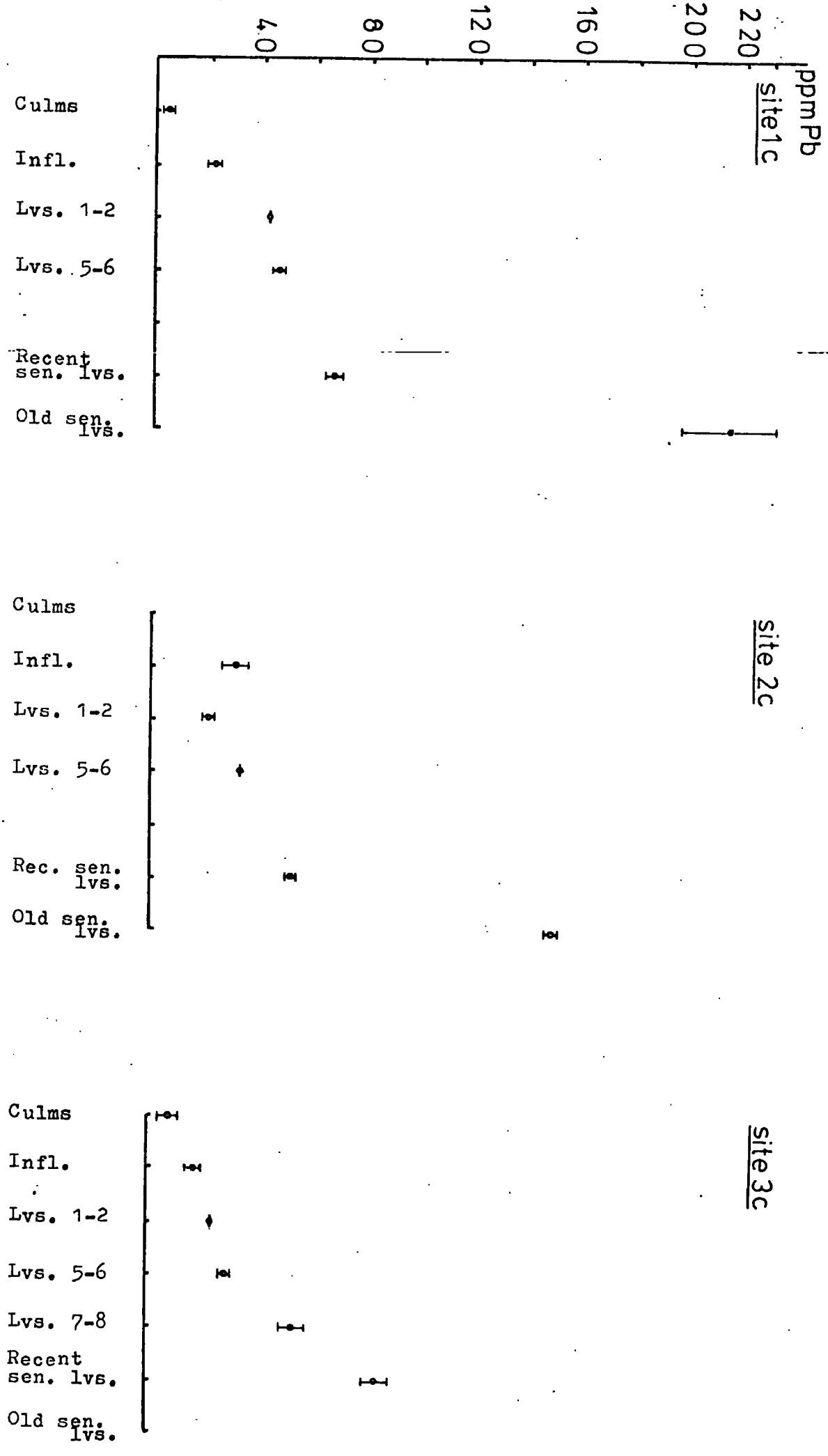


Figure 11 Distribution of lead within plants- A. elatius mean values +2 standard errors

Table 9. Differences between sites - probability levels for significant differences.

Urtica dioica	Sites 1 and 2	Sites 1 and 3	Sites 2 and 3
Tips	<.001	<.001	<.01
Leaves 5 - 7	<.002	<.002	<.02
Leaves 8 - 10	NS	<.01	<.01
Senescent leaves	NS	<.05	NS
Stems	NS	NS	NS
Stachys sylvatica			
Inflorescence	<.001	<.05	<.002
Leaves 1 - 2	<.001	<.001	<.01
Leaf 3	<.001	<.001	<.01
Leaves 4 - 5	<.001	<.001	NS
Senescent leaves	<.02	<.01	<.01
Stems	NS	NS	NS
Arrhenatherum elatius			
Inflorescence	<.05	NS	<.02
Leaves 1 - 2	<.001	<.001	<.05
Leaves 5 - 6	<.001	<.001	<.05
Recent senescent leaves	<.002	<.01	<.001
Old senescent leaves	<.01	-	-
Culms	NS	NS	NS

