THE COMPARATIVE ECOLOGY OF MINUARTIA VERNA (L.) HIERN AND THLASPI ALPESTRE L. IN THE SOUTHERN PENNINES, WITH SPECIAL REFERENCE TO HEAVY METAL TOLERANCE

by

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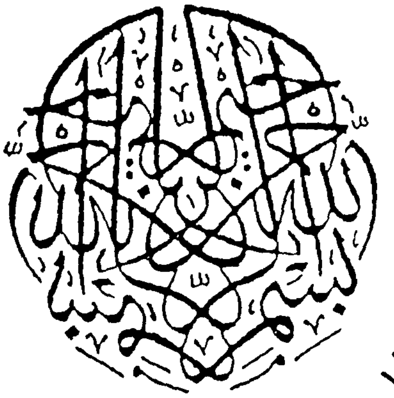
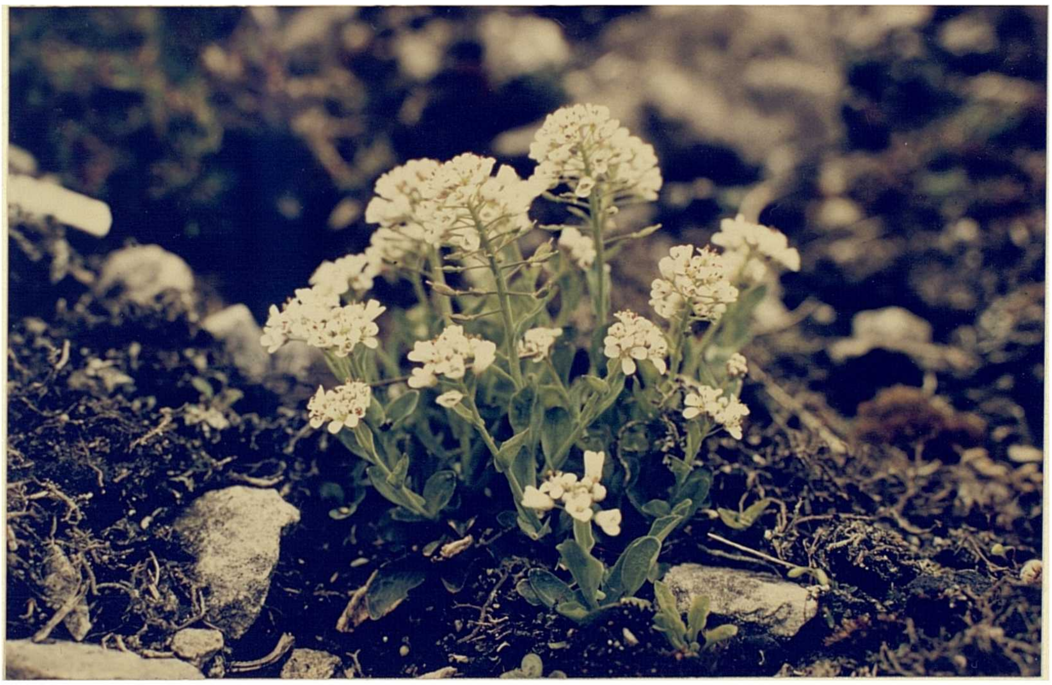


In the name of God Most Merciful Most Gracious

TO

MY FAMILY

Minuartia verna (L.) Hiern (Caryophyllaceae) on open lead mine spoil at Tideslow Rake, Derbyshire.



Thlaspi alpestre L. (Cruciferae) on metal-contaminated ground around lead mine workings at Bonsall Moor, Derbyshire.

(Photographs: A. J. M. Baker)

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ABSTRACT

Minuartia verna (L.) Hiern and Thlaspi alpestre L. are recognized as relict elements of a preglacial montane flora in the British Isles and, as such, display a markedly disjunct distribution pattern. Their present-day distribution shows a close association with metalliferous mine workings, particularly in the Pennines, where both species have become prominent components of the flora of calcareous lead mine wastes. By comparison with M. verna, T. alpestre is more restricted in its distribution and is a rarer species.

The work reported in this thesis attempted to provide an explanation for this pattern of distribution in the southern Pennines on the basis of comparative studies of the autecology and genecology of the species, their population biology and dynamics in the field and their responses to competition from other species.

Both field and laboratory studies confirmed that the two species were highly tolerant of the heavy metals lead, zinc and cadmium. Short- and long-term solution culture experiments on tolerance and uptake of these heavy metals by a range of populations of both species are reported. Tolerance to other toxic metals was also investigated. T. alpestre showed superior tolerance to M. verna in these experiments, and emerged as a metal-accumulating species. M. verna by contrast, operated some degree of metal exclusion, but metal uptake and distribution differed for the various metals in both species and seasonally. Differences in metal tolerance and uptake characteristics alone could not explain the different local distribution patterns in the field, as sites of similar metal status are colonized by both the two species together and by M. verna alone.

A study of inter-population variation in morphological characters showed considerable genetically-based variation in both species. M. verna was however more variable. Some of the variation detected was interpreted as being ecotypic in origin. The contrasting breeding of the two species and their seed biology are considered to be major factors in explaining the present-day distributions of the two species. The tendency for inbreeding in T. alpestre has severely limited its genetic variability within populations and potential for spread. Seedling mortality of both species in their natural habitats is very high, and survival can be related to early shelter effects of neighbouring plants. Both species, however, emerge as weak competitors as predicted from their preference for open habitats with much more bare ground.

The relevance of these studies to the interpretation of the present-day global distribution of the two species and their evolutionary biology is discussed.

ACKNOWLEDGEMENTS

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Many members of the Department of Botany and the Unit of Comparative Ecology (NERC) freely gave their help and advice during the course of this work; to all of them, particularly Dr. R. Hunt, I extend my sincere gratitude.

Finally I wish to thank Mrs. Linda Carter for her care and patience in the preparation of the typescript and Mrs. Dianne Freeman for her skillful presentation of many of the figures.

I wish to take this opportunity to thank all my family especially my parents, my wife and children most warmly for their support, patience and sacrifices.

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CHAPTER 1

INTRODUCTION

Soils may become enriched or polluted with potentially phytotoxic concentrations of heavy metals in the vicinity of ore bodies and other geochemical anomalies as a result of natural weathering processes and dispersion of the particulate and soluble products in soil water. The extent of such affected soils in the British Isles has been greatly increased as a result of metalliferous mining activities dating back as early as Roman times which have created large areas of contaminated spoils frequently containing several percent of metals such as lead, zinc and copper. Such substrates represent a poor medium for plant growth. In addition to heavy metal toxicities, a range of other physical and chemical conditions inimical to plant growth often prevail in mine spoils (Antonovics, Bradshaw & Turner, 1971; Clark & Clark, 1981; Thompson & Proctor, 1983). Surface instability, poor particle size distribution and low organic status often result in drought conditions during summer months. Severe deficiency of macronutrients, particularly N & P, compound the problems of plant colonization of metalliferous mine wastes and tailings. It is not surprising, therefore, that many metalliferous wastes remain almost devoid of any vegetation cover for decades, even centuries.

The process of colonization is innately slow and is effected, at least initially, by those species which have evolved heavy-metal-tolerant mutants which can give rise to specialized races or ecotypes capable of successful growth and reproduction on otherwise phytotoxic substrates. However, with time, the sparse cover may progress to a more continuous vegetation and some spoils can support relatively rich and diverse plant communities which may or may not be distinct phytogeographically from surrounding vegetation on uncontaminated soil. A satisfactory classification of taxa found on metal-contaminated soils in the British Isles and elsewhere in northern Europe is that of Lambinon & Auquier (1963) and is based on the degree of restriction to such sites. Metallophytes (absolute and local) are found only on metal-contaminated soils; pseudometallophytes occur on both contaminated and normal soils in the same region. Within this group can be recognized two classes, electives and indifferents, in order of decreasing abundance and vigour on contaminated soils. Accidental metallophytes usually include ruderals and annuals which appear only sporadically and show reduced vigour.

Metalliferous mine spoils and more naturally metal-enriched soils may thus come to support highly specialized plant communities, frequently including a number of 'indicator' species for the particular type of mineralization concerned. Such species have proved useful in the context of geobotanical prospecting for mineral deposits (see: e.g. Brooks, 1983) or for locating areas of old mining activities. Some metallophyte taxa are now recognized as national rarities of conservational importance in view of the limited range of habitats which has been exacerbated by the episodic reworking of these areas or more active reclamation procedures. Their continued survival _in situ_ or further spread may be limited geographically by proximity and availability of suitable sites or ecologically by changing conditions of stress, disturbance and competition.

The metallophyte flora of lead, zinc and copper mineralization in the British Isles has been classified by Ernst (1976) within the class Violetea calaminariae Tx, 1961. An important association described for the lead/zinc mineralization of the Pennines (north and south), the Mendips and N.Wales is the Minuartio-Thlaspietum alpestris (Koch, 1932; Shimwell, 1968) for which the herbs Minuartia verna (L.) Hiern, (Caryophyllaceae and Thlaspi alpestre L. (Cruciferae) (= T. caerulescens J & C Presl, (Ingrouille & Smirnoff, 1986) are the character species. This association is further classified within the alliance Thlaspion calaminaris (Ernst, 1964) and order Violetalia calaminariae Br.-Bl. et Tx. 1943. It has been described from Britain By Ernst (1968), Shimwell (1968), Shimwell & Laurie (1972), Clark & Clark (1981) and from Ireland by Doyle (1982).

Both M.verna and T.alpestre are considered to represent an alpine element in the flora (Matthews, 1937) but in the British Isles they seem to be more strongly associated with soils contaminated with the heavy metals lead, zinc and copper, than with altitude. The presence of M.verna on metalliferous soils has been noted as early as 1588 by Thalius (Ernst, 1965, cited by Antonovics et al., 1971) and the occurrence of T. alpestre on mine wastes in the Pennines was noted by Windsor in 1865. Both species are considered to be absolute metallophytes (Lambinon & Auquier, 1963) and have been included in lists of potentially useful geobotanical indicators for zinc (Antonovics et al., 1971; Martin & Coughtrey, 1982; Brooks, 1983).

In the Pennine region, as elsewhere in the British Isles, both M. verna and T. alpestre exhibit a markedly disjunct distribution pattern, possibly as a result of the pre- and postglacial history of the two species (Pigott & Walters, 1964. However, T. alpestre appears to be 'rarer' relative to M. verna even in areas where suitable habitats might exist (Clapham, Tutin & Warburg, 1981; Ingrouille & Smirnoff, 1986). The reasons for this 'under-distribution' of T. alpestre have not been investigated but may be of some relevance in defining the conservational status and ecological requirements of this nationally-rare species. This thesis presents the results of descriptive and experimental studies of the comparative ecology of the two species and attempts to explain, at least in part, the differing present-day distributions of M. verna and T. alpestre in the southern Pennines. The rationale for this research was based on various hypotheses summarized in Figure 1.1.

The first hypothesis was that the two species, although strongly associated, may differ in their geographical range and ecological amplitudes as a result of different climatic and microclimatic tolerances and habitat preferences. This aspect was not examined experimentally but inferences were made on the basis of gross and local patterns of distribution (Chapter 2). Community analysis in the S. Pennines was used to define the phytosociological preferences of the two species and to describe their respective niches, both floristically and in terms of some simple environmental variables. Other hypotheses related more to differing intrinsic biological and physiological properties of the two species. Geographical isolation may facilitate population divergence through population differentiation of locally-adapted races or ecotypes possessing distinctive morphological and/or physiological characteristics. Chapter 3 describes an investigation of morphological variation between a small range of populations of the two species from mine sites in the southern Pennines selected to include populations of varying degrees of isolation from each other, in order to test to what extent population divergence has occurred.

Both M. verna and T. alpestre have evolved heavy metal tolerance which must account, at least in part, for their success at the mineralized sites where they are now found. A large proportion of the experimental studies reported in this thesis relates to this aspect. Chapter 4 provides a field-based analytical background to place in context subsequent experimental work (Chapter 5) in which various comparative ecophysiological aspects of metal tolerance have been investigated.

The ability of a species to maintain its presence and distributional range may depend greatly upon its ability to produce sufficient numbers of successful new recruits to the population. Chapter 6 reports the results of an investigation of the population dynamics of *M. verna* and *T. alpestre*, with a major emphasis on seedling recruitment and survival within bare and turfed areas of mine spoil.

The competitive interactions of associated plant species upon establishment and survival is of great importance, and may be responsible for the extinction of a species from areas which could otherwise be suitable for survival. Chapter 7 presents an investigation of the interaction between seedlings of both *M. verna* and *T. alpestre* and established plants of one of the main plant species associated with these two, the grass *Festuca rubra*.

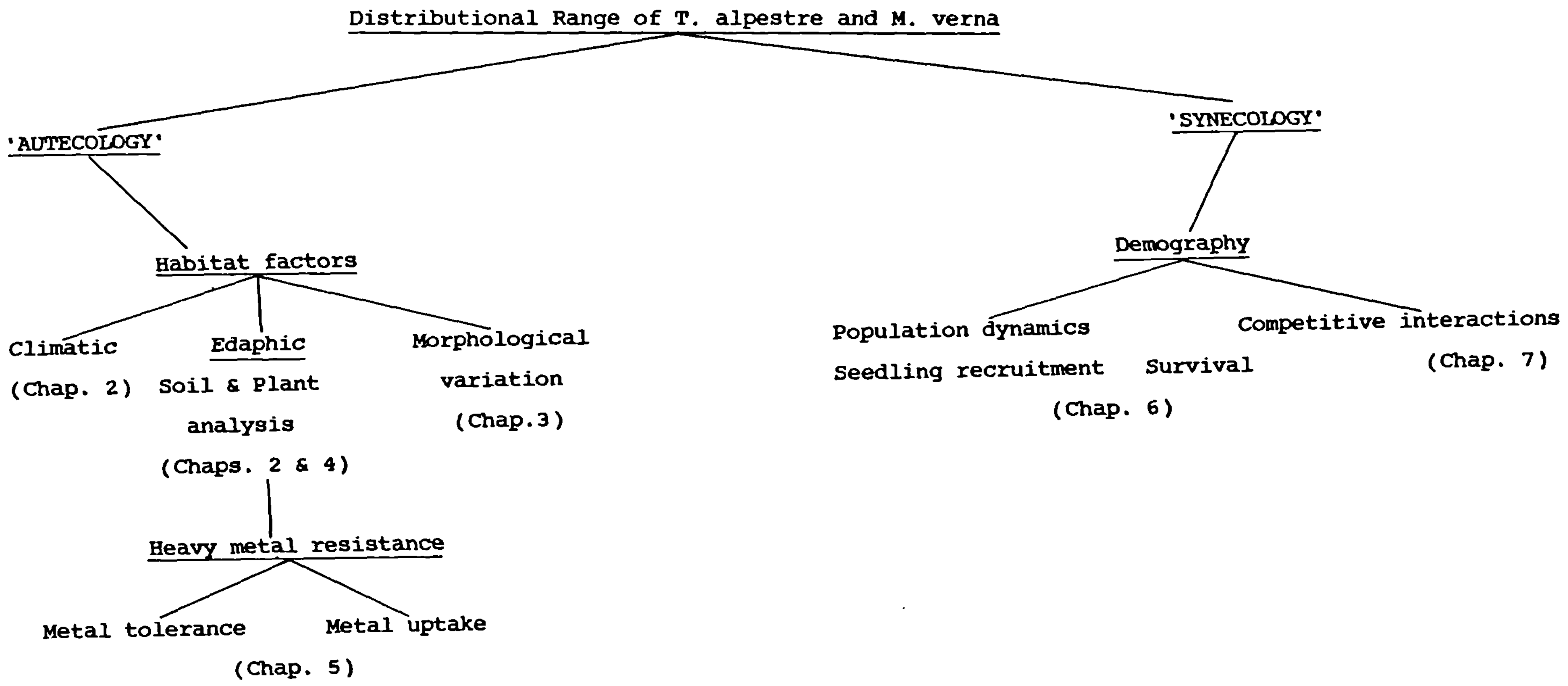


FIG. 1.1 AUTECOLOGICAL AND SYNECOLOGICAL FACTORS INVESTIGATED TO ACCOUNT FOR THE RESTRICTED DISTRIBUTION OF THLASPI ALPESTRE IN RELATION TO THAT OF MINUARTIA VERNA

CHAPTER 2

A PRELIMINARY STUDY OF THE GEOGRAPHICAL AND HABITAT RANGE OF MINUARTIA VERNA AND THLASPI ALPESTRE

2.1 INTRODUCTION

M. verna and *T. alpestre* both occur in metalliferous habitats throughout much of their geographical range (Halliday, 1960; Tutin *et al* 1964; Smith, 1979). This unusual but shared feature tends to overshadow other aspects of the ecological distribution of the two species and it is not clear to what extent the species overlap with respect to other ecological variables. This chapter attempts to identify the similarities and differences between the habitat requirements of the two species.

2.2 THE GEOGRAPHICAL RANGE IN EUROPE

Species with a similar distribution according to habitat may differ considerably in their climatic tolerance and geographical range. Accordingly, the European distribution of *M.verna* and *T.alpestre* has been compared.

There is some disagreement in the assessment of the distributional limits of each species (see Table 2.1). However the maps presented for *M.verna* (Jalas & Suominen, 1983) and for *T.alpestre* (Tutin *et al.*, 1964) may be used to compare the distribution of both species (Figure 2.1). They indicate that *T. alpestre* is much more geographically restricted than *M. verna*.