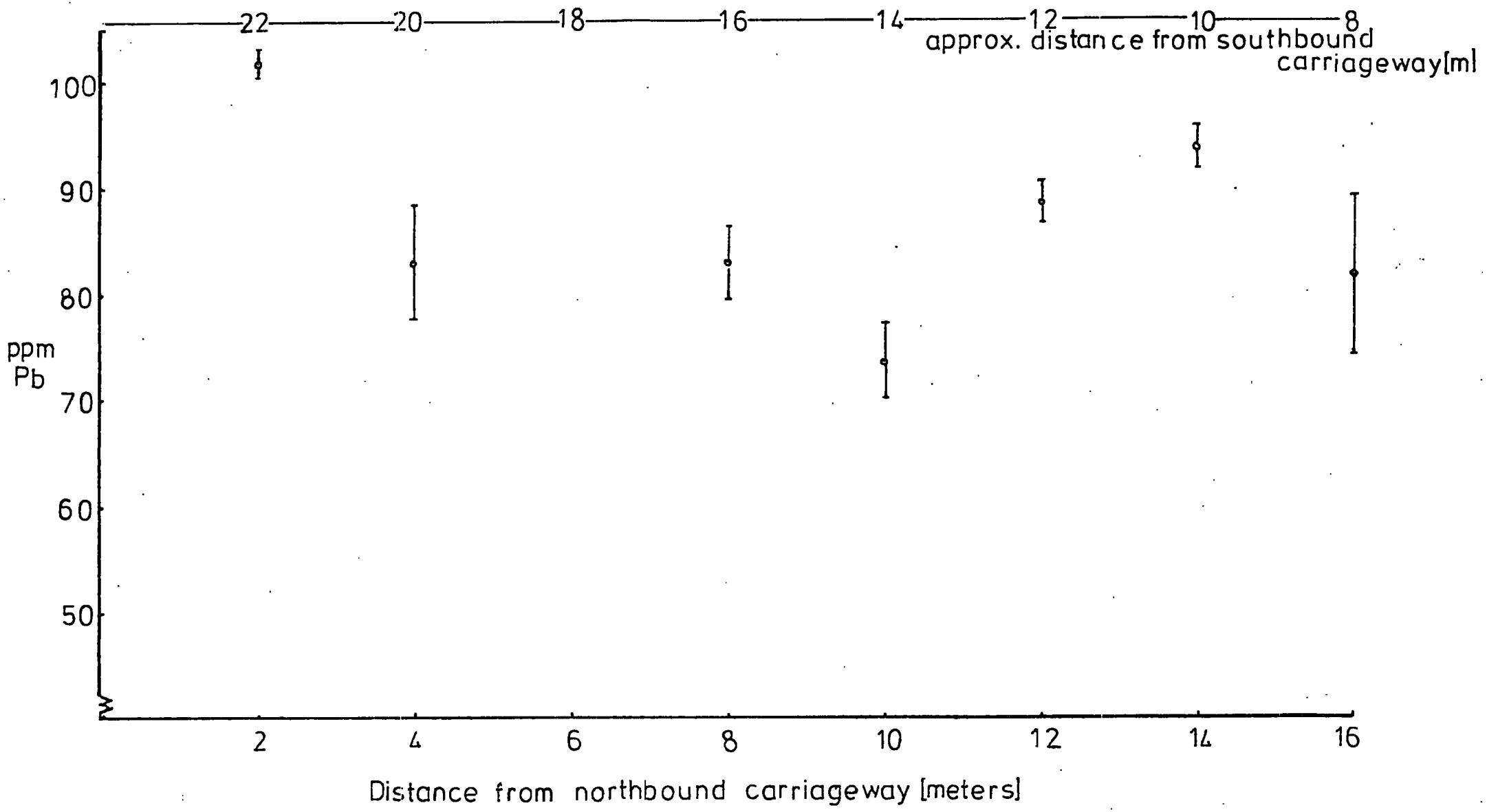
NS = not significantly different.

Table 10. Lead concentrations in Urtica dioica from a transect across the central reservation.

(p.p.m. in dry matter)

Distance from northbound carriageway	Mean lead content p.p.m.	S.E.	Approximate distance from southbound carriageway
2 meters	101.89	0.75	22 meters
4	83.15	2.71	20
6	did not occur		18
8	83.12	1.95	16
10	73.69	1.82	14
12	88.85	0.99	12
14	93.93	0.93	10
16	81.92	3.80	8

Figure12 Transect -mean lead concentrations plus two standard errors



DISCUSSION

9. (i) Introduction.

Heavy metals may accumulate in roadside vegetation both directly from the atmosphere and from the soil. The levels found in the aerial parts of plants will be influenced by four main factors. There are : the concentrations of heavy metals in the air and soil in the immediate vicinity of plants, the duration of exposure to metals from either source, the extent of uptake from the soil and the mobility of the metals within plants, and the ability of foliage to trap and retain topical deposits containing heavy metals.

9. (ii) Evidence for elevated levels of heavy metals in non-senescent leaves.

In the present study, elevated levels of lead were found in non-senescent leaves of all 20 plant species examined. Concentrations were within the range reported in previous studies of contaminated roadside vegetation, but were modest by comparison with those found at some sites. For example, Graham and Kalman (1974) reported 950 p.p.m. in the dry matter of grass from a single Californian site. However, many of the surveys have been carried out on exposed roadside locations, whereas in the present study the sampling area was separated from the carriageways by trees and shrubs. It is probable that these retained a proportion of the airborne load of heavy metals, thereby reducing levels in the immediate vicinity of plants within the enclosed area. This view is supported by work of Smith (1971), in which he showed that roadside pine trees filtered out considerable quantities of airborne contamination, derived from vehicle exhausts.

Zinc concentrations, found in this study, were also elevated above "natural" background levels when compared with those reported by Lagerwerff and Specht (1970), but the evidence is less clear-cut for this element. Zinc is an essential mineral in plant metabolism and a wide range of levels has been reported in uncontaminated plants (Hewitt et al., 1975). Local "background" concentrations of zinc, in plants growing near the study site

but away from the road, are not known. These would provide a useful basis for comparison in future studies.

Little evidence was found of cadmium contamination, but analyses for this element were somewhat inconsistent because levels were close to the limits of detection of the spectrophotometer.

9. (iii) Comparison between heavy metal concentrations in different species.

There was considerable variation in the levels of heavy metals found in the different species sampled. Page et al., (1971) studied 27 crop species grown near Californian highways, and concluded that species with rough, hairy, flexible surfaces accumulated more lead than those with smooth surfaces. Species sampled in the present study included plants with a range of leaf types, although few species were completely devoid of hairs. No clear-cut evidence was found of a correlation between morphological differences and differences in heavy metal concentrations. However, it is possible that morphological characters had some influence on the relatively low levels of lead found in R. obtusifolius, H. helix, and T. communis, as each of these species possessed leaf lamina with relatively smooth upper surfaces.