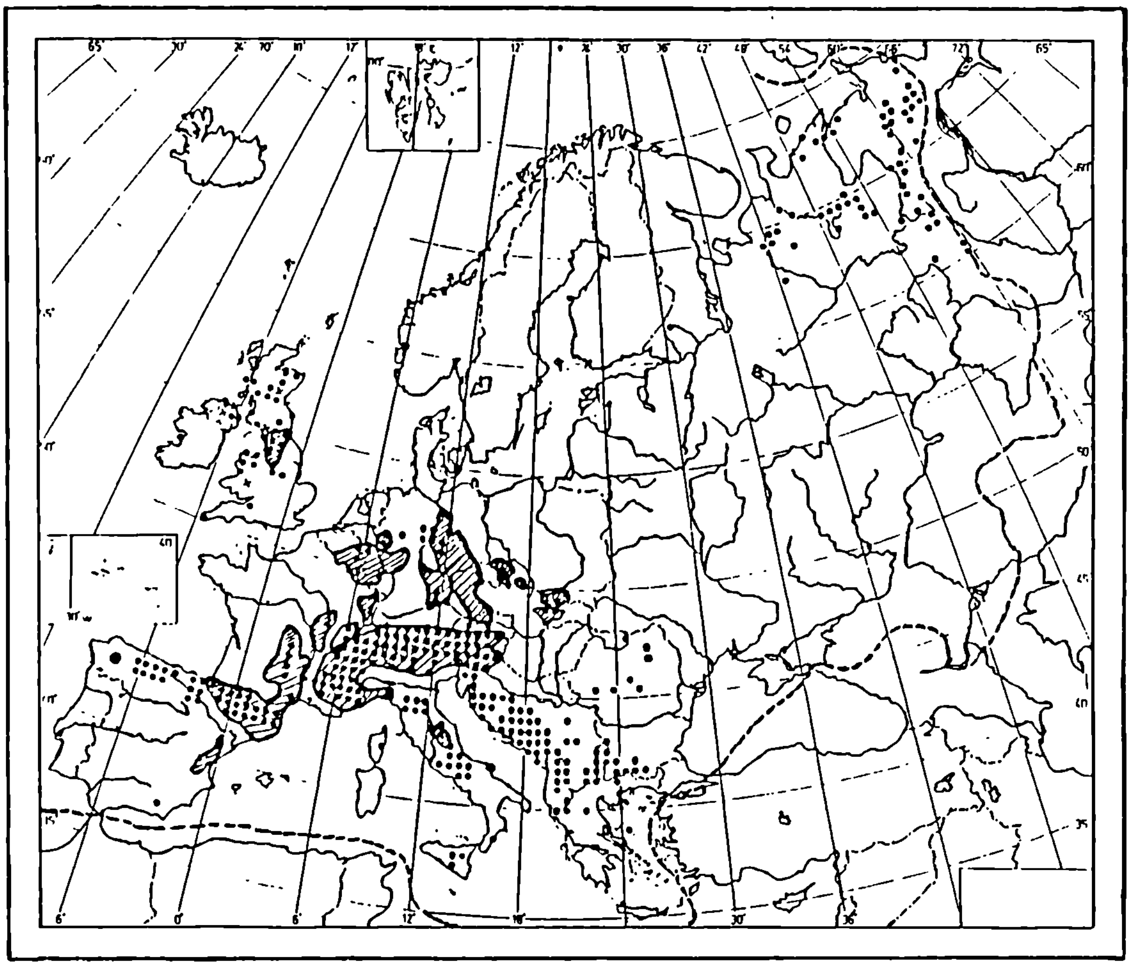
TABLE 2.1 THE GEOGRAPHICAL DISTRIBUTION OF MINUARTIA VERNA AND THLASPI ALPESTRE IN EUROPE, AS REPORTED BY DIFFERENT AUTHORS

Source	*M. Verna*	*T. alpestre*
Tutin *et al.* (1964)	S., W. and C. Europe (Albania, Austria, Belgium & Luxembourg, British Isles, Bulgaria, Corsica, Crete, Czechoslavakia, France, Germany, Greece, Hungary, Ireland, Switzerland, Spain, Italy, Yugoslavia, Poland, Romania, Northern and South-Western Russia, Sicily and Turkey)	Austria, Belgium & Luxembourg, British Isles, Czechoslavakia France, Germany, Greece, Hungary, Switzerland, Spain, Italy, Yugoslavia, Poland, Russia. Also found in Denmark, Holland, Iceland, Norway and Sweden.
Jalas and Suominen (1983)	As Tutin *et al.* (1964) except not recorded from Crete, Hungary, South-Western Russia and Turkey.	No information available for *T. alpestre*.
Hegi (1954)	Additional records in Iceland, Finland and Portugal.	The species has not been reported in localities differing from those reported by other authors.
Ingrouille & Smirnoff (1986)		Restricted to the area from Central Czechoslovakia west-wards to the Rhine and South to Lyons in France.

Figure 2.1 Distribution of Minuartia verna ssp. verna and Thlaspi alpestre in Europe. Data abstracted from Jalas & Suominen (1983) and Tutin et al. (1964).

M. verna ⊡

T. alpestre ▨



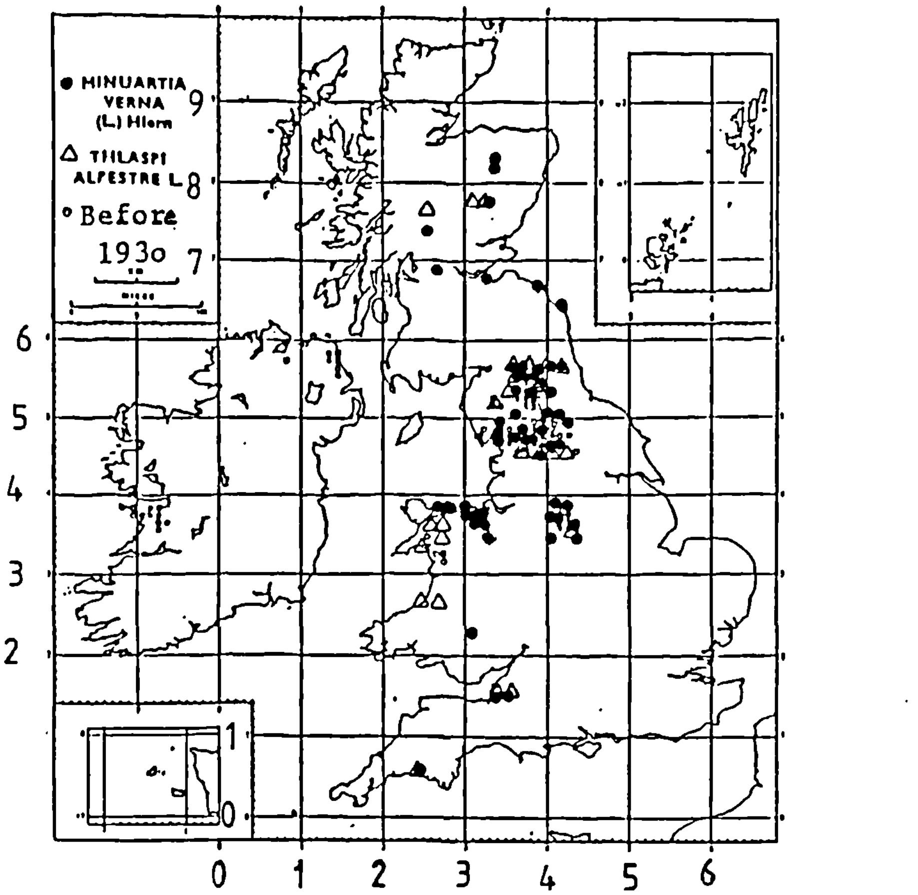
Although the general distribution of M. verna and T. alpestre (Figure 2.1) indicates a montane distribution for both species (up to over 3000 m; Hegi 1958, 1963) both species are also found in lower altitude metalliferous habitats (Hegi 1958, 1963; Halliday, 1960; Shimwell, 1968; Ernst, 1976; Smith, 1979). This makes a simple climatic interpretation of the data in Figure 2.1 impossible. However the distributional range of M. verna spans regions characterized by extreme dry cold in the north to those with relatively hot summers in the south of Europe (Figure 2.1). This suggests that M. verna has a greater tolerance of extremes of climate. The reasons for this tolerance may perhaps have two components, one relating to an initial ability to colonize different sites and another enabling the rapid formation of ecotypes adapted to the new conditions.

2.3 THE GEOGRAPHICAL RANGE IN THE BRITISH ISLES

The distribution of M. verna and T. alpestre in the British Isles is shown in Figure 2.2a. Both species have a disjunct distribution, and are strongly associated with metalliferous sites (Figures 2.2a, b) but as in Europe, M. verna is more widely distributed than T. alpestre. It is scattered through the W and N of the country (19% of British Vice-counties) (Clapham, Tutin & Warburg, 1981). By contrast T. alpestre is restricted to the Mendips, parts of mid - and North Wales, scattered localities along the Pennines and a few sites in Scotland (11.9% of British Vice-counties) (Ingrouille & Smirnoff, 1986).

The distribution of both species within Derbyshire, presented in Figure 2.3a, also shows that even within a local area T. alpestre is more geographically restricted. The number of recorded sites for T.

Figure 2.2a Distribution of Minuartia verna and Thlaspi alpestre in The British Isles on a 10 km grid square basis. Data abstracted from Perring & Walters (1976).



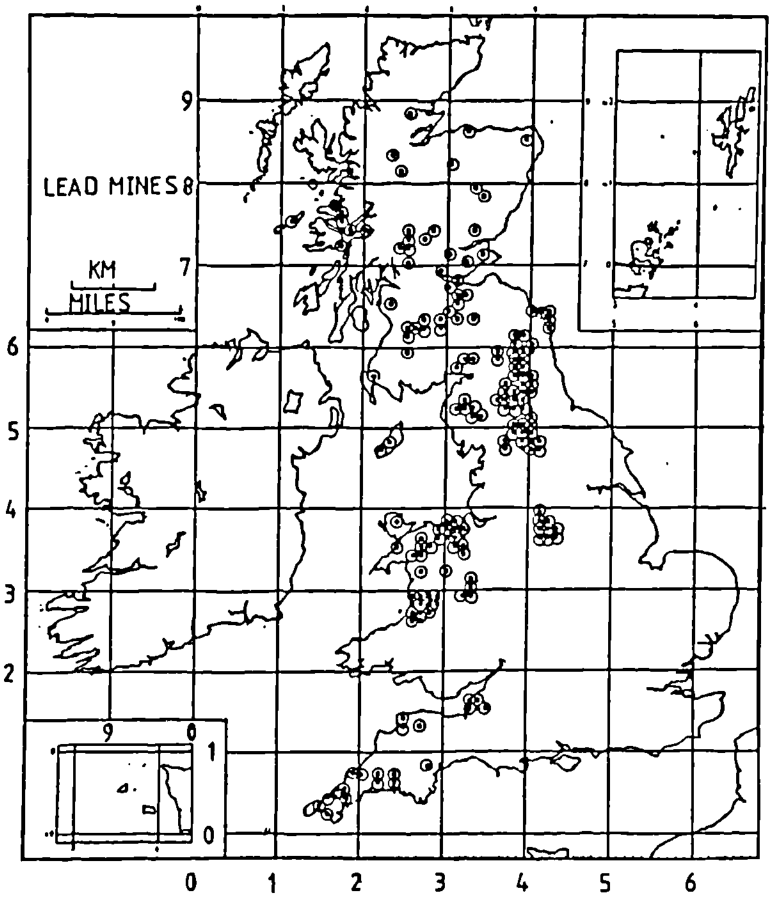


Figure 2.2b Distribution of lead mines in The British Isles (from Halliday, 1960).

alpestre is about half of the number recorded for *M. verna* (Smith, 1979). Both species are confined to metalliferous sites (Figures 2.3a, b) and *M. verna* was found on most of the spoil heaps in the area.

As in the previous section an attempt has been made to correlate the geographical distribution of the two species with climatic variables using climate maps presented by Perring & Walters (1976) for the British Isles and the climate data from Clapham (1969) for Derbyshire. However the results are unsatisfactory and there is no convincing evidence that the differences in distribution between the two species may be simply accounted for by reference to crude climate estimates. The identification of climatic effects on plant distribution by experimental means or by means of measurement is not easy (Grace, 1987) and even on-site measurements of climate were unfortunately too time-consuming to be included in this study. Consequently, the remainder of the chapter will consider the nature of any differences in the habitat requirements of *M. verna* and *T. alpestre*.

2.4 A COMPARISON OF THE HABITAT RANGE OF *M. VERNA* AND *T. ALPESTRE*

2.4.1 Rationale

T. alpestre has a more restricted geographical range than *M.verna*, but has it also a narrower ecological range ?. In an attempt to answer this question, some habitat characteristics of the two species have been compared within a local flora (Derbyshire).

2.4.2 Material and Methods

Field data were abstracted from two general vegetation surveys carried out by the Unit of Comparative Plant Ecology (NERC) and described by Hodgson (1986a) and Grime, Hodgson & Hunt (1987). Subsequently, additional data have been collected with the assistance of Dr.

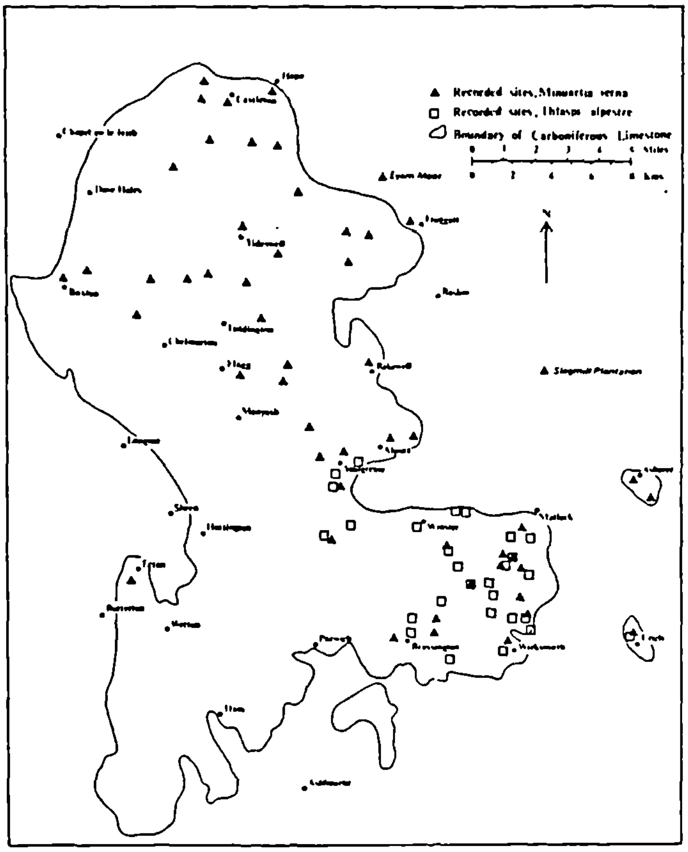


Figure 2.3a Distribution of <u>Minuartia verna</u> and <u>Thlaspi alpestre</u> in the Southern Pennines. (from Smith, 1979).

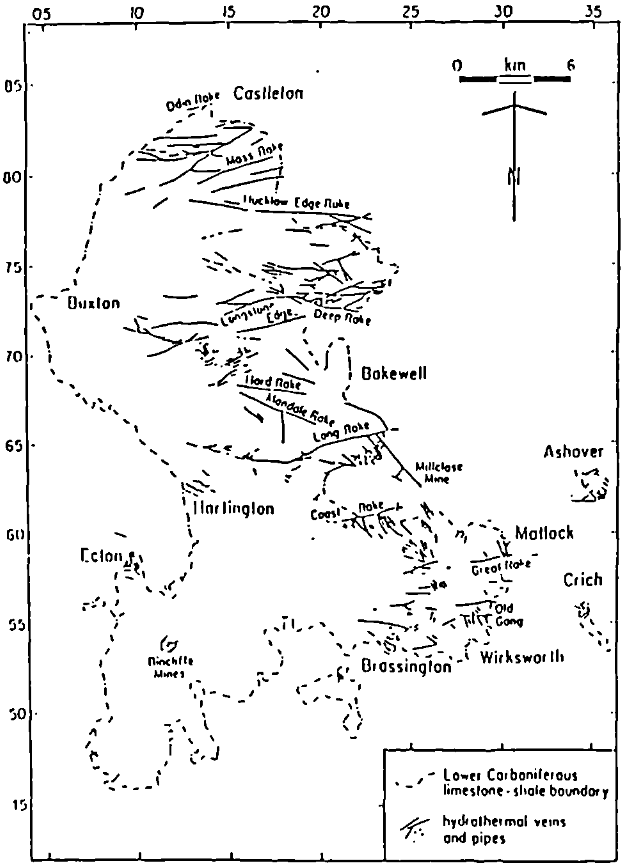


Figure 2.3b Sketch map of some of the major lead ore veins in the South Pennine orefield, (from Ineson, 1981).

J. G. Hodgson in areas not sampled during these studies. In all, 42 sites were samples (Table 2.2). A 1 m^2 metre quadrat was used to sample the vegetation. This size of quadrat has already been used successfully in the limestone grasslands of Derbyshire (Grime & Lloyd, 1973) and was also adopted during the subsequent surveys mentioned above. Such a quadrat size has disadvantages when surveying mine spoil, since the size of the quadrat is large relative to the scale of vegetation pattern found on mine spoil heaps. Differences in vegetation associated with changes in the size of rock fragments or with variation in distribution of heavy metals (see Shaw, 1984) may be obscured by the large size of the quadrat. However the potential advantages of having access to a large quantity of field data counterbalances these limitations.

Rooted-frequency within the 100, 10 cm^2 sub-units of the quadrat was used to assess the distribution and abundance of species. This measurement has the advantage of being more stable than shoot frequency or cover during the fieldwork season. Bare soil, pH at 0-3 cm, slope and aspect, the extent of the exposed soil, rock fragments and bryophyte cover were also scored. These criteria were used because they are both of ecological significance and easy to measure. Notes on grazing and other site features were also taken.