Differences in metal burdens, between species, may have resulted in part from variations in the duration of direct exposure to airborne heavy metals. Samples comprised pooled collections of the lowest 2 or 3 non-senescent leaves from a number of plants and, whilst these were the most mature, living leaves in each case, there is no reason to suppose that they were of identical age for each species. This interpretation is supported by the finding that leaves of all grasses analysed contained lower concentrations of the metals than those of other plants. Grass leaves are short lived by comparison with those of many dicotyledons (Langer, 1972); indeed, Bean (1964) showed that the average life of leaves of the grass Dactylis glomerata was only 8 - 10 weeks, and data present by Langer (1954) may be analysed to show that leaves of Phleum pratense may remain alive for only 4 - 5 weeks.

It is unlikely that such differences in growing seasons could have produced variation in lead and zinc concentrations, between species, of the magnitude found in this study, and other factors must also have been involved. It is possible that airborne heavy metal concentrations, in the immediate vicinity of the lower leaves of plants, may be influenced by the degree of protection afforded by the canopy of the ground-layer vegetation. It may be argued that the lower leaves of species with "open" growth habits, or which grow in exposed situations, are more vulnerable to fall-out of heavy metals. Thus the large difference between lead levels in leaves of R. obtusifolius and U. dioica, the species at the extremes of the range of concentrations found, may have resulted, in part, from differing exposures within the ground layer canopy. The lowest, non-senescent leaves of R. obtusifolius originated from nodes near ground level, where they may have been protected from the full effects of contamination. However, equivalent leaves of U. dioica usually originated from nodes which were quite high up on the tall stems of this species.

9. (iv) Comparison of lead and zinc levels in leaves of the same species.

A comparison of lead and zinc concentrations (fig. 8) showed that a high lead concentration did not necessarily entail a high concentration of zinc in leaves of the same species, values for the two metals were not significantly correlated. It is possible that compounds of the two metals, deposited on leaf surfaces, differ in their solubility and that different proportions of each are removed by rainfall. In addition, the extent of foliar penetration differs between the two metals. Little (1973) reviews studies which suggest that zinc and cadmium are more likely to achieve foliar penetration than lead. Arvik and Zimdahl (1974) demonstrated that little foliar uptake of lead occurs.

An alternative explanation is that the two metals differ in their origins, and have different distributions in the leaves of plants. Much of the lead in soils is thought to be unavailable to roadside plants (Smith, 1975), and only a small portion, of that taken up by roots, is translocated to the shoot (Jones et al., 1973a). Zinc is more readily taken

up by roots and is more mobile within plants: the zinc present in leaves may thus be derived from both soil and atmospheric sources. It is probable, therefore, that lead concentrations in leaves are largely controlled by factors governing the extent of aerial deposition, but that concentrations of zinc are influenced by these factors and also, independently, by those which influence the extent of uptake from the soil. Species differ in their abilities to accumulate elements from the soil; Peterson (1971) draws attention to unusual accumulations of elements, including zinc, found in some plant species.

9. (v) The effect of washing on heavy metal levels in leaves.

Limited evidence for differing distributions of lead and zinc on and within leaves, was provided by the comparison of concentrations in unwashed and washed foliage. Significant amounts of zinc were removed from only 4 species, but of lead, from 7 species. In species from which a proportion of both metals was removed by washing, a greater percentage of lead than zinc was lost. This suggests that the latter was more tightly bound on, or within, the leaf. However, neither metal was lost in significant quantities from living leaves of 5 species and senescent material of a sixth. The non-significant differences between the metal levels, in these washed and unwashed materials, included both apparent increases and decreases; it is not know to what extent these results were influenced by the small sample sizes (3 replicates).

Percentages of lead removed were generally within the range reported in the literature, but differences could not be correlated with differences in leaf morphology, as found by Page et al., (1971). However, comparisons with reported values are complicated by the variations in washing procedures used in different studies; those employed in the present study were mild by comparison with those used by some workers.

Comparisons between washing "experiments", carried out on plants sampled on different occasions, may be misleading because of the effects of variations which may have occured in the weather. It may be hypothesised that there is an upper limit to the absolute amount of lead (X) which a

particular leaf may carry, and also that there is a limited, absolute amount of lead (Y) which cannot be removed from a leaf during normal rainfall, because it is too tightly bound. Some evidence in support of the latter is provided by Little (1973), who showed that washing "smelter-contaminated" leaves with acid removed an additional 10% of the lead, which could not be removed by prolonged washing in deionised water.

If leaves are sampled and washed after a wet period, then much of the removable lead will already have been lost, and the amount present will be approaching Y. However, if leaves are sampled and washed after a dry period, then the amount of lead will be approaching X, and more removable lead is present. The percentages of lead removed by washing in the laboratory will differ between the two samples. This is illustrated in figure 13.