2.4.3 Results and Conclusions

The distributions of M. verna and T. alpestre, according to the % of bare soil and pH are illustrated in Figures 2.4a and b respectively. Slope-aspect polarographs for M. verna and T. alpestre are presented in Figure 2.5. The results are similar for both species. Each has a peak in pH about 7 and is more frequent on north-facing slopes. These similarities result in part from the fact that M. verna

TABLE 2.2 A LIST OF THE DERBYSHIRE LEAD MINE SPOIL HEAPS SAMPLED DURING THE VEGETATION SURVEY

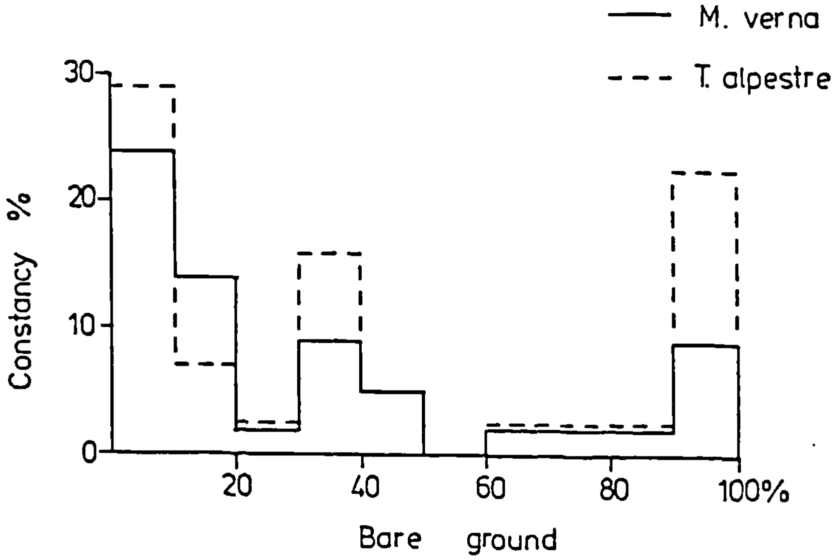
Site	Grid Ref.	Altitude (metres)
Alport	SK224644	168
Black Rocks	SK293558	244
Blue John	SK134834	229
Bonsall Moor	SK237600	335
Bradford Dale	SK218643	137
Burfoot, Millers Dale	SK162728	259
Clough Wood(Mill Close Mine)	SK258618	137
Cockington	SK344616	244
Combsdale	SK223743	244
Dirtlow Rake	SK155821	320
Dovegang Mine	SK287556	244
Eyam	SK216768	274
Fallgate Quarry	SK354621	152
Grattondale	SK207607	259
Hassop	SK236735	229
High Rake, Windmill	SK162778	335
High Tor	SK299588	137
Lathkill Dale	SK134658	183
Longcliffe	SK234552	335
Longstone Edge	SK200731	305
Magpie Mine, Sheldon	SK173682	320
Moss Rake	SK161806	320
Near Alport	SK228645	152
Near Ible	SK265573	290
Near Overton Hall	SK348623	213
Nether Wheal	SK156694	351
Northern Dale	SK269605	183
Odin Mine, Castleton	SK138833	244
Old Mines (Youlgreave)	SK191642	290
Pikehall	SK193595	274
Rainster Rocks	SK221553	305
Rough Side	SK220744	244
Sir William Hill	SK228782	335
Steeplehouse Quarry*	SK287554	229
Stone Edge	SK334668	305
Tansleydale	SK173744	244
Tideslow Rake	SK159778	335
Water Grove	SK189757	290
Wensleydale	SK265603	244
White Rake	SK146782	381
Winnats Pass	SK136827	305
Winster	SK245603	274

* Disused Limestone Quarry

Figure 2.4a Distribution of *Minuartia verna* and *Thlaspi alpestre* relative to bare soil. Data abstracted from vegetation surveys 2 & 3 and the additional records (see 2.4.2).

——— *M. verna* (59 records)

- - - *T. alpestre* (31 records)



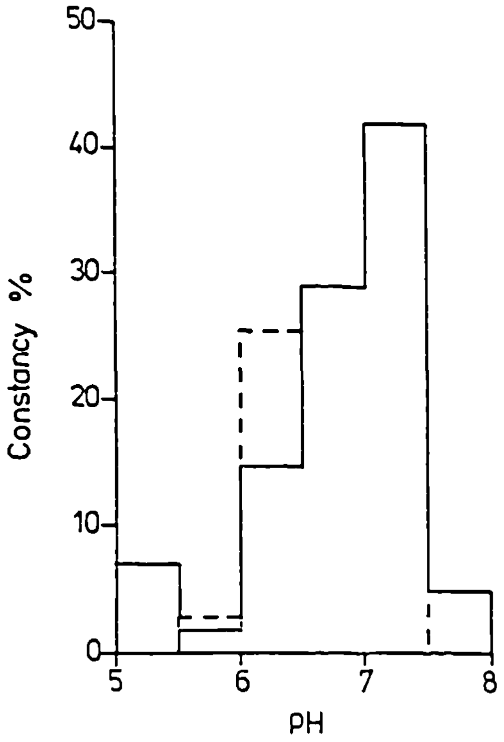
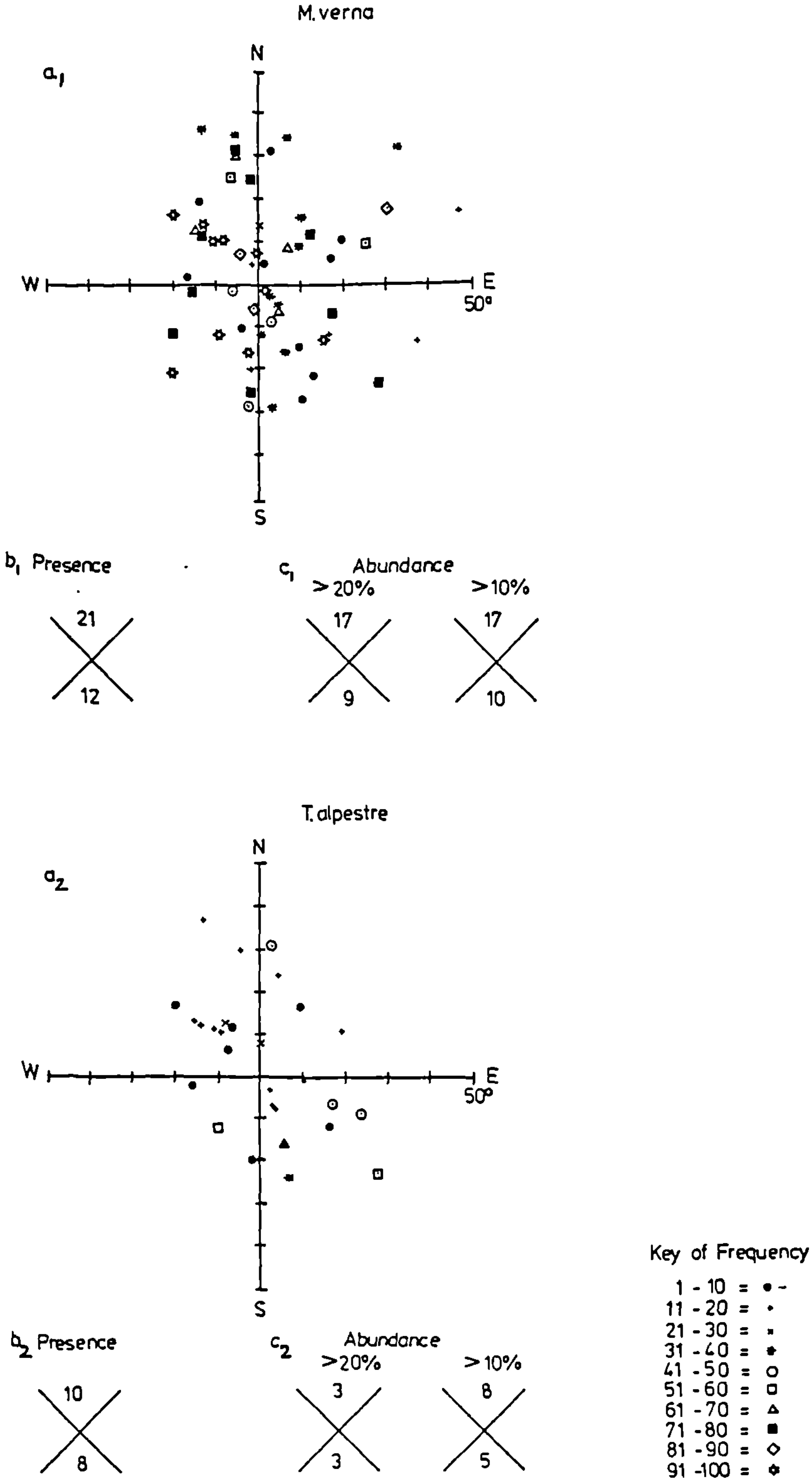


Figure 2.4b Soil pH histograms for *Minuartia verna* and *Thlaspi alpestre*.

FIGURE 2.5 SLOPE-ASPECT POLAROGRAPHS FOR MINUARTIA VERNA AND THLASPI ALPESTRE

The pattern of occurrence of M.verna (a$_1$) and T.alpestre (a$_2$) is shown in relation to slope and aspect. [Increased distance from the centre of the polarograph indicates an increased angle of slope.] A summary of the distribution of the two species with respect to north-and south-facing slopes is included by means of smaller diagrams (b$_1$, b$_2$ & c$_1$, c$_2$). As in Grime & Lloyd (1973), in these latter diagrams records are excluded if the slope angle is less than 5°.



was recorded from all the quadrats containing T. alpestre. There are however also some differences between the two species. T. alpestre tends to be more commonly associated with bare soil and M. verna is found over a wider range of soil pH than T. alpestre. The data in Figure 2.5 also suggest that M. verna is more restricted to north-facing slopes than T. alpestre but additional sampling is required to verify this relationship.

In conclusion, this study has revealed only slight differences with respect to habitat characteristics between the two species and it is not clear whether these minor differences are of ecological significance.

2.5 PHYTOSOCIOLOGY

2.5.1 Rationale

In the previous section an unsuccessful attempt was made to identify differences in the habitat requirements of M. verna and T. alpestre. An alternative approach using the quadrat data already collected, will therefore be adopted which takes into account the ecological attributes of the species with which M. verna and T. alpestre are associated. These species are not restricted to metalliferous sites and their ecological distribution is better known than that of M. verna and T. alpestre. They may therefore be used as ecological marker species and an analysis which relates the distribution of M. verna and T. alpestre to that of other species may be used to detect more clearly any differences between the two in habitat requirements. Therefore the rooted frequency data collected for M. verna, T. alpestre and their associated species were subjected to multivariate analysis using detrended correspondence analysis (DECORANA); (Hill, 1979), and subsequently to the seriation method for summarizing vegetation-environment relationships devised by Dargie (1986). I was advised that these programs were the most efficient methods for analysing the present data (personal comm., Dr. T.C.D. Dargie).

2.5.2 DECORANA (DCA)

In the process of the analysis of the rooted frequency data, an ordination program DCA was used to group together species with similarities. The ecological attributes of species (Tables 2.3 a & b) were examined in relation to this analysis to determine the similarity and differences between species.

DECORANA was originally written by Hill (1979a) as a Fortran program for detrended correspondence analysis and reciprocal averaging (RA). The program is an improvement of the widely-used RA method produced by Hill (1973). Such a method is non-centred, i.e. it is efficient in handling a heterogeneous data set. The double standardization of RA gives emphasis upon rarer species and poorer sites, especially where the two coincide representing a distinctive nodum (Noy-Meir, 1974). Nevertheless, RA has two main problems. Firstly, the 'arch-effect' (Gauch, Whittaker & Wentworth, 1977) or the 'horseshoe effect' (Kendall, 1971). Such effects distort the linear gradient of composition into an arch in two dimensions of the ordination. Secondly, sites of similar differences in composition are not expressed as the same differences on the first axis, resulting in a bunching effect of co-ordinates at the ends of axes. Hill & Gauch (1980) and Gauch (1982) have examined these problems and proposed an alternative analysis, DCA, which eliminates the 'arch-effect' by adjusting loadings on the second axis to zero mean value within predefined segments of axis one. This method has been shown to give

TABLE 2.3 A Summary of Environmental Characteristics of Species Associated with M. verna and T. alpestre in the vegetation survey.
Species are listed according to their rank order on the seriation arrays of the first two ordination axex.

Nomenclature follows Clapham, Tutin & Warburg (1962).

Environmental indices

a. axis one

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dis	Str	S.W	Calc	N.Calc	pH	Sc	L.M.	B.S.	As.	LH	Species	GRC	SL
-	-	1	-	-	-	-	-	-	-	b	Gentiana amarella	1	*
-	-	3	30.5	0	7.5-7.9	25	6.0	1-10	(N)	P	Helictotrichon pratense	1	**
2	4	2	39.5	0.3	7.5-7.9	37	5.0	11-25	(N)	P	Carex flacca	1	**
2	4	3	11.0	0	5.5-5.9	5.6	0.0	51-75	S	P	Primula veris	1	*
2	5	2	35.0	0	5.5-5.9	8.3	2.0	11-25	(N)	P	Briza media	1	**
1	5	2	8.0	86.3	< 3.5	0.0	16.0	Nil	(N)	P	Deschampsia flexuosa	1	**
-	-	4	-	-	-	-	-	-	-	P	Knautia arvensis	1	*
3	4	4	7.5	0	5.5-5.9	0.0	2.0	11-25	(N)	P	Succisa pratensis	1	**
3	3	3	-	-	5.5-5.9	2.8	4.0	51-75	(S)	P	Prunella vulgaris	1	*
2	4	4	25.5	0.7	5.5-5.9	38.9	10.0	1-10	(N)	P	Viola riviniana	1	**
2	5	4	19.0	0	7.5-7.9	11.1	2.0	1-10	(N)	P	Scabiosa columbaria	1	**
-	-	2	20.5	0	7.5-7.9	22.2	6.0	Nil	(N)	sh	Helianthemum chamaecistus	1	*
2	4	4	27.0	0.3	5.5-5.9	11.1	2.0	11-25	(N)	P	Carex caryophyllea	1	**
2	5	4	12.0	0.3	5.5-5.9	8.3	4.0	51-75	S	P	Polygala vulgaris	1	**
-	-	4	24.0	0	5.5-5.9	11.1	10.0	1-10	(N)	P	Poterium sanguisorba	1	**
2	4	4	36.0	0	7.5-7.9	0.0	2.0	1-10	(S)	P	Centaurea nigra	1	*
3	4	3	35.0	0	7.5-7.9	25.0	20.0	1-10	(N)	P	Leontodon hispidus	1	**
1	5	3	4.5	0	7.0-7.4	8.3	6.0	26-50	(N)	P	Brachypodium sylvaticum	1	**
2	5	4	26.0	0	7.5-7.9	2.8	2.0	11-25	(S)	P	Pimpinella saxifraga	1	*
3	4	1	8.5	0	7.5-7.9	13.9	0.0	1-10	(N)	m	Arabis hirsuta	1	**
2	5	2	48.5	62.3	3.5-3.9	52.8	48.0	1-10	(S)	P	Festuca ovina	1	*
-	-	1	15.0	0	7.5-7.9	25	26.0	1-10	(N)	sh	Thymus drucei	1	**
2	5	2	22.5	0	7.5-7.9	38.9	24.0	1-10	(N)	P	Galium sterneri	1	**
-	-	2	35.5	0	7.5-7.9	19.4	14.0	1-10	(N)	P	Koeleria cristata	1	**
3	4	2	38.5	0	7.5-7.9	19.4	30.0	26-50	(N)	b	Linum catharticum	1	**
2	4	2	32.5	7.7	5.5-5.9	22.2	18.0	1-10	(N)	P	Anthoxanthum odoratum	1	*
3	4	3	26.0	18.7	5.0-5.4	8.3	16.0	1-10	(N)	P	Luzula campestris	1	**
3	4	2	10.0	0	7.5-7.9	5.6	34.0	51-75	(S)	P	Euphrasia officinalis	3	*
3	4	1	52.5	3.7	7.5-7.9	0.0	0.0	Nil	(N)	P	Campanula rotundifolia	3	**
3	4	1	-	-	6.5-6.9	0.0	50.0	51-75	(S)	P	Minuartia verna	3	**
3	3	3	21.5	6.0	5.0-5.4	5.6	64.0	1-10	(N)	P	Rumex acetosella	3	**
-	-	2	-	-	5.5-7.5	-	-	-	(S)	P	Thlaspi alpestre	3	**
3	3	3	76.0	9.0	7.5-7.9	66.7	56.0	1-10	(N)	P	Festuca rubra	4	**
3	3	2	-	-	5.5-5.9	2.8	46.0	51-75	(N)	P	Cerastium fontanum	5	**
-	-	1	-	-	-	-	-	-	-	P	Dianthus deltoides	3	*
4	2	3	21.5	1.7	5.5-5.9	5.6	24.0	11-25	(N)	P	Trifolium repens	5	**
4	2	3	-	-	6.5-6.9	0.0	20.0	26-50	(N)	P	Leontodon autumnalis	5	*
3	4	2	-	-	6.0-6.4	16.7	6.0	1-10	(N)	P	Veronica chamaedrys	5	**
4	3	2	-	-	7.5-7.9	0.0	4.0	26-50	S	a	Crepis capillaris	5	*
-	-	2	38(41.5)	10.3	7.5-7.9	11.1	10.0	11-25	(S)	P	Poa pratensis	5	**
5	2	2	-	-	7.5-7.9	0.0	8.0	76-100	(N)	a	Poa annua	5	*
4	1	4	-	-	6.0-6.4	0.0	6.0	26-50	(S)	P	Rumex crispus	5	*
4	1	4	-	-	5.5-5.9	0.0	4.0	76-100	(S)	P	Ranunculus repens	5	*
3	1	2	-	-	7.0-7.4	8.3	4.0	76-100	(N)	P	Urtica dioica	5	*
3	2	3	-	-	7.0-7.4	0.0	2.0	76-100	(S)	P	Glechoma hederacea	5	*
4	1	1	5.5	0	6.5-6.9	5.6	10.0	51-75	(N)	P	Poa trivialis	5	**