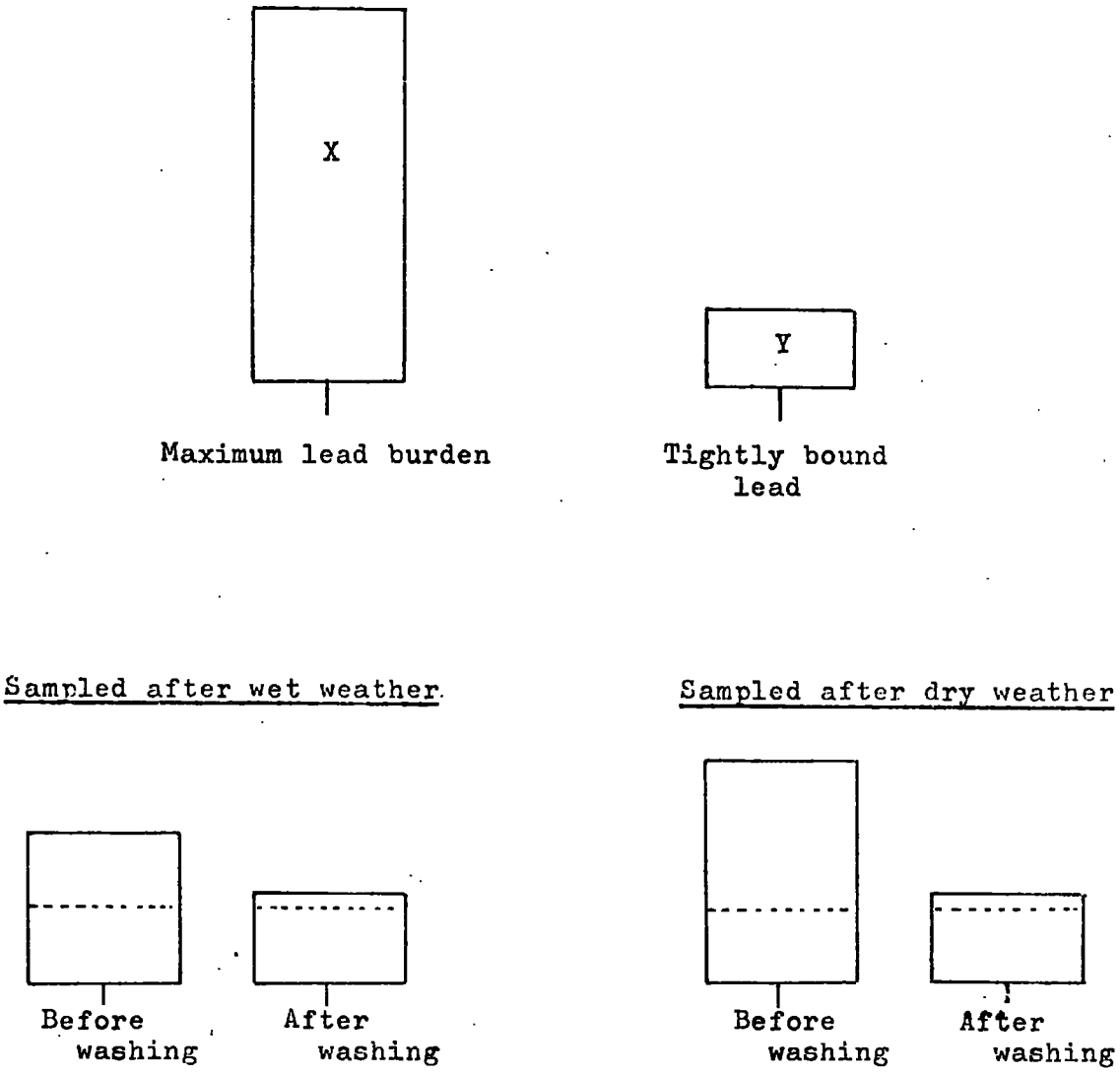
In the present study, it was observed that the amount of wilting which had occured between collection in the field and washing in the laboratory, varied between species. Thin, relatively wilted, leaves appeared to offer less physical resistance to wetting than others, and also tended to become folded up during agitation. It is possible that this affected the removal of surface deposits, and it is recommended that, in future studies, washing should be carried out immediately after sampling, or, if possible, in the field before collection.

9. (vi) <u>The effect of rainfall on heavy metal levels determined in vegetation.</u>

It is difficult to relate the results of washing experiments to the behaviour of plants in the field, where "canopy effects" may cause variations in the amount of rainfall reaching different plants, or different parts of the same plant. Furthermore, it is possible that precipitation may enhance the fall-out of heavy metals from the air.

The amount of lead present on a leaf, at a particular time, will be influenced by characteristics of that leaf, but will be in balance between the rate of accumulation from the atmosphere and the rate of removal by

Figure 13 Model comparing results of washing a leaf after both wet and dry weather



Note: Figures are hypothetical and included for illustration purposes only.

wind and rain. Both rates vary with time, and rainfall may produce a rapid increase in the rate of removal of lead from leaves. Davies and Holmes (1972) found that the lead content of grass, sampled after wet weather, was only half of that sampled after dry weather.

In the present study, some of the variables which affect the net accumulation of lead, by plants, were held constant by completing the sampling within one day, on each occasion.

10. Distribution of lead within plants of U. dioica, S. sylvatica and A. elatius.

The distribution of lead within plants of non crop species, from roadside sites, appears to have received little previous attention, although data recorded by Lagerwerff et al. (1973) showed more lead present in lower than upper leaves of corn, growing 400 m from a busy road. However the plants were pot grown and not subject to "canopy effects".

In the present study, a similar trend in lead distribution was observed in all three species examined; upper, aerial parts of the plant contained small lead concentrations than lower, aerial parts. Much of the difference may be explained by the difference in ages of these parts of plants: lower leaves had been exposed to airborne lead for a longer period of time. However, the relationship between age and lead concentration is not straightforward. Young parts of plants are also undergoing relatively rapid growth and both the amount of lead and plant dry matter are accumulating with time. It is the ratio of these two which determines the measured concentration of metal. Thus differences between lead concentrations in plant materials, of dissimilar ages, may be altered by differences in their growth rates.

The differences between concentrations found in living grass leaves, of different ages, was less pronounced than in those of other species examined. However, their lead concentrations were lower, and it is probable that the mean duration of exposure of grass leaves is less than that of dicotyledons, as previously discussed.

Inflorescences of the dicotyledon S. sylvatica and the grass A. elatius contained lower levels of lead than those contained in leaves, at two of three sites. Foliage leaves and inflorescences differ in their morphology, and this may influence their relative abilities to retain lead deposits. Also, in grasses, the inflorescence is protected, during much of its early growth, by enclosure within leaf bases.

Stems of all three species contained lower lead concentrations than any other parts of plants sampled. The amount of lead deposited on stems may be reduced by their upright growth habit, and also by protection afforded by leaves, particularly so in grasses, in which the stem does not emerge from leaf bases until the flowering stage is reached. Concentrations of lead in dicotyledonous stems may be influenced by a lower receptive surface area : weight ratio than that of leaves.

10. (i) Concentration of lead in senescent leaves.