b. axis two

2	5	2	35.0	0	5.5-5.9	8.3	2.0	11-25	(N)	P	Briza media	1	**
2	4	2	39.5	0.3	7.5-7.9	37	5.0	11-25	(N)	P	Carex flacca	1	**
-	-	2	-	-	-	-	-	-	-	P	Plantago media	1	*
2	5	4	19.0	0	7.5-7.9	11.1	2.0	1-10	(N)	P	Scabiosa columbaria	1	**
3	4	4	7.5	0	5.5-5.9	0.0	2.0	11-25	(N)	P	Succisa pratensis	1	**
2	4	4	27.0	0.3	5.5-5.9	11.1	2.0	11-25	(N)	P	Carex caryophyllea	1	*
-	-	4	24.0	0	5.5-5.9	11.1	10.0	1-10	(N)	P	Poterium sanguisorba	1	*
3	3	1	28.0	0	7.5-7.9	36.1	16.0	26-50	(N)	m	Senecio jacobaea	1	**
3	4	3	35.0	0	7.5-7.9	25	20.0	1-10	(N)	P	Leontodon hispidus	1	**
3	4	2	10.5	0	5.5-5.9	0.0	0.0	11-25	(S)	P	Galium verum	1	*
3	4	2	38.5	0	7.5-7.9	19.4	30.0	26-50	(N)	b	Linum catharticum	1	**
3	4	2	34.5	0.7	7.5-7.9	27.8	20.0	26-50	S	P	Hieracium pilosella	1	*
-	-	-	-	-	7.5-7.9	11.1	26.0	26-50	(S)	P	Hieracium spp	1	*
3	4	4	53.5	1.3	7.5-7.9	8.3	26.0	11-25	(S)	P	Lotus corniculatus	1	**
2	5	2	22.5	0	7.5-7.9	38.9	24.0	1-10	(N)	P	Galium sterneri	1	**
3	4	2	-	-	7.5-7.9	5.6	34.0	51-75	(S)	P	Euphrasia officinalis	1	**
-	-	1	15.0	0	7.5-7.9	25.0	26.0	1-10	(N)	sh	Thymus drucei	1	**
4	3	4	46.0	1.3	7.5-7.9	5.6	36.0	26-50	S	P	Plantago lanceolata	1	**
-	-	2	-	-	5.5-7.5	-	-	-	(S)	P	Thlaspi alpestre	2→	*
3	3	3	76.0	9	7.5-7.9	66.7	56.0	1-10	N	b	Festuca rubra	2	**
3	4	1	-	-	6.5-6.9	0.0	50.0	51-75	(S)	P	Minuartia verna	2→	**
3	4	2	9.5	0.7	6.0-5.4	16.7	6.0	1-10	(N)	P	Veronica chamaedrys	3	*
3	3	2	-	-	5.5-5.9	2.8	46.0	51-75	(N)	P	Cerastium fontanum	3	**
3	4	1	52.5	3.7	7.5-7.9	0.0	0.0	NIL	(N)	P	Campanula rotundifolia	1	**
3	2	4	25.5	0.7	7.5-7.9	2.8	2.0	26-50	(S)	m	Heracleum sphondylium	5	*
3	3	3	21.5	6	5.0-5.4	5.6	64.0	1-10	(N)	P	Rumex acetosa	3	**
2	5	2	48.5	62.3	3.5-3.9	52.8	48.0	1-10	(S)	P	Festuca ovina	5	**
4	1	1	5.5	0	6.5-6.9	5.6	10.0	51-75	(N)	P	Poa trivialis	4	*
-	-	2	-	-	5.5-5.9	0.0	4.0	76-100	(N)	P	Agropyron repens	5	*
5	1	4	-	-	7.5-7.9	0.0	6.0	76-100	(N)	P	Rumex obtusifolius	5	*
5	1	1	32.0	38.7	4.5-4.9	0.0	44.0	26-50	S	P	Agrostis tenuis	5	**
3	1	4	-	-	7.0-7.4	0.0	4	26-50	(S)	a	Galium aparine	5	**
2	4	3	-	-	7.5-7.9	30.6	14.0	26-50	(S)	P	Teucrium scorodonia	5	**
1	5	2	8.0	86.3	< 3.5	0.0	16.0	NIL	(N)	P	Deschampsia flexuosa	5	**

Key and significance of characters

*1 - Index of disturbance (Dis) 1-5, where (1) the lowest and (5) the highest degree of disturbance.

*2 - Index of stress (Str) 1-5 scale; 1 is the lowest and 5 the highest degree of stress.

Characters 1 & 2 relate to theory of plant strategies (Grime, 1979).

'Disturbance' factors (such as grazing and soil erosion) cause a loss of plant biomass.

'Stress' restricts plant growth, e.g. nutrient deficiency.

*3 - Seed weight (S. W) 1-5 scale,

1 = <0.2 mg
2 = 0.2-0.5 mg
3 = 0.6-1.0 mg
4 = 1.1-5.0 mg
5 = >5.0 mg

[There is a basic conflict in resource allocation between seed size and seed number (Salisbury, 1942; Harper _et al._, 1970; Kawano, 1981). For species exploiting disturbed sites the production of large numbers of small seeds may be advantageous allowing a wide dispersal of seeds in space or time (through the formation of buried seed bank -Thompson and Grime, 1979; Grime _et al._, 1981). In sites very unfavourable for seedling establishment (including toxic mine waste) the production of a smaller number of large seeds, with large seed reserves, may reduce the risk of seedling mortality. Thus seed weight may be used to give an indirect assessment of 'disturbance' and 'stress' _sensu_ Grime (1979)].

4 - Degree of association with substrate calcareous soil (Calc): the average of the percentage constancy of the species in semi-natural grasslands on the Carboniferous and Magnesian Limestones.

5 - Degree of association with substrate Non-calcareous soil (N.Calc): the average of the percentage constancy of the species in semi-natural grasslands on the Bunter Sandstone, Coal Measures and Millstone Grit.

4 & 5 were calculated from Grime & Lloyd (1973).

*6 - Soil pH at 0-3 cm depth. Values refer to pH class with which species most frequently associated.

Characters 4 to 6 relate to edaphic factors, which affect plant growth (see Jefferies & Willis 1964; Grime & Hodgson, 1969; Rorison 1973.)

*7 - Scree (Sc): the percentage constancy on limestone scree. This substrate shares many of the physical characters of lead-mine spoil but is non-toxic.

*8 - Lead mine spoil (L.M.): the percentage constancy on lead mine spoil.

*9 - Bare soil classes (percentage)
1-10, 11-25, 26-50, 51-75, 76-100.

These values refer to the bare ground class with which species most frequently associated.

10 - Aspect (As)
a - North-facing = N
b - South-facing = S

a and b refer to species with statistically significant bias to north or south. If distributing bias is not statistically significant the result is included in parentheses.

Temperature and humidity vary according to aspect (Rorison _et al_., 1986). In consequence, species which exploit dry warm habitats tend to show a south-facing bias and those from moist or shaded habitats are more frequently on north-facing slopes.

*11 - Life history (L. H.)
a = Annual
b = Biennial
p = Polycarpic perennial
m = Monocarpic to polycarpic perennial
sh = Shrub

Short-lived species are typically associated with disturbed habitats.

12 - The species associated with _M. verna_ and _T. alpestre_. These were filtered out by the DCA (Hill, 1979) and then by a seriation method (Dargie 1986). The latter technique grouped species according to gradient restriction code (G.R.C.) see 13.

13 - Gradient restriction code (G.R.C.) (from Dargie, 1986).
0 = Species not significant for either tests (these were excluded).
1 = Species with an average or lower score and only significant for the first test.
2 = Species with average or lower score and significant for both tests.
3 = Species significant only for the second test and largely restricted to the central section of the gradient.
4 = Species with higher than average scores and significant for both tests.
5 = Species with higher than average scores and significant for the first test only.

14 Significance level (S.L.): refers to the significance level for a species (1% or 5%) according to X^2 (Dargie, 1986).

---

* Data abstracted from Grime _et al_. (1987)

good quality site co-ordinate sets (Hill & Gauch 1980. & Gauch, 1982) and to be a powerful method to ordinate the data plots on separate axes (Kershaw & Looney, 1985).

Both RA and DCA are part of a program package DECORANA and they produce ordinations of both sites and species for four axes.

2.5.2.1 Results

On the DCA species ordination for axes one and two, presented in Figure 2.6, M. verna and T. alpestre occupy a similar position on the diagram. An attempt was made to identify the ecological significance of the two axes using environmental indices which relate to the autecology of the species being studied and which are presented in Table 2.3. No simple patterns could be visually detected and no ecological separation of M. verna and T. alpestre has been achieved.

2.5.3 Seriation Method

The rooted-frequency data analyzed by DCA were also subjected to a more rigorous method of analysis. This method presents results as one or more two-way site-species tables, termed seriation arrays, which order species according to the main environmental trends in sample sites (Dargie, 1986).

SERIATE is a Fortran program written by Dargie (1984, 1986), devised to "seriate" and summarize site-species patterns derived from ordination analysis including DCA. The method is principally a way of re-ordering data into a more understandable form, but also involves a χ^2 procedure for sorting floristic data and isolating species significantly restricted to segments of a site sequence. Dargie (1986) outlines the seriation technique which can be applied to three types of site sequencing, termed summarization modes. First: single habitat

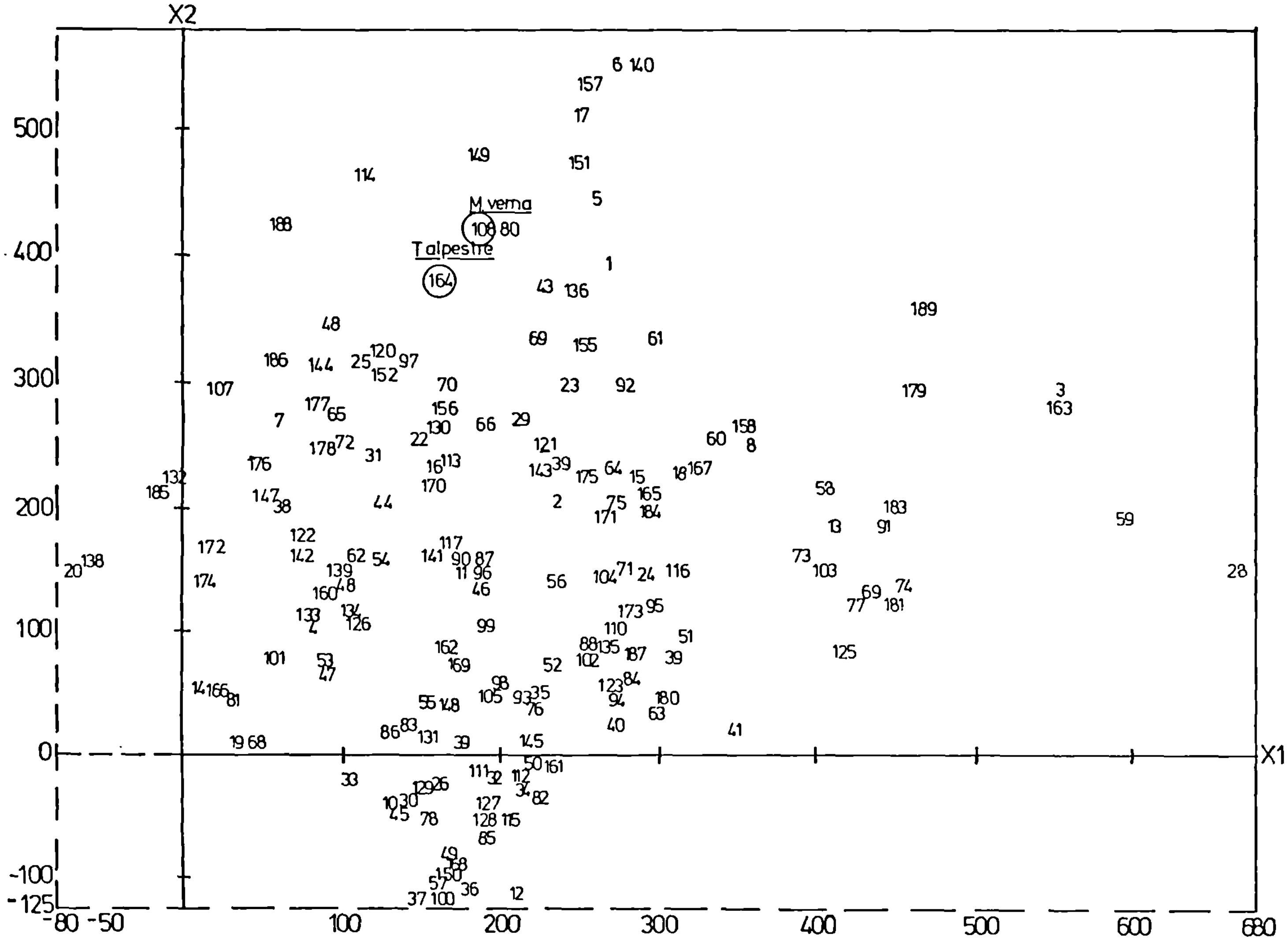


Figure 2.6 The DECORANA ordination of species with respect to their frequency in 134 quadrats of lead mine vegetation. Only the first two axes are been plotted.

variables are used as a form of direct gradient analysis. Second: trends on the original axes of indirect site ordinations can be examined. Third: trends oblique to axes of indirect site ordinations are examined. For each seriation two χ^2 tests are applied to each species. Test 1 dichotomizes the axis into upper and lower halves to see if the species is restricted to the lower or upper part of the axis at 5% and 1% significance levels of χ^2. Test 2 divides the axis into three parts, a central and two outer zones to see if the species is restricted to the central zone at 5% and 1% significance levels. A location code (0, 1, 2, 3, 4 and 5) is then attached as appropriate to each species. According to χ^2 results at $\chi^2 < 3.84$, $p = 0.05$, these codes can be listed as follows:

Code 0 = Species not significant for either tests.

1 = Species significant only for the first test, largely confined to lower half of site gradient.

2 = Species significant for both tests, mainly on lower half of gradient in the first test.

3 = Species significant only for the second test, largely restricted to the central section of the gradient.

4 = Species significant for both tests, come on upper half of the gradient in the first test.

5 = Species significant for the first test only, largely confined to the upper half of the gradient.

Non-significant species (coded 0) are eliminated from seriation output. Hence the analysis acts as a filter to retain only those species significantly restricted to parts of the site gradient on the axis. However in the present study only mode 2 (original ordination axes) was used because of the nature of the investigation. For more details of the method and the way of constructing the diagrams see Dargie (1984, 1986).

2.5.3.1 Results

Seriation arrays of species and site groups for axes one and two are presented in Figures 2.7 a & b. As in the previous analysis, M. verna and T. alpestre occur in a similar position on each axis. However the two axes are much more readily interpreted in ecological terms than those of the DCA ordination. The first portion of axis one comprises mainly species of north-facing slopes with infertile calcareous soils, and the latter portion of the axis consists of species of more fertile, often disturbed sites. The middle area contains species of relatively infertile but disturbed sites. This part of the axis includes both M. verna and T. alpestre. Axis two seems to be similar to axis one except that there is a mixture of species from disturbed and from acidic soils along its latter part.

The data from both axes suggest that M. verna and T. alpestre have a similar ecological niche (infertile but somewhat disturbed sites with broken vegetation on metalliferous mine spoil) but do not identify any clear-cut ecological differences between the two species.

2.6 DISCUSSION

In this chapter the geographical and aspects of ecological distribution of M. verna and T. alpestre have been studied by a variety of methods. Both species appear to be similar in their habitat requirements. However a few differences could be detected between the two species. These are (1) T. alpestre is much more restricted than M. verna in Europe, Britain and Derbyshire. (2) in Derbyshire M. verna

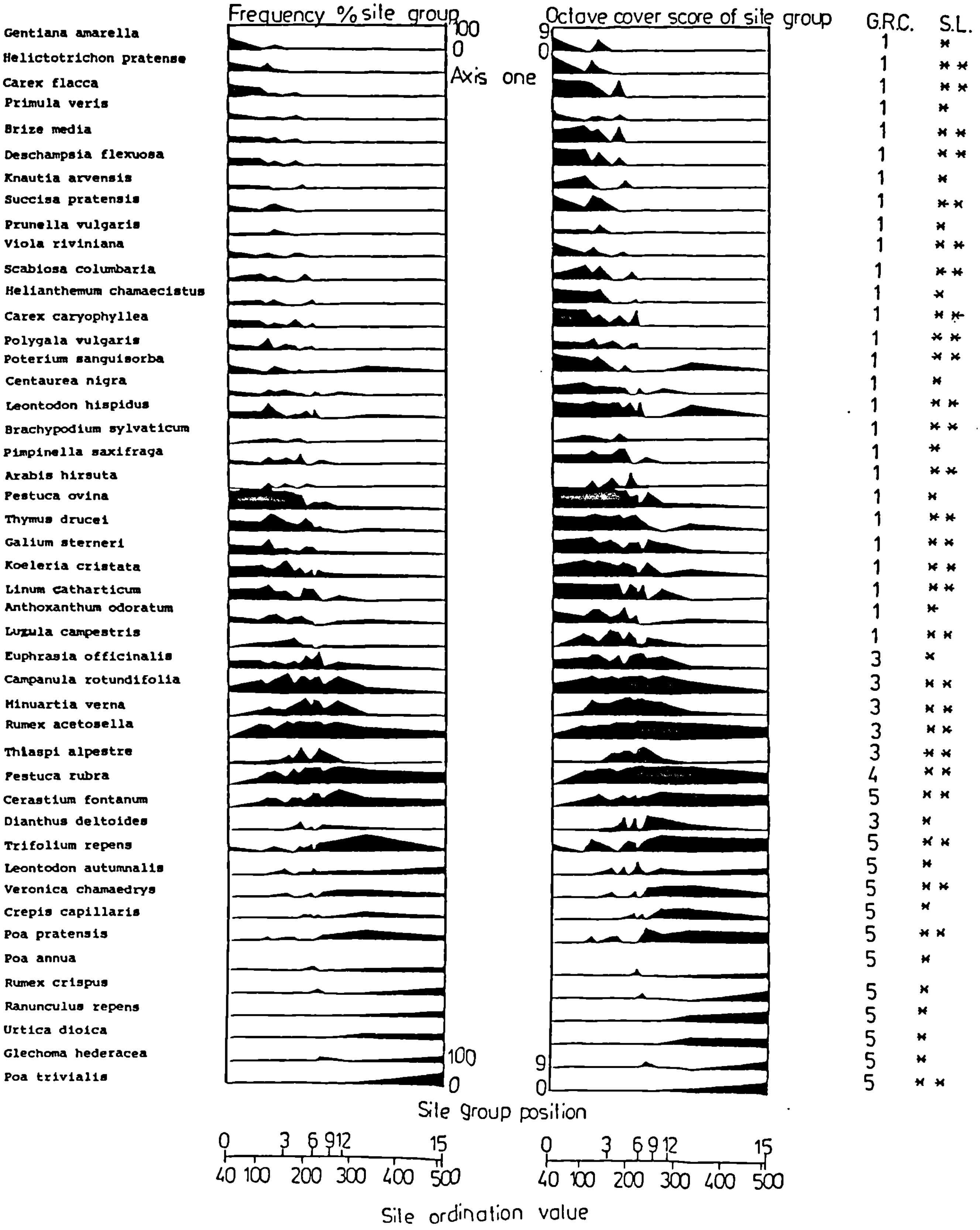
Figure 2.7 a & b Seriation arrays of species and site groups for axes one and two.

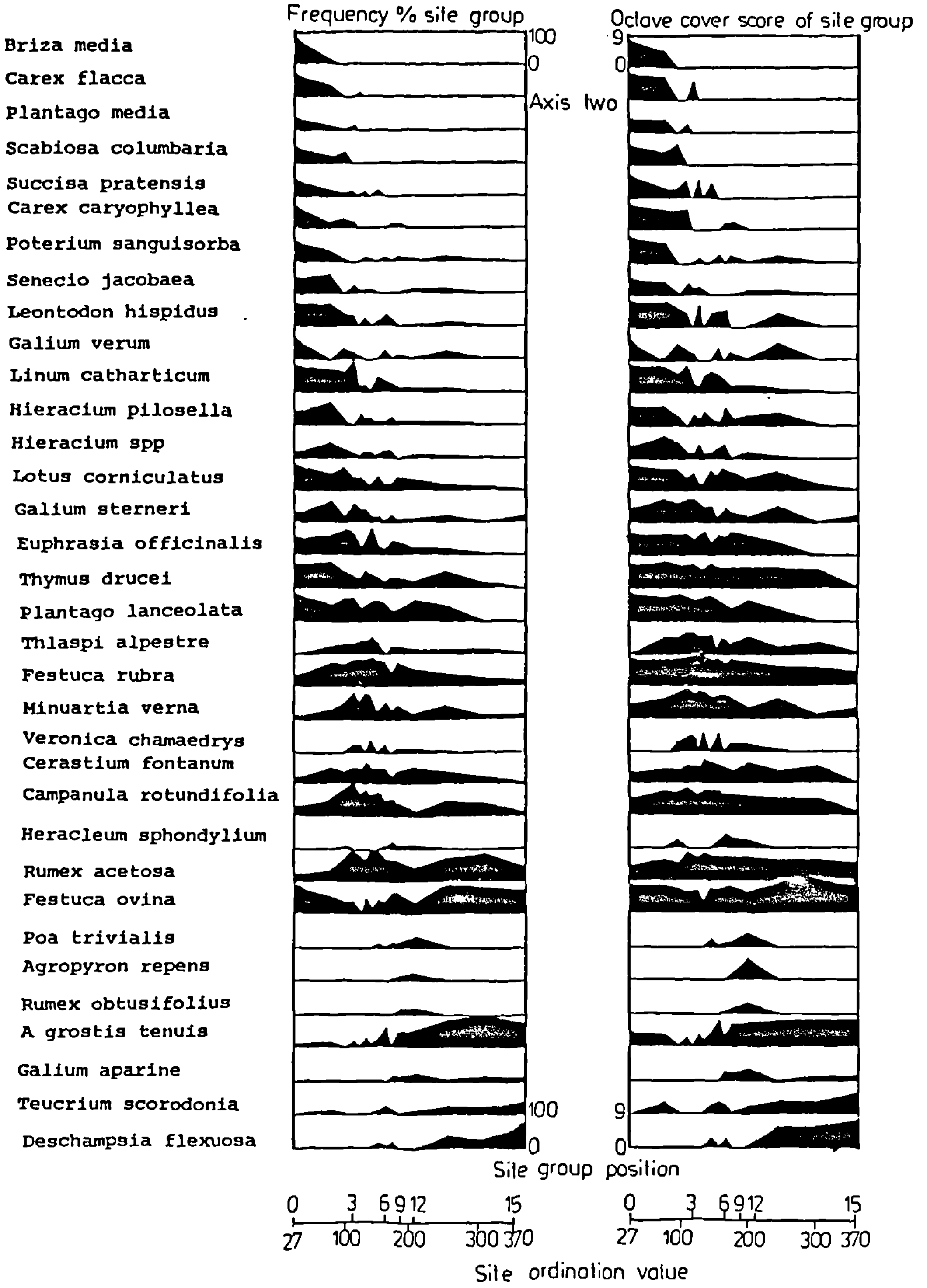
Data have been summarized according to:

(a) Percentage frequency in site group (0 - 100%), and

(b) Octave cover score of site group.

(Index of the octave cover score 0 - 9).





	G.R.C.	S.L.
Briza media	1	* *
Carex flacca	1	* *
Plantago media	1	*
Scabiosa columbaria	1	* *
Succisa pratensis	1	* *
Carex caryophyllea	1	*
Poterium sanguisorba	1	*
Senecio jacobaea	1	* *
Leontodon hispidus	1	* *
Galium verum	1	*
Linum catharticum	1	* *
Hieracium pilosella	1	*
Hieracium spp	1	*
Lotus corniculatus	1	* *
Galium sterneri	1	* *
Euphrasia officinalis	1	* *
Thymus drucei	1	* *
Plantago lanceolata	1	* *
Thlaspi alpestre	2	*
Festuca rubra	2	* *
Minuartia verna	2	* *
Veronica chamaedrys	3	*
Cerastium fontanum	3	* *
Campanula rotundifolia	1	* *
Heracleum sphondylium	5	*
Rumex acetosa	3	* *
Festuca ovina	5	* *
Poa trivialis	4	*
Agropyron repens	5	*
Rumex obtusifolius	5	*
A grostis tenuis	5	* *
Galium aparine	5	* *
Teucrium scorodonia	5	* *
Deschampsia flexuosa	5	* *

Key:

0 = 0
1 = >0.01
2 = 0.5-1
3 = 1-2
4 = 2-4
5 = 4-8
6 = 8-16
7 = 16-32
8 = 32-64
9 = 64+

is found over a wider range of soil pH. (3) T. alpestre is particularly associated with high exposure of bare soil. (4) T. alpestre may be less restricted to north-facing slopes.

These small differences do not provide a satisfactory basis for ecologically separating the two species. Therefore the following questions will be posed in later chapters.

(A) Relating to the contrasted geographical range of the two species:

(1) Do the two species differ in their capacity to form populations adapted to new environments?

(2) Do the two species differ in their ability to colonize new sites?

(B) Relating to the ecological "niche" each species occupies on metalliferous spoil:

(1) Do the two species differ in their tolerance of heavy metals and consequently occupy microsites differing in toxicity?

(2) Do the two species occupy sites differing in other respects, e.g. in the degree of disturbance or vegetation cover?

CHAPTER 3

MORPHOLOGICAL VARIATION BETWEEN POPULATIONS OF MINUARTIA VERNA AND THLASPI ALPESTRE FROM THE SOUTH AND NORTH PENNINES

3.1 INTRODUCTION

In the previous chapter it became apparent from both the broad geographical and local distributions of M. verna and T. alpestre, that the latter species is considerably 'rarer' relative to the former, even in areas where suitable habitats might exist. In the Pennine region, as elsewhere in the British Isles, both species exhibit a markedly disjunct distribution pattern. The consequences of this geographical isolation of local populations, presumably at least at some relict sites since glacial times, have a major bearing on population divergence and further evolution within the species (Pigott & Walters, 1964).

Geographical isolation may facilitate population divergence through the differentiation of locally-adapted races or ecotypes possessing distinctive morphological and physiological characteristics. However, such genecological divergence requires the necessary genetic variability within the gene pools of populations to allow the operation of the processes of natural selection. Small, reproductively-isolated populations of predominantly inbreeding species like T. alpestre may possess restricted gene pools largely as a result of their origins from a very limited number of establishing individuals (the founder principle). Divergence may result from the effects of subsequent random genetic drift. Species showing more tendency to outbreeding, such as M. verna, may accordingly show more genetic variability within populations. Some degree of crossing between geographically-separated populations may even occur depending on the range of pollen vectors. Contrasting breeding systems may then be another factor implicated in the interpretation of the present-day distributions of both species.

Regrettably, it is impossible to trace the course of recent evolutionary change in plant populations. All that is possible is to observe the results of such processes a posteriori, by comparing and contrasting morphological and physiological traits of individuals within population samples. Features of adaptive significance may thus be revealed. The extent of inter - and intra-population variation in such characteristics may throw some light on plasticity of the species as a whole and the ease with which adaptive variants are selected.

This chapter describes an investigation of morphological variation between a small range of populations from metalliferous mine sites within the study area, selected to include populations of varying degrees of geographical isolation from each other. Chapters 4 and 5 discuss similar studies on physiological properties (heavy metal tolerance).

3.2 MATERIALS AND METHODS

3.2.1 Experimental Rationale

Field observations and the reports of other workers suggest that populations of both M. verna (Halliday, 1960) and T. alpestre (Riley, 1955; Ingrouille & Smirnoff, 1986) exhibit some degree of variation in morphological characteristics between sites. The more obvious include plant height, leaf size, floral and fruit dimensions. Comparisons of population samples collected in the field may be confounded by the effects of different habitat conditions resulting in some degree of phenotypic modification. This is frequently the case for vegetative characters which may prove highly responsive to climatic factors. In order to detect genotypic differences, plants must be raised from seed under standardized environmental conditions. A protocol for this type of genecological study was established by the classic work of Turesson in the 1920's (Jones & Wilkins, 1971). By contrast to vegetative characters, floral and fruit characters are generally implastic and remain unaffected by environmental conditions. Comparison of material collected in the field is therefore justified. In the present study a combination of approaches was employed, dictated largely by problems of the flower initiation in T. alpestre. Plants of both species were grown from large seed samples collected at the field sites listed in Table 3.1. They were raised in trays of John Innes Potting Compost No. 2 (JIPC) in a glasshouse at Tapton Experimental Gardens. Supplementary day-light was supplied for 16 hours per day from 100W Phillips mercury lamps. Daytime temperatures did not generally exceed 25°C or the night temperature fall below 18°C. Thirty plants of any one species/population (10 per tray) were grown on from seedling stage to maturity under these conditions. For M. verna, vegetative and floral characters were recorded when each population sample reached the flowering stage. For T. alpestre, however, vegetative characters were recorded when it was felt that they were most fully expressed, i.e. when leaf rosettes had reached maximum dimensions; this was at approximately 36 weeks after sowing. In view of the problems mentioned above, these plants were not grown on to flowering but fruiting inflorescence axes were collected from 30 individuals from the field for all populations in this study.

3.2.2 Populations Sampled

Sites of origin of populations used in the morphological study is given in Table 3.1.

Table 3.1 Sites of origin of populations sampled

Site	**Bradford Dale**
Locality	S. Pennines
Grid. Ref.	SK 202637
Altitude	152 m
Slope	12°
Aspect	340°
Soil pH	6.7
Species	M. verna & T. alpestre
Grazing	Not grazed by sheep
Common associates	Festuca ovina; Festuca rubra Rumex acetosa
Substrate	Lead mine spoil
Principal metals	Pb; Zn; Cd & Fe
Disturbance	Low
Site	**Black Rocks**
Locality	S. Pennines
Grid. Ref.	SK 293557
Altitude	244 m
Slope	14°
Aspect	320°
Soil pH	7.1
Species	M. verna & T. alpestre
Grazing	Not grazed by sheep
Common associates	Festuca rubra & Rumex acetosa
Substrate	Lead mine spoil
Principal metals	Pb & Zn
Disturbance	Very high
Site	**Clough Wood**
Locality	S. Pennines
Grid. Ref.	SK258618
Altitude	168 m
Slope	9°
Aspect	160°
Soil pH	7.3
Species	T. alpestre
Grazing	Not grazed by sheep
Common associates	M. verna, F. rubra & Rumex acetosa
Substrate	Lead mine spoil
Principal metals	Pb & Zn
Disturbance	High

Site	**Whitesike Mine (Garrigill)**
Locality	N. Pennines
Grid. Ref.	NY 751425
Altitude	131 m
Slope	0°*
Aspect	-*
Soil pH	6.9*
Species	M. verna & T. alpestre
Grazing	Grazed by sheep
Common associates	Armeria maritima, F. ovina & Rumex acetosa Cochleavia pyrenaica, Viola lutea
Substrate	Lead mine spoil
Principal metals	Pb & Zn
Disturbance	High

Site	**Grattondale**
Locality	S. Pennines
Grid. Ref.	SK207607
Altitude	259 m
Slope	20°
Aspect	315°
Soil pH	7.3
Species	M. verna
Grazing	Not grazed by sheep
Common associates	F. rubra, Rumex ocetosa
Substrate	Lead mine spoil
Principal metals	Pb & Zn
Disturbance	Low

Site	**Tideslow Rake**
Locality	S. Pennines
Grid. Ref.	SK 159778
Altitude	335 m
Slope	13°
Aspect	45°
Soil pH	7.7
Species	M. verna
Grazing	Not grazed by sheep
Common associates	F. ovina, Koeleria macrantha, Plantazo lanceolata, Thymus praecoc ssp. arcticus
Substrate	Lead mine spoil
Principal metals	Pb, Zn, & Cd
Disturbance	High

Site	**Eller Beck**
Locality	N. Pennines
Grid. Ref.	SD 984900
Altitude	61 m
Slope	0°*
Aspect	-*
Soil pH	6.7*
Species	T. alpestre
Grazing	Grazed by sheep

Common associates	*M. verna*; *A. maritima* & *Cochlearia pyrenaica* *Festuca rubra*
Substrate	Lead mine spoil
Principal metals	Pb & Zn
Disturbance	Low

* Dale (1974)

3.2.3 Choice of Morphological Characters

The characters selected for scoring all individuals were chosen on the following criteria:

(a) previous observations suggested some degree of inter-population variation.

(b) they were expressed in all individuals of a population sample at the same time.

(c) they could be simply and accurately recorded.

(d) a range of vegetative and reproductive characters were included for each species.

The characters chosen are listed below, together with the abbreviations used elsewhere in the chapter.

Minuartia verna

1. Vegetative characters

(a) Plant height (P.Ht.), measured from the ground to the apex of the highest inflorescence axis.

(b) Plant breadth (P.Br.), an estimate of maximum plant width measured across the widest dimension.

(c) Leaf length (Lea.L.), recorded as the length of the longest leaf pair, measured to the nearest millimetre.

2. Floral characters

(a) The structure of the inflorescence was noted according to the complexity of branching (simple or compound dichasium).

All observations on floral characters were made on one fully-opened flower, taken from the centre of one inflorescence on each individual. Preliminary observations suggested that this flower generally showed the fullest expression of all the characters scored and that there was little variation from one inflorescence to another. Thus, although it was realized that a single flower per individual was a very limited sample, it was deemed to be a representative sample, at least for comparative purposes. The following were scored on each flower *in situ*

(b) Flower diameter (Flo.Br.) was measured using a calliper gauge

(c) Stamen number (number of filaments)

(d) Anther position

(e) Anther colour

(f) Number of style branches

(g) Number of stigma lobes

(h) Presence of nectaries

The sample flower on each plant was then dissected and one petal and one sepal were mounted in a drop of water on a microscope slide. The following dimensions were then recorded under low power magnification, using a micrometer eye-piece and graticule.

(i) Petal length (Pet.L.)

(j) Sepal length (Sep.L.), both recorded as the maximum length from the base to the most distal point.

The ovary was sectioned using a razor blade and then the additional characters below scored:

(k) Number of carpels

(ℓ) Number of septa.

3. Fruit characters

One fully-developed capsule was selected from near to the centre of the largest inflorescence on each individual plant. This was used to provide measures of the following characters:

(a) Capsule length (Cap.L.), measured using a calliper

(b) Number of capsule valves.

(c) Using a bulk sample of mature seed from each population, mean seed weight (Seed W) was determined from the average of six weight determinations on sub-samples (approx. 0.1 g) of counted numbers of seed.

4. Phenology

The time for germination after initially sowing the seed samples was recorded as was the time taken for flowering to commence in each population.

T. alpestre

1. Scape and plant size at the fruiting stage.

(a) Plant height (P.Ht.) was measured from the ground to the apex of the highest raceme.

(b) Raceme length (Rac.L.) was measured from the apex to the lowest pedicel.

(c) Stem length (Ste.L.) was measured as the distance from the insertion of the pedicel of the lowest capsule to the base of the stem.

(d) Rosette breadth (Ros.Br.) was measured across the widest point.

(e) Leaf length (Lea.L.) from the apex to the point at which the petiole joins the base and leaf breadth (Lea.Br.) across the widest point were measured for the largest leaf.

2. Fruit (see Figure 3.1).

One fully-developed capsule, from the middle of the largest raceme of each individual in the sample was measured for the following parts, using a binocular microscope with micrometer eye-piece:

(a) Capsule length (Cap.L.): measured from the line level with the junction of the capsule and the receptacle to the apex of the lobe of one of the 'wings'.

(b) Capsule breadth (Cap.Br.): measured at the widest point.

(c) Breadth index (Bd.I.): the distance from the junction of the capsule and the receptacle to an imaginary line joining the widest points across the capsule.

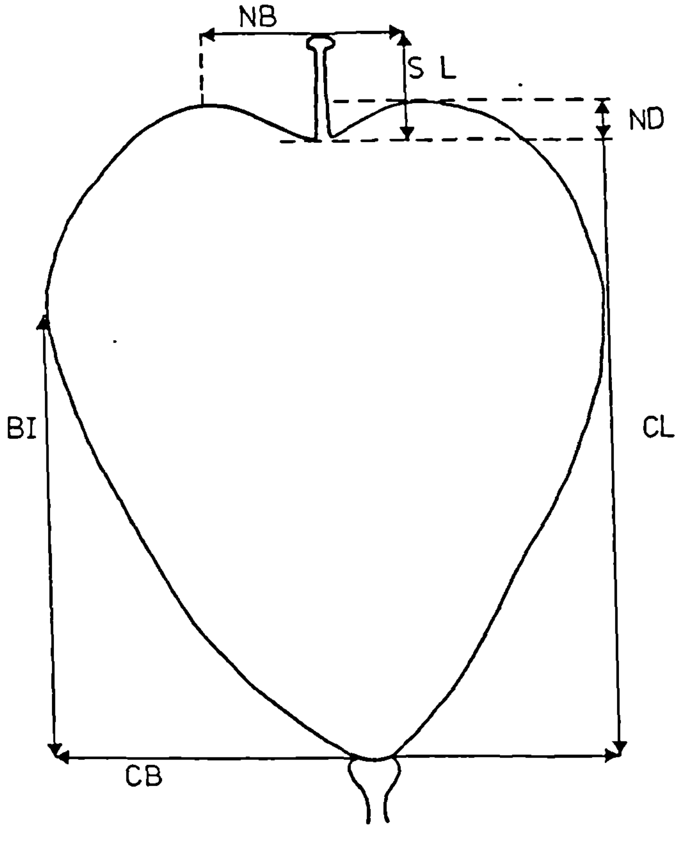
(d) Notch breadth (Not.Br.): measured as the distance between the apices of the 'wings'.