The high concentrations of lead found in senescent leaves, in both the general survey and the study of lead distribution within plants, are of particular interest. All senescent leaves examined contained more lead than living leaves of the same species. This confirms the findings of Mitchell and Reith (1966) for uncontaminated pasture grasses, and of Rains (1975) for Avena fatua growing near a smelter complex.

Recently senesced leaves of U. dioca and A. elatius contained approximately $1\frac{1}{2}$ times the lead concentration of the oldest, living leaves, whilst those of S. sylvatica contained $1\frac{1}{2}$ - 3 times the lead concentration of oldest, living leaves. Senescent material from the previous season's growth was analysed in the case of A. elatius, and contained almost 3 times the lead concentration of recent senescent material.

In the general survey, it was also found that concentrations of zinc were significantly greater in senescent than living leaves.

Several factors may be responsible for the concentration of these metals during senescence. Many elements are mobilised from leaves and translocated to other parts of plants, during senescence and before leaves

are shed, but Guha and Mitchell (1966) produced evidence that zinc and lead were relatively immobile in leaves, at this time. Breakdown and loss of organic matter from leaves would produce an apparent rise in concentration of immobile metals in the remaining material. However, it is difficult to believe that this could account entirely for the difference between lead concentrations in mature and recently senesced leaves of S. sylvatica; a loss of more than $\frac{2}{3}$ of the organic matter would have been required to produce the magnitude of this difference found at one site.

Senescent leaves have received a longer period of exposure to exhaust emission than other leaves, and it seems probable that such material can continue to accumulate lead from the atmosphere. This is the explanation favoured by Rains (1975), but Mitchell and Reith (1966) considered that accumulation of lead by senescent grasses might be caused by a translocation of lead accumulated in roots during active growth. However, they were concerned with accumulations, which occured in the Autumn, in senescent plants, after active growth had ceased in the whole plant.

In the present study, "old senescent leaves" from grasses were collected from the base of clumps, where contamination by soil could have taken place. If this had occured, washing could be expected to reduce very substantially the concentrations of lead present. This was not found in the limited number of replicates of the washing experiment.

10. (ii) Comparison between sites.

A comparison of lead concentrations, in different parts of plants (U. dioica, S. sylvatica and A. elatius), between sites, showed that there were significant differences between sites. This suggests that the fall-out of lead particulates was not uniform over the study area. It is probable that the amount of airborne lead available at any precise location within the study area is modified by factors which operate at two "levels".

At the first level, borders of trees and shrubs reduce the amount of fall-out on the enclosed, central area, and variations in the degree of protection afforded produce variations in the magnitude of lead deposits.

In addition to a direct shelter effect, trees and shrubs may also modify local air currents.

At the second level, variations in the canopy of the ground-layer vegetation influence the amount of airborne lead in the immediate vicinity of plants, or parts of plants. It is probable that both sets of factors were partially responsible for the inconclusive results of the transect study.

11. Importance of the variation found in heavy metal levels of roadside vegetation.

The considerable variations found, throughout this study, in concentrations of lead, and to a lesser extent zinc, in roadside vegetation are of considerable significance. Firstly, such variations may have a considerable effect on the intake of these metals by invertebrates which feed on plants of the roadside environment. Results of this study suggest that herbivores, which show preferences between plant species, may differ considerably in their intake of lead, zinc, and perhaps cadmium, although evidence for the last element is inconclusive. In addition, the portion of the plant eaten by herbivores will have a considerable influence on the amount of lead which they ingest. In particular, decomposers and other organisms which feed on senescent plant material, can be expected to have much higher intakes of lead than those which feed on living plant material.

Secondly, the variations found highlight the problems involved in designing monitoring programmes for heavy metal contamination of roadside environments. In any such programme, great care must be taken to collect samples which are not biased by localised variations in the extent of contamination. Spot sampling may produce very misleading results, which may be avoided by sampling more extensively. Comparisons based on different species, the same species sampled after different weather conditions, or on different parts of plants of the same species, are all of doubtful value.

For the plants examined in this study, it would appear that the most reliable indication of the heavy metal burden of a site would be gained by sampling whole plants, distributed throughout the site, or pooled collections of leaves from such plants. The "moss bag" technique for routine monitoring,

described by many workers, appears to offer an attractive alternative to the sampling of indigenous vegetation, but its discussion is beyond the scope of this study.