(e) Notch depth (Not.D.): measured from the base of the notch to the point level with the apex.

(f) Style length (St.L.): measured from the base to the apex including the stigmatic surface.

(g) Fruit (≡ flower) number (Fru.N.) was taken as the total number of fully-developed + non-developed or sterile capsules.

Figure 3.1 Diagrammatic representation of the *T. alpestre* capsule showing the measurements used to describe capsule size and shape.



KEY

CL	Capsule length
CB	Capsule breadth
SL	Style length
ND	Notch depth
NB	Notch breadth
BI	Breadth Index

(h) 'Fertility' was estimated from the mean seed set of five fully developed capsules taken from the centre of the largest raceme.

(i) Seed weight was determined in an identical manner to that for M. verna.

3. Ratios were derived from the above measurements, as these may serve to exaggerate the differences between populations. These were: raceme L/stem L, raceme density (Rac.D, estimated from the ratio of fully-developed capsule number/raceme length), Cap.L/Cap. Br, Bd. I/Cap.L, Bd.I/Cap.Br, Cap.L/Not.D, Not.D/Not.Br, St.L/Not.D. and Cap.Br/Not.Br.

3.3 RESULTS

Seeds from all population samples of both M. verna and T. alpestre were germinated at the same time in the glasshouse as already described. Differences in growth habit between individuals were noted as they became apparent.

Minuartia verna

The following characters did not vary either within or between populations of M. verna, and will not be discussed further: (characters coded as above)

2(a) The branching pattern of the inflorescences.
2(d) Anther position (dorsifixed).
2(e) Anther colour (light purple).
2(f) Number of style branches (3).
2(g) Number of stigma lobes (2).
2(h) Nectaries (present in all flowers).
2(k) Number of carpels (3).

2(l) Number of ovary septa (one locule for all).

3(n) Number of capsule valves (3).

Although germination occurred in all population samples up to 9 days from sowing, populations showed considerable variation in the time at which flowering commenced. This was 15 weeks from germination for Bradford Dale and Black Rocks and 20, 25 and 30 weeks for the Tideslow Rake, Grattondale and Whitesike populations respectively. A difference of 15 weeks thus separated the first and last populations to commence flowering.

The summarized data for the morphological characters scored for the five population samples are shown in Figure 3.2. All data were subjected to a full analysis of variance for each character; LSD (5%) values for comparison of population means, derived from these ANOVA tables, are shown beside each set of bar-charts. Standard error bars are also attached to the population means to provide a measure of within-population variation.

For some characters scored, such as plant height, plant breadth, flower breadth and seed weight, there was considerable and significant, inter-population variation detected. Others, such as leaf length, petal, sepal and capsule lengths showed less variability both within and between populations. To allow easy visual comparison of the inter-population variation in the characters scored, the number of significant (5%) differences between population means for each character is shown above each population bar on all bar-charts presented in Fig. 3.2. The overall degree of inter-population variation has also been summarized for each character as a percentage score out of a maximum of 20 possible significant differences. Plants from the Tideslow Rake population showed slow growth (reflected both in terms of plant height and breadth) and maintained a compact growth habit

Legend to Figures

Figures 3.2 - 3.5

Inter-population variation in a range of morphological characters between five populations of M. verna and five of T. alpestre.

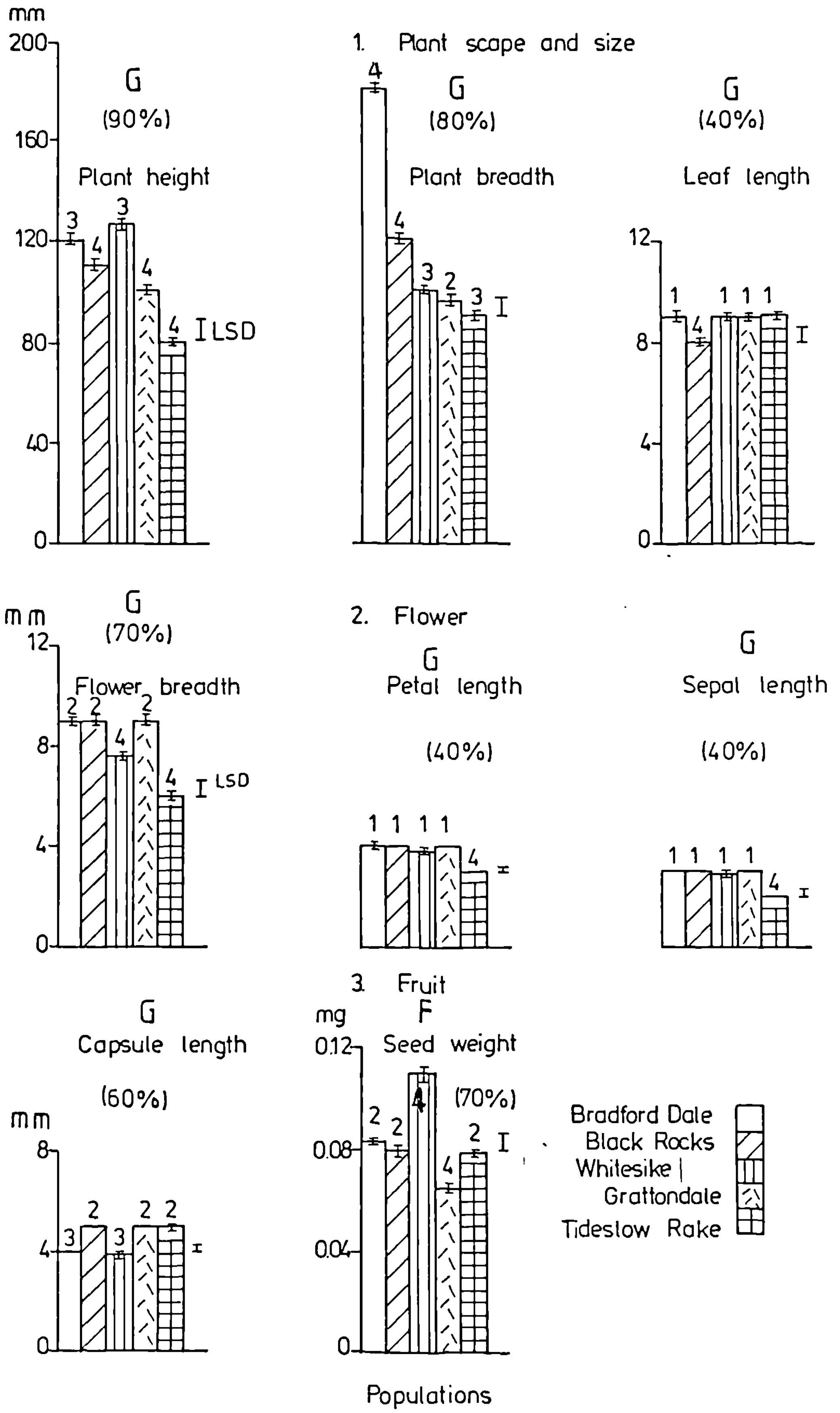
Each bar represents the mean for the population sample (n = 30) for either glasshouse-grown (G) or field-collected (F) plants. Standard errors are attached to each mean, and the LSD (5%) values from the ANOVA tables are shown for each population comparison.

The numbers shown above each bar (score/4) are the number of significantly-different population comparisons between the respective population mean and those of the other four populations in each data set.

The percentage value in parentheses above each bar-graph is an index of overall variability for each character; it has been calculated by summing the total number of significant population comparisons and expressing this total as a percentage of the sum of the maximum number possible (20).

Figure 3.2

<u>M. verna</u> - Variation in vegetative, floral and fruit characters



throughout the period of study; these plants also possessed the smallest floral dimensions. Plants from the Bradford Dale population were, on average, significantly larger than all other population samples, as is apparent from the plant breadth data. In general, however, differences in growth habit between populations were not as marked as might have been predicted from observations in the field.

Maximum leaf length showed very little overall variation which is surprising as vegetative characters are generally the most plastic phenotypically and variable genotypically. The converse was also true for flower breadth.

The mean seed weight for most populations is about 0.08 mg, but seed from the only population from the N. Pennines included in this study (Whitesike) proved to be significantly heavier than from all the S. Pennine populations.

Thlaspi alpestre

Seed of all population samples of T. alpestre readily and rapidly germinated under the glasshouse conditions. The Eller Beck sample was however slightly delayed in its germination. Subsequent seedling growth resulted in the formation of a basal rosette of leaves. In no case was flowering initiated, probably as a result of the combination of daylength and temperature conditions prevailing in the glasshouse. The data assembled for the five T. alpestre populations (summarized in Figures 3.3, 3.4 and 3.5) are thus a composite derived from the glasshouse and field-collected plants. The data have been summarized in the same way as for M. verna, Figures 3.3 and 3.4 presenting the primary information for whole plant and capsule characters respectively and Figure 3.5 some derived ratios.

Figure 3.3

T. alpestre - Variation in vegetative characters and raceme dimensions

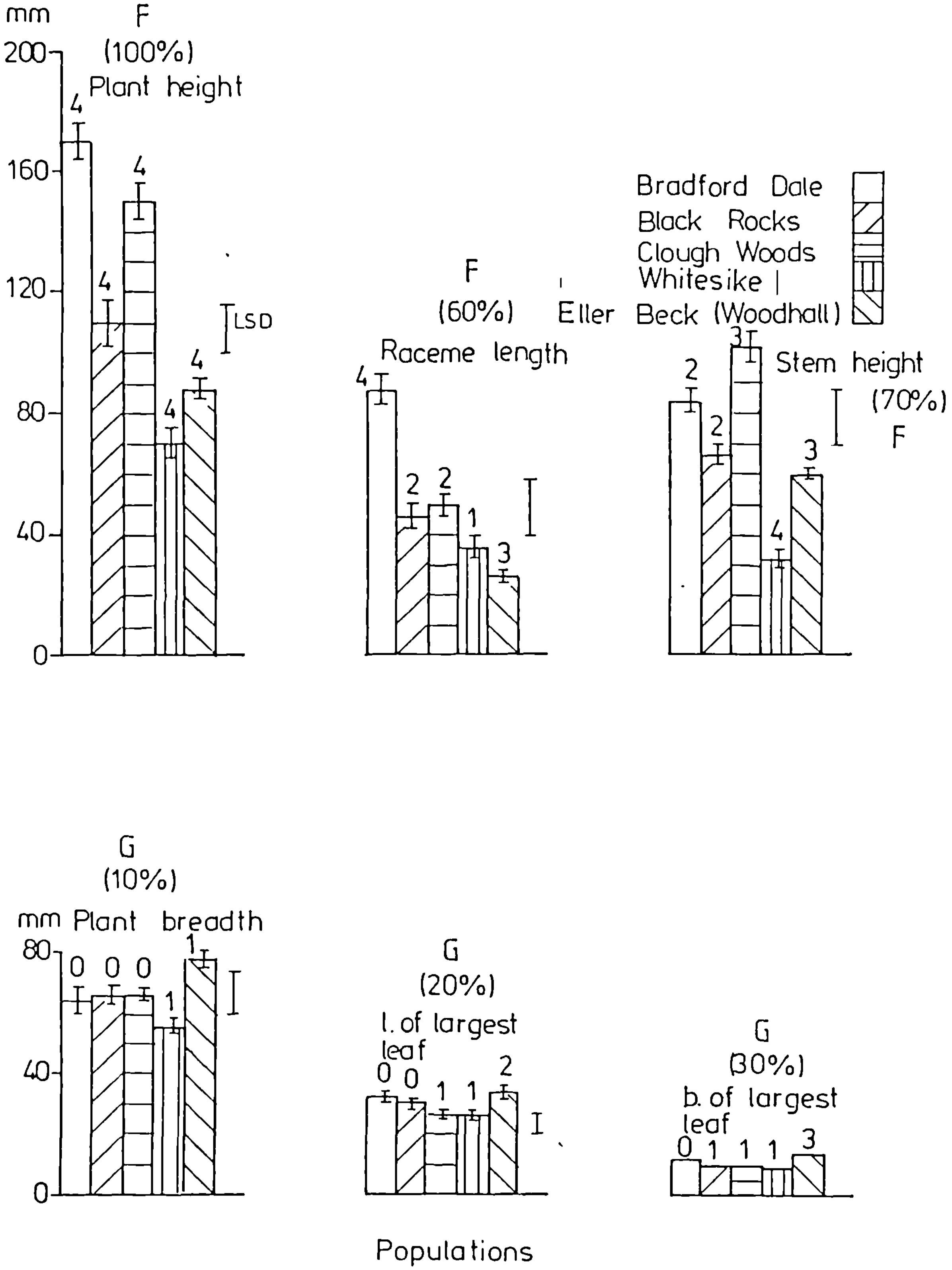


Figure 3.4

<u>T. alpestre</u> - Variation in capsule dimensions and seed weight (F)

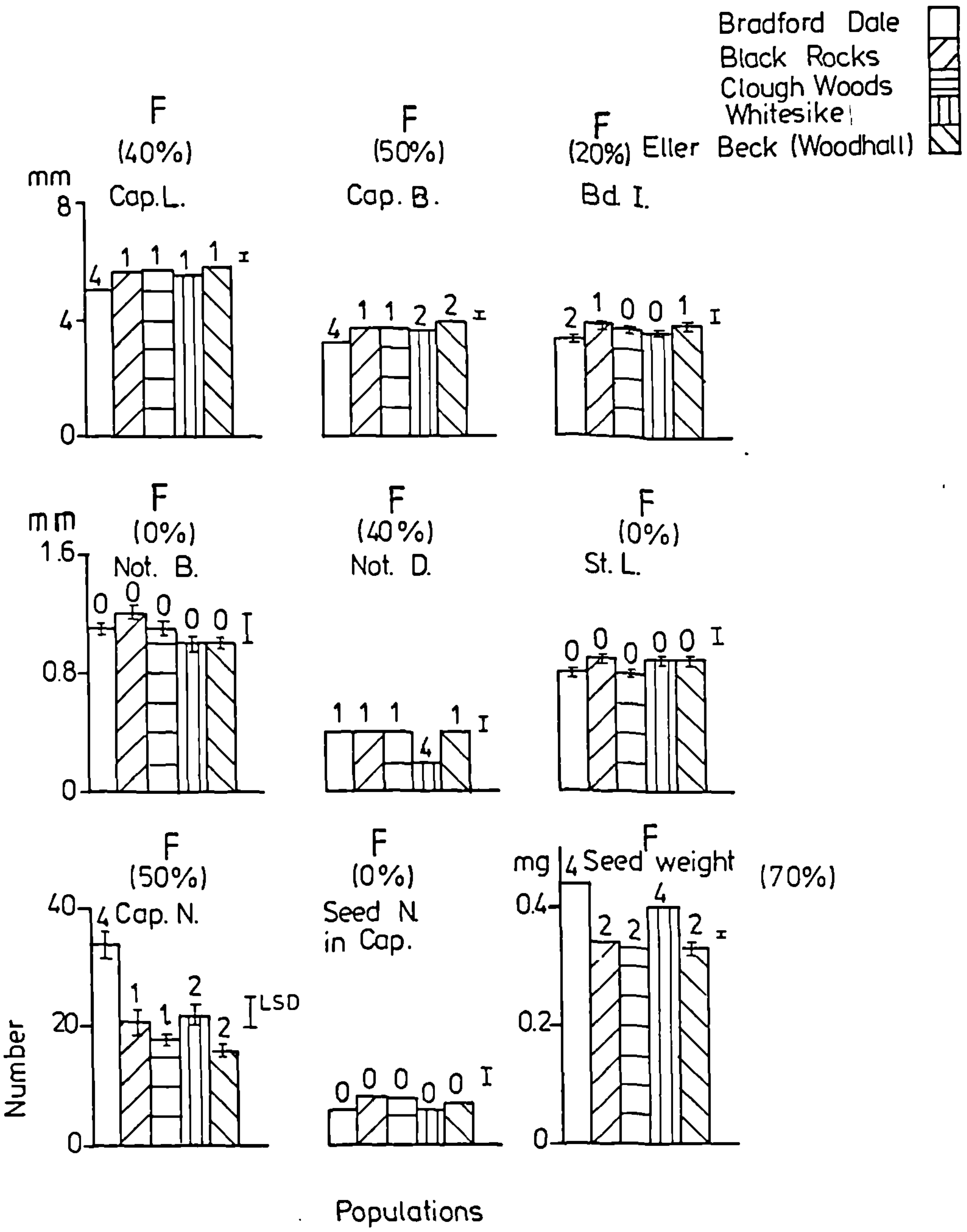
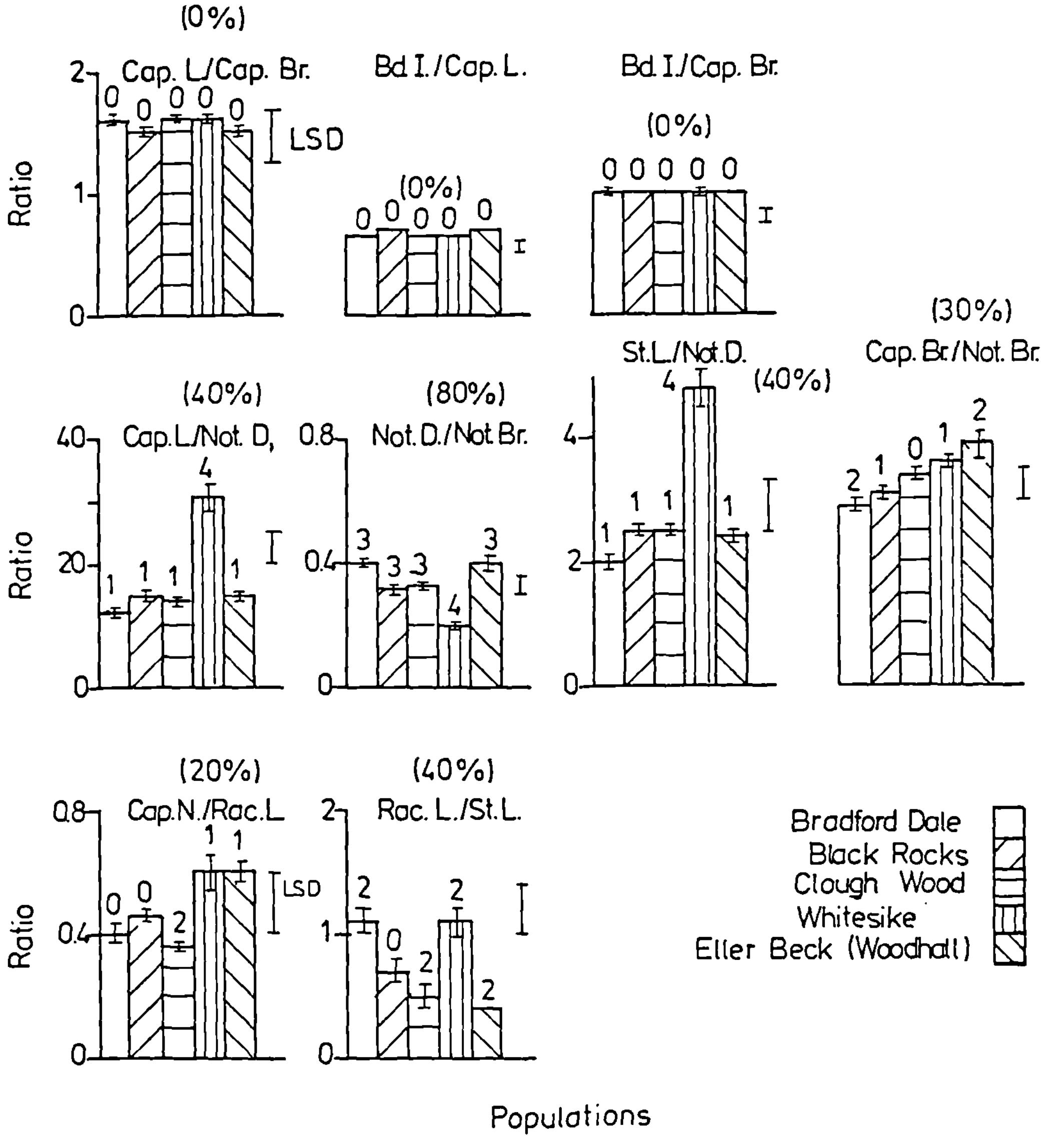