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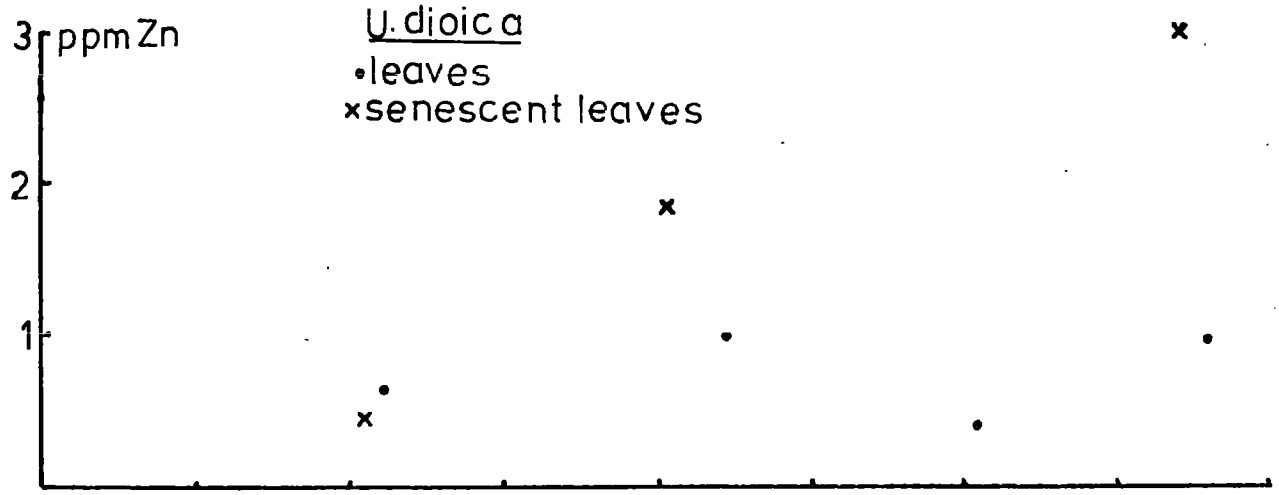
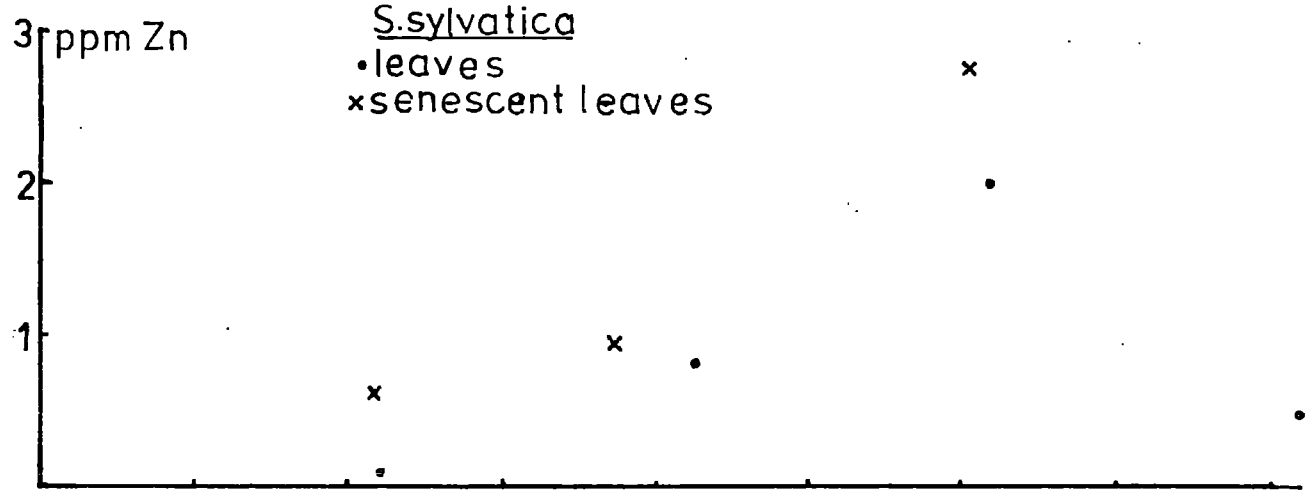
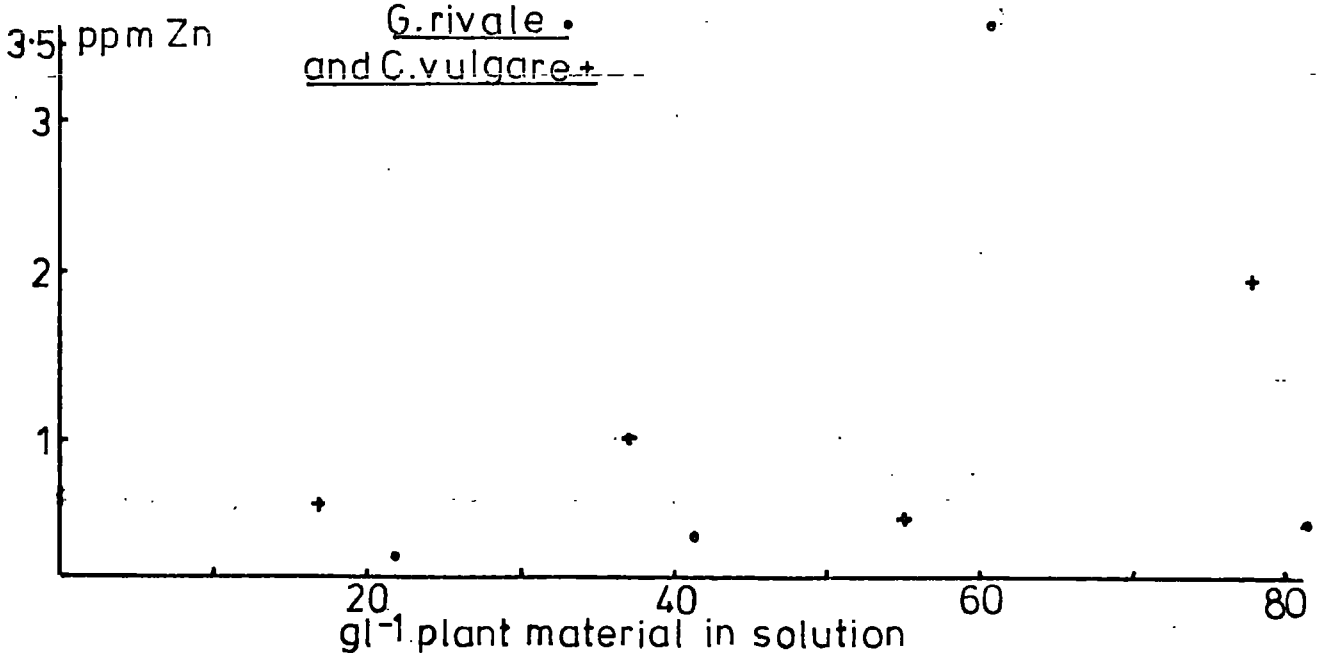
Appendix 1