Figure 3.5

T. alpestre - Variation in some derived ratios from capsule dimensions



The analyses of variance for all the characters scored suggested considerable inter-population variation in morphology, particularly in the vegetative characters such as plant height, stem height and also in raceme length, as measured on plants collected from the field. Rosette and leaf dimensions for glasshouse-grown plants were less variable. The Whitesike population was characterized by plants of small dimension which contrasted markedly with typical plants from Bradford Dale and Black Rocks. Capsule characters, in general, showed little variation between populations, but some dimensional ratios (e.g. notch depth/notch breadth) accentuated small-scale interpopulation variations. The Whitesike population again emerged as extreme in several ratios (notably capsule length/notch depth and style length/notch depth). Mean seed weight also proved to be variable, the highest mean weight (o.44 mg) being for the Bradford Dale population, which also had the largest number of capsules/inflorescence and the largest plant height and raceme dimensions.

Overall comparison of population variability in the two species

It is difficult to assess the overall degree of similarity (or difference) between the population samples of the two species used in this comparative study, in view of the range of morphological characters employed. However, an attempt to provide such a single measure is presented in Table 3.2. Here, an index of variability for each population has been calculated by summing the number of significant differences between any one population and the remaining four for all characters scored, and expressing this number as a percentage of the maximum total number of significant comparisons possible. This calculation has been performed for each population of each species in

TABLE 3.2 INDICES OF VARIABILITY (%) FOR THE POPULATIONS OF M. VERNA AND T. ALPESTRE USED IN THE MORPHOLOGICAL STUDY

Species/Population		Index of variability*
M. verna		
1.	Tideslow Rake	74.9
2.	Black Rocks	62.6
3.	Whitesike (Garrigill)	62.6
4.	Grattondale	53.1
5.	Bradford Dale	53.1
T. alpestre		
1.	Whitesike (Garrigill)	42.8
2.	Bradford Dale	39.6
3.	Eller Beck (Woodhall)	36.5
4.	Black Rocks	23.0
5.	Clough Wood	17.8

*Index values have been calculated by summing the number of significant differences between any one population and the remaining four for all characters scored, and expressing this value as a percentage of the maximum total number of significant comparisons theoretically possible.

turn, and the populations ranked in decreasing numerical value of indices. A high index value thus suggests much (significant) inter-population variation in morphological traits.

It is noteworthy that indices are on average nearly 30% higher for *M. verna* than for *T. alpestre* suggesting that, at least for the range of populations examined in this study, *M. verna* shows greater overall inter-population variability. Whilst direct comparison with *M. verna* is not strictly possible in view of the different range of populations employed, it is clear that *T. alpestre*, at least from the sites common to both species, is markedly less variable at the population level than *M. verna* at these sites. The Tideslow Rake population of *M. verna* emerges as the most extreme in terms of morphological variation but, on average, each population proves to be significantly different from at least two others for every character scored numerically. The most distinct population of *T. alpestre* morphologically is Whitesike, the most geographically-isolated.

Population variability in relation to site characteristics

The morphological study reported in this chapter does reveal differing degrees of population divergence in both species which can be considered further in the light of some site features which could be important in contributing to reproductive isolation and opportunities for population differentiation. In this context, it is probably meaningful to consider only the S. Pennine populations as so few N. Pennine population samples were included in the study.

Table 3.3 summarizes some of the most important site characteristics for these populations, tabulated alongside recalculated indices of population variability, having omitted the N. Pennine material. Site size (which may be considerably larger than population

TABLE 3.3 POPULATION VARIABILITY IN RELATION TO SOME S. PENNINE SITE CHARACTERISTICS

Site	Approx. Size (km^2)	Period worked	Nearest spoil heaps (km)	Index of Variability (%)	Index of floristic similarity (%)
M. verna					
Tideslow Rake	>.1	Since at least 12th.C. but worked periodically until 1780's-90's.	0.46	71	55
Black Rocks	<1	Early working by 1680's but most recent workings date from early 19th.C.; no definite closure date.	0.4	63	62
Bradford Dale	0.001	Worked during second half of 17th.C. ending 1760-70.	0.8	63	66
Grattondale	<0.001	Working during mid-late 18th.C.; possible working up to *c.* 1830.	0.55	54	58
T. alpestre					
Bradford Dale	0.001	see above	0.8	31	69
Black Rocks	<1	see above	0.4	21	64
Clough Wood	0.001	First working early 18th.C. until closure *c.* 1750. Re-opened and worked more extensively between 1850-1876.	0.27	17	63

size) has been estimated from direct measurements from the relevant 1:25,000 scale maps. The nearest lead mine spoil heaps have also been located and distances read off from these maps. Historical aspects of mining activities have been abstracted from Ford & Rieuwerts (1983) and presented together with additional information kindly provided by Mr. S. R. Band (UCPE, Sheffield). Information available often relates purely to the major periods of mining activity, particularly the opening and closing of the most economical mines. Subsequent small-scale mining activities and sporadic reworking have not generally been documented in the literature. Such historical notes can therefore only be used as a broad indicator of the age of the site and the period over which colonization and spread of metallophyte species can have taken place. An index of floristic similarity between sites has also been tabulated. This has been obtained directly from the appropriate quadrat records for the sites as used in Chapter 2 (see also Appendix 2 for the primary floristic data) by calculating similarity coefficients for these vegetation stands based on the number of shared and different species they include. For any stand comparison, the coefficient was obtained from the formula $(2w/a + b)$, where w is the number of 'shared' species, a the total number in stand 1 and b the total number in stand 2. These values were then averaged for each site and indices expressed as a percentage value.

For M. verna, it emerges that the population from the oldest site (Tideslow Rake) is also the most morphologically distinct (as evidenced by the highest index of variability). There is also a possible relationship with the size of site; Grattondale is the smallest site (and population) and shows the lowest index of variability. Although Tideslow Rake ranks only number two in the order of size of site, it is probably the largest in terms of a continuous population and numbers of individuals. Black Rocks is nominally the largest site as a result of the apparent fusion of a number of small workings, the spoil from which collectively presents a large number of potential M. verna sites. However, not all are presently colonized by M. verna (or T. alpestre), and the sub-population from which seed was collected for this study was certainly smaller than Tideslow Rake. The size of the Bradford Dale population relates closely to the small size of this site.

The same trend in index of variability and age of site is also apparent for the three T. alpestre populations; Bradford Dale, the oldest having a convincingly higher index value. There is no clear relationship with size of site here. All the M. verna sites are within 1 km of other mine spoil areas, supporting similar populations; there is no apparent trend in these distances (extent of geographical isolation) with indices of variability. For the three T. alpestre populations however, there is a trend of increasing morphological distinctness with increasing isolation. Little variation is apparent between the indices of floristic similarity derived for all sites confirming that, in spite of age and size differences, the communities supporting both species are very similar, a conclusion reached in Chapter 2. Community characteristics cannot therefore be related directly to morphological traits in these populations of M. verna and T. alpestre.

3.4 Discussion

It must be stressed at the outset of any discussion of the ecological and evolutionary significance of the results of this morphological study that caution must be exercised in comparing the two species as:

(a) It was not possible to grow both species to maturity (flowering and fruiting) under the glasshouse conditions available,

(b) Only five populations of each species were studied; these were not selected to be the most geographically-isolated or extreme, but to be representative of the range of populations within the study area,

(c) A limited range of morphological characters was employed to assess inter - and intra-population variation. Some of these had potential ecological significance (e.g. plant height) others were mere markers, which could easily be scored,

(d) Some characters, particularly those associated with flower or fruit dimensions and geometry, would be expected to show some degree of correlation.

With the constraints mentioned above, it is therefore surprising to find the significant, albeit small in many cases, morphological variation between populations of both species reported in this chapter. It is also apparent that intra-population variation in the characters scored is generally small relative to inter-population differences, as evidenced by the generally small values for standard errors for population means.

Some characters scored for both species showed little, if any, inter-population variation; others on the other hand, showed considerable genetically-based variation. The growth conditions employed in the glasshouse used for the study were generally considered to be 'productive' and to enable the fullest expression of genotypes in terms of characters related to growth potential. In this context, the limited variation in maximum leaf dimensions of both species can be mentioned. The significance of variation in seed weight, both between species and at the population level, will be discussed elsewhere.

Other workers have commented upon morphological variation between isolated populations of M. verna and T. alpestre. According to Halliday (1960), in its natural habitats, the insect-pollinated and potentially outbreeding M. verna shows considerable polymorphism. This situation in the field can arise as a result of an interaction between genetic diversity and extreme phenotypic plasticity (Briggs & Walters, 1969; Jones & Wilkins, 1971). The expression of such polymorphism may thus depend on the environment in which individuals are cultivated and individuals of the same biotype may be phenotypically different when grown in contrasting environments (Halliday, 1960). Local variations between British and European populations of M. verna have been observed and reported in the literature and Halliday (1960) has shown the existence of significant genetically-based differences, even between populations which are geographically close. This is in agreement with the findings of the present study. Here it is concluded that there is significant variation between both geographically-close (S. Pennine) and isolated (N. Pennine) populations of M. verna. Some of the characters which vary (e.g. growth habit, time of flowering) are of clear ecological significance and suggest the existence of ecotypic differentiation between at least some of the populations. Variation in floral dimensions (flower diameter, petal length, etc.) may have implications in terms of attraction of pollinating insects, which could have similar genecological repercussions. The most markedly different population described from the British Isles is that growing on the serpentine soils of the Lizard Peninsula, Cornwall. Plants from this geographically-isolated and ecologically-extreme habitat show a characteristic cushion growth form and are more glaucous and ciliate, the lower leaves being closely appressed to the stems (Halliday, 1960). The Lizard population has been given sub-specific status, ssp, gerardii

(Willd.) Graebn, (Clapham, Tutin & Warburg, 1962), although the expression of these distinctive characters can be modified in cultivation (Steel, 1955; Clapham, Tutin & Warburg, 1962). No local populations in the present study showed comparable morphological divergence.

Populations of T. alpestre in the study area, and indeed in the British Isles generally, are considerably more isolated geographically (and hence reproductively) than those of M. verna (Chapter 2); they may also consist of a very small number of individuals. T. alpestre is a predominantly inbreeding species - Riley (1956) quotes an overall figure of about 5% outbreeding - and consequently populations may possess restricted gene pools, largely as a result of their origins (a limited number of establishing individuals) and their reproductive isolation. In these circumstances, one might expect to find extensive morphological divergence between isolated populations of T. alpestre and the fixation of certain morphological traits due to genetic drift. One could argue that such morphological variation would thus be unlikely to be linked to ecotypic differentiation in view of the limited variation present within population gene pools on which natural selection might operate. The present study suggested significant inter-population variation in a range of characters relating to both plant habit and the reproductive parts of the plant (inflorescence and fruit dimensions). Some variation can be linked to prevailing ecological conditions in the field. For example, the reduced plant height, stem height and raceme length of plants from the two N. Pennine populations (Whitesike and Eller Beck) can be related to the grazing regimes at these sites. Both are entirely open to grazing (as is apparent in the field from the closely-cropped turf) whereas the S. Pennine sites are not grazed by sheep and the vegetation is markedly taller. This suggests that ecotypic differentiation can occur in T.

alpestre. These findings are consistent with the reports of other workers (Riley, 1956; Ingrouille & Smirnoff, 1986) who have examined populations of T. alpestre in the field. The diversity of capsule form and its distinctness in relation to geographical locality in the British Isles has been stressed (Riley, 1955; 1956) and illustrated in the Flora of the British Isles (Clapham, Tutin & Warburg, 1962). Both Riley (1956) and Ingrouille & Smirnoff (1986) have shown wide-scale population variation in the length of the style and the depth and breadth of the fruit notch (and the relevant ratios). Although there is apparently some geographical pattern in this variation within the British Isles, there exists a complex series of variants with few discontinuities (Ingrouille & Smirnoff, 1986). Riley (1956) concluded that in spite of the existence of these considerable differences between populations, no clear morphological separation could be made on the basis of fruit types. Such continuous variation does not allow the recognition of segregate taxa, although there have been a number of attempts to provide a classification of seemingly distinct inbred lines of the T. alpestre complex (see e.g. Jordan, 1846). The problem of population variation and isolation has also confounded the taxonomy of the species - T. alpestre L, has recently (Ingrouille & Smirnoff, 1986) been shown to be an illegitimate name and, according to these authors, should be replaced by T. caerulescens J and C. Presl.

The correlations reported between indices of population variability and some important site characteristics, although only tentative, can be interpreted as potentially causal. It is clear that both age and size of site can affect population development and variability and that proximity to other sites will determine the degree of reproductive isolation, particularly in M. verna. For both species, the populations at the oldest mine sites show the highest degree of morphological distinctness; for M. verna, the two largest sites also support populations with high indices of variability. The time factor is clearly crucial when populations may be established from a limited number of founder individuals on a newly-created or newly-exposed habitat. Founder effects may thus determine morphological distinctness in small inbred populations such as those of T. alpestre. A large site can allow the possibilities for population spread and some degree of ecotypic differentiation as a result of local divergence between sub-populations separated spatially within the site. Such could be the case for M. verna at Black Rocks. All the populations scored in this study occur within a maximum of 1 km from other suitable (and generally colonized) mine spoil sites. It is then perhaps an important conclusion that population differentiation is apparent even with the restricted geographical area studied in the S. Pennine orefield.

In any consideration of population biology, a crucial difference between M. verna and T. alpestre is the nature of the breeding systems alluded to above. This is probably of major importance in understanding the present-day distribution of the two species and the extent of population variability which is detectable. M. verna is predominantly outbreeding, although not self-incompatible. Additionally, there is an extended flowering season (June - September in most northern situations) when suitable pollen vectors are abundant. The flowers are slightly protandrous, a feature favouring out-crossing. According to Riley (1956) however, T. alpestre has a protogynous floral mechanism and artificial pollinations have shown that it is both self-compatible and freely cross-compatible within populations, although there is some incompatibility in inter-population crosses. T. alpestre, by contrast then, is predominantly inbreeding, flowering very early in the season (April - May) when pollen vectors are scarcer. Riley (1956) believes that visits of insects to the flowers of *T. alpestre* are nevertheless adequate to ensure some cross-pollination. However, it is estimated that pollen is transported only short distances, so that gene migration by pollen is slight. Thus whilst the breeding system of *T. alpestre* can maintain genetic stability within populations and ensure high seed production and thus be regarded as of selective advantage, it limits the potential genetic variation upon which selection may operate. The limitations caused by gene migration (by out-crossing) can be exacerbated further by limited seed dispersal. Seed weight of *T. alpestre* is on average about five times heavier than for *M. verna*. Longer range dispersal of seed of the latter species is likely and easier, allowing the possibility for movement from one to another and the establishment of populations *de novo* from a small number of founder individuals. This aspect, and its potential affects on population dynamics, is discussed further in Chapter 6.

A further difference between the two species relates to vegetative regeneration. *M. verna* is a patch-forming polycarpic perennial while *T. alpestre*, which is occasionally monocarpic (Clapham, Tutin & Warburg, 1962), lacks the capacity for the same degree of vegetative spread. In consequence, *T. alpestre*, because of its greater dependence upon regeneration from seed, is perhaps less invasive when a colonist and less resilient when facing extinction than *M. verna*. This aspect, together with cytological factors, will be amplified in the Final Discussion (Chapter 8).