Figure 1 Results of Correction Factor Determinations for Zinc

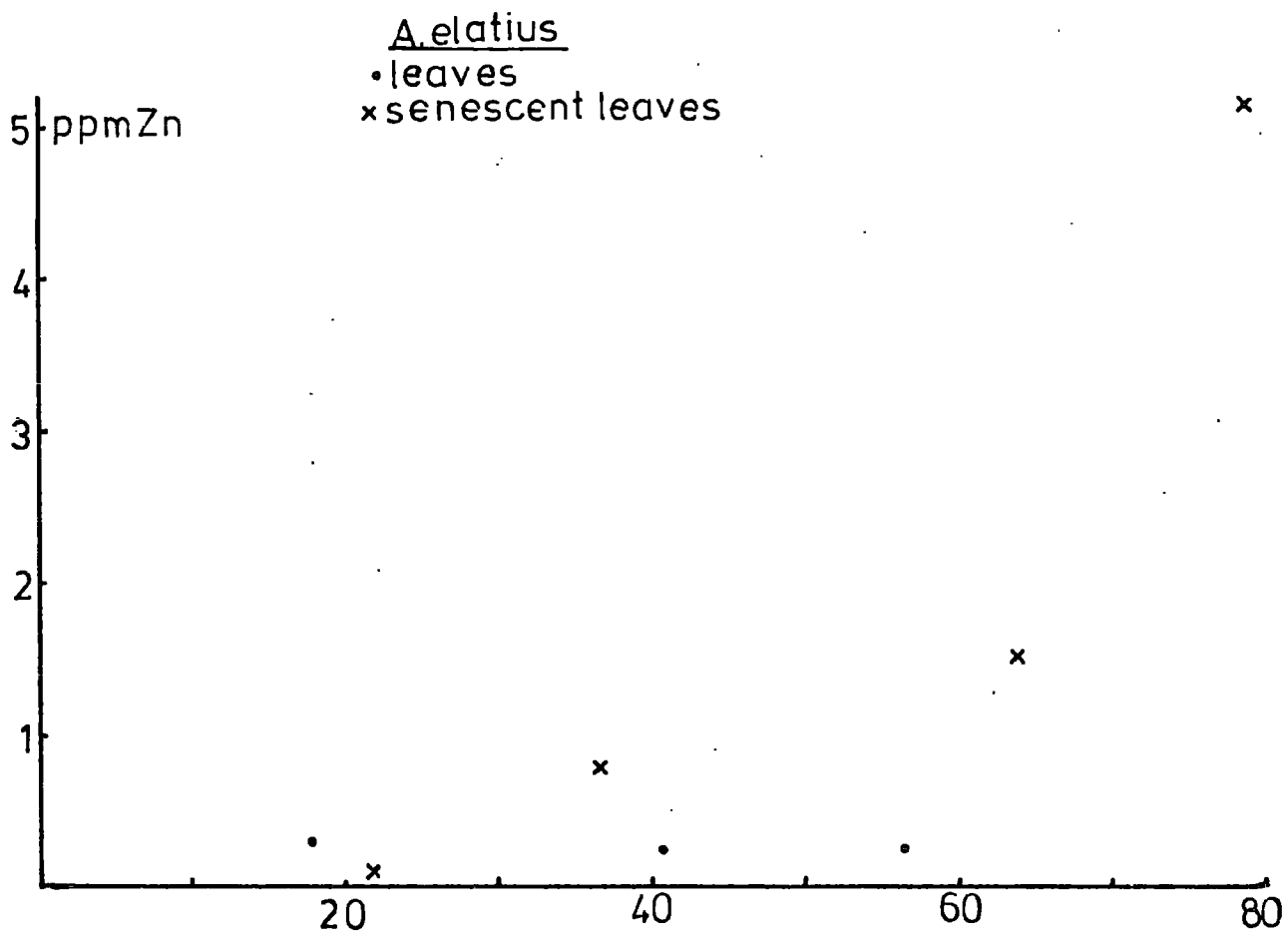


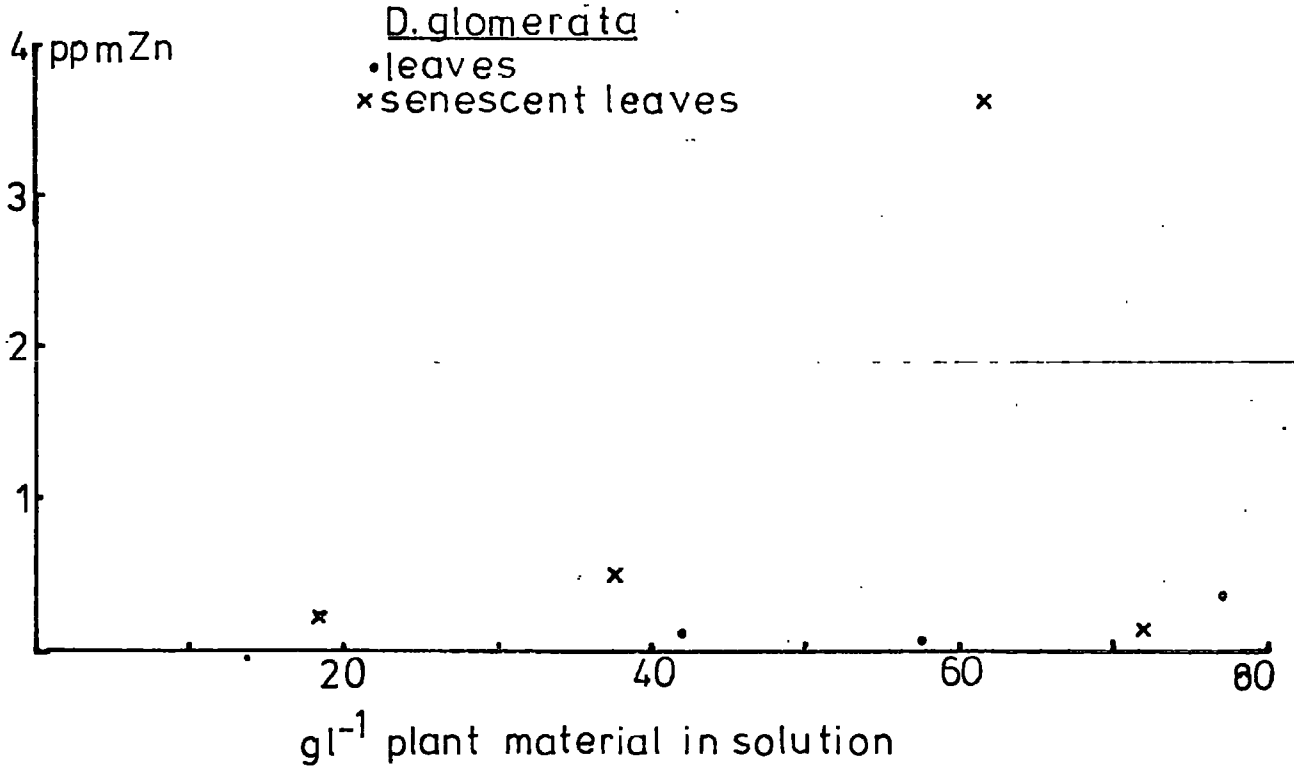




Appendix 1

Figure 2 Results of Correction Factor Determinations for Zinc





APPENDIX 1

Table 1 Results of Correction factor determinations for Cadmium

	Concentration of plant material in solution g l^{-1}	Absorption expressed as p.p.m. Cd in soln.
Urtica dioica	22.22	.02
	44.69	.05
	60.82	.105
	76.22	.125
Stachys sylvatica	42.67	.01
	62.09	.03
	82.11	.03
Stachys sylvatica senescent	21.05	0
	37.24	.04
	60.51	.06
Geum rivale	22.12	.01
	41.25	.02
	81.65	.05
Cirsium vulgare	16.95	.09
	55.08	.06
	77.81	.14
Arrhenatherum elatius	18.0	0
	40.70	.01
	56.43	.02
Arrhenatherum elatius senescent	36.78	.02
	63.87	.01
	78.91	.04
Dactylis glomerata	41.98	.04
	57.49	.04
	76.92	.02
Dactylis glomerata senescent	18.52	.03
	37.77	.01
	72.16	.03

Appendix 2. Distribution of lead within plants of selected species.

(p.p.m. in dry matter)

Urtica dioica.

Site 1a.

	Mean	S.E.	Apparent corrected mean
Tips	45.82	0.75	36.59
Leaves 5 - 7	49.57	1.30	40.00
Leaves 8 - 10	76.87	2.03	67.96
Senescent leaves	109.63	2.32	82.25
Stems	10.04	1.86	1.48

Site 2a.

Tips	54.77	0.18	45.75
Leaves 5 - 7	61.81	0.84	51.92
Leaves 8 - 10	73.69	1.75	64.71
Senescent leaves	118.28	2.35	95.77
Stems	6.18	0.94	0

Site 3a.

Tips	66.10	1.57	57.04
Leaves 5 - 7	73.45	2.69	63.52
Leaves 8 - 10	95.52	3.17	87.00
Senescent leaves	136.10	8.62	113,62
Stems	11.04	0.51	1.06

Note : The "apparent corrected mean" is derived by applying possible correction factors for background interference (see figures 6 and 7).

Appendix 2 (Continued)

Stachys sylvatica.

Site 1b.

	Mean	S.E.	Apparent corrected mean
Inflorescence	28.37	0.19	19.15
Leaves 1 - 2	88.49	0.57	79.20
Leaf 3	125.95	0.77	116.69
Leaves 4 - 5	115.98	1.85	106.50
Senescent leaves	180.20	2.50	168.34
Stems	11.50	0.61	2.16
Site 2b.			
Inflorescence	75.23	2.10	65.82
Leaves 1 - 2	54.39	0.68	44.72
Leaf 3	65.97	0.99	56.66
Leaves 4 - 5	76.86	0.93	67.51
Senescent leaves	252.45	17.75	236.96
Stems	9.65	0.63	0.69
Site 3b.			
Inflorescence	40.78	3.55	31.39
Leaves 1 - 2	44.10	1.54	34.78
Leaf 3	50.51	2.20	41.05
Leaves 4 - 5	73.00	2.81	63.00
Senescent leaves	145.89	5.06	134.06
Stems	9.86	1.93	1.40

Appendix 2 (Continued)

Arrhenatherum elatius.

Site 1c.

	Mean	S.E.	Apparent corrected mean
Inflorescence	21.33	1.56	13.14
Leaves 1 - 2	42.08	x	33.66
Leaves 5 - 6	46.02	1.30	37.91
Leaves 7 - 8	-	-	-
Recent senescent leaves	67.34	1.63	66.40
Old senescent leaves	212.88	9.33	211.86
Culms	4.91	0.80	0

Site 2c.

Inflorescence	30.99	2.85	22.86
Leaves 1 - 2	20.66	0.86	12.68
Leaves 5 - 6	32.91	0.59	24.75
Leaves 7 - 8	-	-	-
Recent senescent leaves	52.23	0.90	51.40
Old senescent leaves	149.80	1.19	148.70
Culms	3.35	0.62	0

Site 3c.

Inflorescences	16.86	1.35	8.72
Leaves 1 - 2	23.55	0.27	15.32
Leaves 5 - 6	29.16	0.89	20.84
Leaves 7 - 8	54.21	2.59	44.67
Recent senescent leaves	85.39	2.70	84.39
Old senescent leaves	-	-	-
Culms	7.49	1.80	0.69

- indicates material not available at this site.

Appendix 3 Values of "t" for comparison of lead concentrations in plants from three sites

Urtica dioica

	Site 1a and 2a	1a and 3a	2a and 3a
Tips	11.62	11.65	7.17
Leaves 5-7	7.90	7.99	4.13
Leaves 8-10	1.19	4.96	6.03
Senescent leaves	2.62	2.96	1.99
Stems	1.86	0.11	0.54

Stachys sylvatica

	Site 1b and 2b	1b and 3b	2b and 3b
Inflorescence	22.21	3.49	8.36
Leaves 1-2	38.40	27.06	6.12
Leaf 3	47.98	32.38	6.41
Leaves 4-5	18.89	12.79	1.30
Senescent leaves	4.03	6.08	5.77
Stems	2.10	0.81	0.10

Arrhenatherum elatius

	Site 1c and 2c	1c and 3c	2c and 3c
Inflorescence	2.97	2.17	4.49
Leaves 1-2	24.70	65.20	3.21
Leaves 5-6	9.16	10.73	3.50
Recent senescent leaves	8.12	5.73	11.63
Old senescent leaves	6.70	-	-
Culms	1.54	1.31	2.17