What emerges from the exploratory morphological study presented in this chapter is that both *M. verna* and *T. alpestre* do show some degree of inter-population variation in morphological characteristics. It remains to be determined to what extent similar variations in physiological properties can be detected and related to prevailing environmental conditions. This aspect is considered in Chapters 4 and 5.

3.5 SUMMARY

1. This chapter presents the results of a study of variation in morphological characteristics between a range of five populations of M. verna and T. alpestre from the South and North Pennines.
2. Individuals of each population were raised from seed under glasshouse conditions. Flowering specimens of T. alpestre were also collected from the field as, under the glasshouse conditions, flowering did not occur in this species.
3. Plants of both species were scored for a range of vegetative and floral characters.
4. For M. verna, the greatest inter-population variation occurred in plant height, plant breadth and seed weight; for T. alpestre most variation was in plant (and stem) height, raceme length, in certain capsule dimensions (notch depth/notch breadth ratio) and mean seed weight.
5. Considerable variation in the time to flowering for the M. verna populations was noted.
6. The findings of this study are considered in the light of some important site characteristics (age, size and geographical isolation) and the ecological and evolutionary implications discussed.

CHAPTER 4

HEAVY METAL ACCUMULATION BY MINUARTIA VERNA AND THLASPI ALPESTRE IN RELATION TO SOIL METAL STATUS

4.1 INTRODUCTION

It is well established that both M. verna and T.alpestre are frequently, if not exclusively, associated with heavy metal mineralization (Chapter 2). For both species, there is ample evidence of this association in the extensive geobotanical literature. As early as 1588, Thalius noted M. verna as a metal indicator (Ernst, 1965 cited in Antonovics et al, 1971). Similarly, Windsor (1865) comments on the occurrence of T. alpestre on lead and calamine mine workings in the Pennines where it grows in association with M. verna. Both species have been designated absolute metallophytes (Lambinon & Auquier, 1963) in view of this strong association and have been included in lists of potentially useful geobotanical indicators by several authors (e.g. Antonovics et al. 1971; Martin & Coughtrey, 1982; Brooks, 1983). The detailed distributions of the two species and their ecological and evolutionary consequences have been considered in Chapter 2. It is apparent that at least in the British Isles, the majority of sites at which the two species were recorded are heavily contaminated with lead and zinc which may also be accompanied by cadmium or copper. The implication is that both plants have evolved heavy metal tolerance which accounts, at least in part, for their success at the mineralized sites where they are now found. A large proportion of the experimental studies reported in this thesis relates to this aspect.

The present chapter provided a field-based analytical background to place in context subsequent experimental work in which various ecophysiological aspects of the metal tolerance mechanisms have been investigated.

It is possible to gain some insight into gross aspects of metal tolerance mechanisms by employing a biogeochemical approach and analyzing plant and soil samples collected from sites of a range of metal status. Such paired analyses can be used to quantify plant-soil relationships and hence reveal differing patterns of metal uptake and accumulation (Baker, 1981). Metal concentrations in the above-ground plant dry matter can thus suggest where accumulation or exclusion phenomena are involved. Biogeochemical investigations of plants colonizing metalliferous mine wastes in the U.K. and elsewhere in Europe (e.g. Ernst, 1965b; 1968; 1974; Shimwell & Laurie, 1972; Johnston & Proctor, 1977; Barry & Clark, 1978; Smith, 1979) provide some information on the heavy metal status of mine soils. However there are rather fewer reports of metal concentrations in the plant dry matter of *M. verna* and *T. alpestre*. Table 4.1 presents a summary of this information available in the literature.

Metal concentrations within plant parts may show substantial seasonal variation as a result of changes in the availability of metals within the soil system and the subsequent effects on uptake. Few studies on metalliferous soils have considered this aspect (see e.g. Shaw, 1984). The magnitude of these seasonal variations was studied in detail at one site with a view to gaining information about possible differences in behaviour between *M. verna* and *T. alpestre*.

Table 4.1 Heavy metal concentrations ($\mu g.g^{-1}$) in soils, M. verna and T. alpestre dry matter from various metalliferous sites

a - Soil

LOCALITY	Pb	Zn	Cd	REFERENCE
British Isles				
S.Pennines	(75-1500)	(1900-35000)	-	Shimwell & Laurie, 1972
	50000-80000	1550-1600	-	
	56200-95000	7100-14900	-	
	13000-67500	74000-181000	-	
Tideslow Rake	39970-53300	4220-4560	75-92	Shaw, 1984
Bonsall	(278)	(278)		Ingrouille & Smirnoff, 1986
Matlock High Tor	(1766)	(700)		Ingrouille & Smirnoff, 1986
Slaley	310	36000	-	Ernst, 1968
Grassington Moor	120-31250	320-1660	-	Barry & Clark, 1978
	8200-78100	4600-8900	-	Clark & Clark, 1981
	(311-21800)	(65-128)		
	2471-7583	282-493	3-5	Rother,
	434-1143	134-193	2-3	Millbank &
	373-407	74-120	1.7-2	Thornton, 1982
Killhope Wheel	(301)	(541)		Ingrouille & Smirnoff, 1986
Moorhouse	(382)	(1389)		Ingrouille & Smirnoff, 1986
Flinty Fell	(420)	(910)		Ingrouille & Smirnoff, 1986
Wylam	(185)	(181)		Ingrouille & Smirnoff, 1986
Grizedale	62-1300	460-14700	-	Ernst, 1968
Langthwaite	250	4420	-	Ernst, 1968
Mendips	2400-8500	10000-40000	-	Smith, 1979
Yarnbury mine	(1475)	(414)		Ingrouille & Smirnoff, 1986
Charterhouse	(1860)	(285)		Ingrouille & Smirnoff, 1986
Trelogan	460	2150		Johnston & Proctor, 1977
Europe				
Greece				
Leodikino-kato,	<100	<100		Kelepertsis & Andrulakis, 1983
Polykastro,	>1500	>1500		Kelepertsis & Andrulakis, 1983
Italy				
Alps	776	107700		Ernst, 1965

* Exchangeable values are in parenthesis

b - *M. verna*

British Isles				
S. Pennines	60-500	1100-7500	-	Shimwell & Laurie, 1972
	3734	1303	18	Martin & Coughtrey, 1982
Tideslow Rake	5000	700		Shaw, 1984
Slaley	17	2150	-	Ernst, 1968
Grinton	-	1695	-	Ernst, 1968
Grizedale	162-363	286-1600	-	Ernst, 1968
Copperthwaite	-	1320	-	Ernst, 1968
Langthwaite	41	870	-	Ernst, 1968
Grassington Moor	100-20000	160-2130	-	Barry & Clark, 1978
Grassington	174	3170	-	Ernst, 1968
Mendip Hills	-	820	-	Ernst, 1968
Trelogan	700-1580	2200-3200	-	Johnston & Proctor, 1977
Dolfrwynog	-	41	-	Ernst, 1968
Europe	-	3007	-	Ernst, 1965 b
Greece				
Leodikino-Kato,	-	380	-	Kelepertsis & Andrulakis, 1983
Polykastro,	7600	3300	-	Kelepertsis & Andrulakis, 1983
Italy				
Alps	226	3497	-	Ernst, 1965

c - *T. alpestre*

British Isles	1167	11758	37	Martin & Coughtrey, 1982
Pennines	100-2740	1200-25000	-	Shimwell & Laurie, 1972
Slaley	86	19050	-	Ernst, 1968
Grassington	77	23240	-	Ernst, 1968
Grinton	-	11330	-	Ernst, 1968
Copperthwaite	-	11280	-	Ernst, 1968
Grizedale	335-640	5500-8400	-	Ernst, 1968
Langthwaite	44	8070	-	Ernst, 1968
Mendip Hills	-	5760	-	Ernst, 1968
Europe	-	1400	-	Reeves & Brooks, 1983
	-	5220-39600	-	
	-	7757	-	Ernst, 1965 b
Germany	1600	10000	550	Ernst, 1975

4.2 MATERIALS AND METHODS

4.2.1 Analysis of plants & soils from different sites

Eight plants of either or both species were collected at random, together with replicate, 3 cm soil cores from the rooting zone from a range of eight calcareous lead mine spoil sites in the north and south Pennines. These sites were selected to provide soil with a wide range of heavy metal status (Table 4.2).

Prior to analysis, samples were stored overnight at 5°C then for each, the aerial plant parts were carefully washed in a dilute detergent solution (0.1% Decon 90) and thoroughly rinsed in distilled water, then oven-dried at 80-85°C overnight and left to cool in a desiccator. Up to 100 mg crushed plant material was weighed into porcelain crucibles and transferred into a cold muffle furnace set at 475°C and ashed overnight. After the samples were cooled, the ash was carefully moistened with a drop or two of distilled water directed down the side of the crucible, dissolved in 1 ml N/10 'Analar' HNO3 and washed into 15 ml capacity auto-sampler tubes and finally made up to 10 ml with distilled water. Samples were analyzed for Pb, Zn, Cd, and Ca by flame Atomic Absorption spectrophotometry (Perkin-Elmer 3030). For Ca, 9 ml 5% lanthanum chloride were added to one ml of each sample to minimize phosphate interference.

It did not prove possible to carry out similar analyses on root samples as these could not be cleaned free of all surface contamination with any degree of confidence.

The soil samples were freed of any root fragments and thoroughly mixed before being passed through a 2 mm stainless steel sieve. All samples were then air-dried in the laboratory. For estimation of exchangeable metals 5 g sub-samples of soil were placed into 100 ml centrifuge tubes with 25 ml 1M ammonium acetate pH 7, shaken for

Table 4.2 Sampling sites

Site	O.S. Grid reference	Altitude	pH	Species
Black Rocks	SK 296559	800'	7.2	*M. verna*, *T. alpestre*
Bonsall Moor	SK 249593	1100'	6.6	*M. verna*, *T. alpestre*
Bradford Dale	SK 218643	450'	7.5	*M. verna*, *T. alpestre*
Dirtlow Rake	SK 155821	1050'	7.3	*M. verna*
Whitesike	NY 751425	1450'	6.8	*M. verna*, *T. alpestre*
Grattondale	SK 205604	850'	7.3	*M. verna*
Tideslow Rake	SK 159778	1100'	7.7	*M. verna*
Wensleydale	SK 265603	800'	7.3	*M. verna*, *T. alpestre*

4 hours on an end-over-end shaker and centrifuged for 10 minutes at 4000 rpm at 20°C. After filtration through Whatman No.1 papers into appropriately labelled polythene bottles, the filtrates were analyzed for Pb, Zn, Cd and Ca. For 'total' metal estimation 0.5 g air-dried soil samples, which had been ground down by pestle and mortar to pass through 80 mesh sieve, were weighed into glass boiling tubes and 25 ml 4M 'Analar' HNO3 added. The digests were allowed to stand for a few minutes in order to allow any effervescence to subside and then the samples were digested further in a water bath at 60°C for two hours, allowed to cool and then filtered into labelled polythene bottles. The digests were analyzed for total Pb, Zn, Cd, and Ca, as above.

4.2.2 Seasonal variation in the accumulation of lead, zinc and cadmium

A plot of 10 x 10 m was chosen on mine spoil at the Dovegang lead mine, Derbyshire, (Grid ref. SK 287556). The site is described in more detail in Chapter 6. The area chosen was observed to have rather uniform topography, with both bare areas and patches of continuous vegetational cover and presence of the two species at high enough frequency to enable repeated sampling. The corners were marked out to allow relocation on successive sampling occasions. On each visit, not less than 12 points within the sampling grid were located using random-number tables, and for each species the nearest plant to the point was collected together with a soil core from the plant rhizosphere. Soil and plants were stored overnight at 5°C prior to analysis.

The samples were prepared for analysis by using the procedure described above with the exception that when possible the above-ground plant parts were divided into upper (young) and lower (older) shoot parts for M. verna, and for T. alpestre leaf rosette, stem and inflorescence or fruit. Total soil metal concentrations were estimated on the first occasion only. Exchangeable metals however were measured at every sampling. Sampling was carried out on 6 occasions between 31.5.1983 and 17.5.1984. Climatic information (Figure 4.1) was abstracted from weather data produced by the Meteorological Office (Bracknell, Berks) for Ashover (SK 539128, 178 m a.s.l.) about 5 miles north of the sampling site.

4.3 RESULTS

4.3.1 Analysis of soils & plants from different sites

Soils

t-tests of differences between means showed no significant differences between metal concentrations of soil samples collected for each species suggesting that the soil data for each site could be pooled. Therefore the data presented in Table 4.3 represent the means of 16 samples for each site. For each site, mean total and exchangeable soil concentrations of Pb, Zn, Cd and Ca are presented.

The analyses suggest widescale variation in total content between the soils from the eight sites. The exchangeable metal concentrations however, show rather less variation and the rank order of sites on this basis does not always coincide with rank order on total metal contents. Ca status is high for all sites other than Whitesike and there is little variation between most sites in extractable Ca.

Figure 4.1 Climatic data for sampling period June 1983 to May 1984.

a - Snow: Mean number of days snow lying at 0900 h.

b - Temperature: Mean monthly temperature

c - Rainfall: Monthly mean.

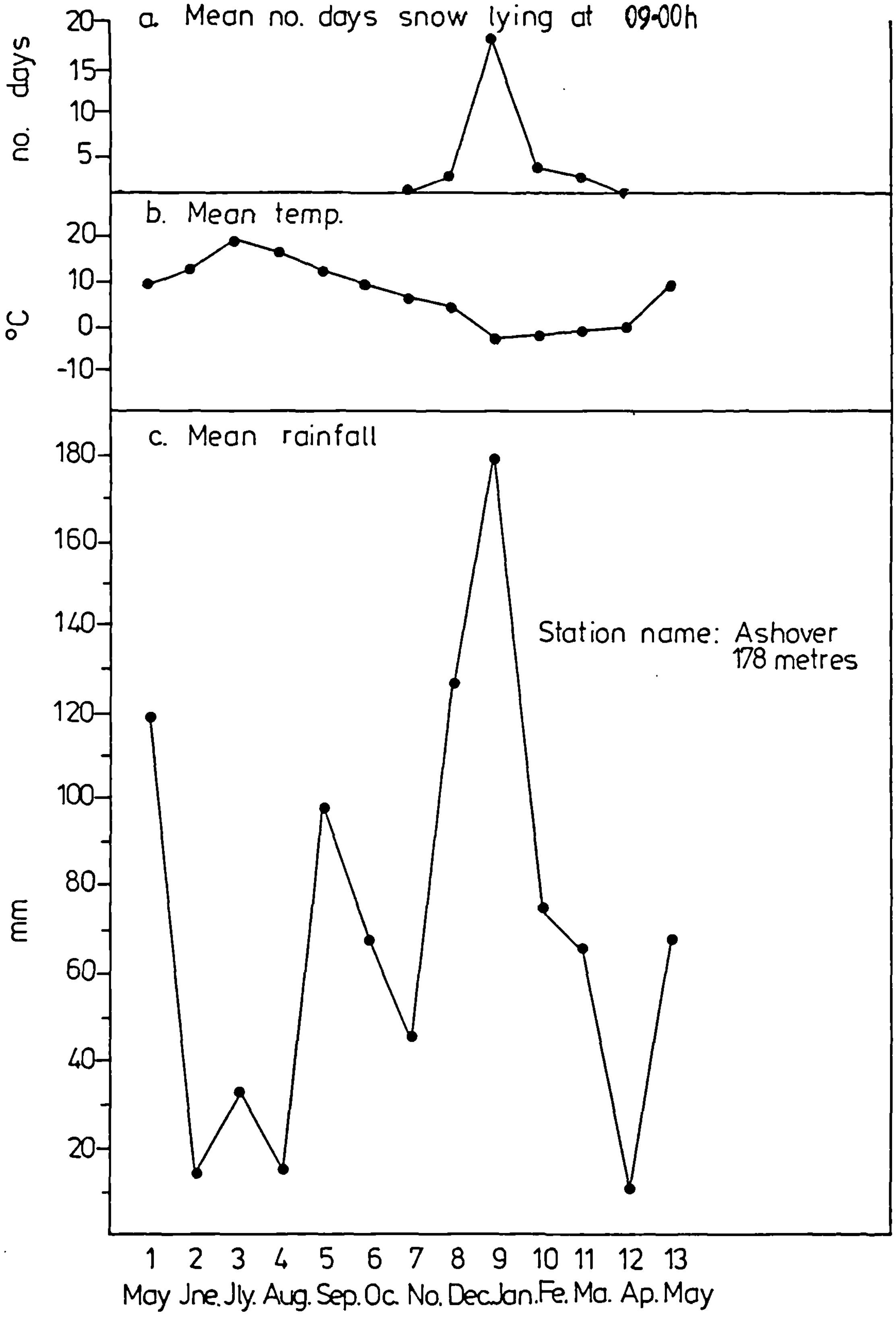


Table 4.3 Total and exchangeable soil metal concentrations $\mu g.g^{-1}$ at the eight lead mine sites sampled. (means ± S.E. , n = 16).

LOCALITY	Pb	Zn	Cd	Ca
Black Rocks	84920 ± 7020	16240 ± 1610	91 ± 7	88350 ± 4090
	(2820 ± 340)	(2650 ± 290)	(30 ± 5)	(420 ± 40)
Bonsall Moor	17400 ± 4440	20160 ± 4590	292 ± 70)	149370 ± 9250
	(970 ± 260)	(390 ± 50)	(16 ± 3)	(800 ± 50)
Bradford Dale	59150 ± 5290	10730 ± 1111	86 ± 7	28120 ± 1890
	(3870 ± 440)	(450 ± 60)	(20 ± 3)	(750 ± 110)
Dirtlow Rake	55560 ± 3480	3640 ± 552	36 ± 6	112490 ± 6950
	(1510 ± 140)	(110 ± 15)	(5 ± 1)	(660 ± 60)
Whitesike	28030 ± 6890	5890 ± 960	12 ± 1	4890 ± 790
	(3040 ± 480)	(650 ± 130)	(4 ± 1)	(580 ± 60)
Grattondale	7730 ± 1110	25700 ± 3610	280 ± 42	63670 ± 3000
	(340 ± 70)	(730 ± 110)	(70 ± 10)	(880 ± 90)
Tideslow Rake	21330 ± 1870	6420 ± 340	124 ± 5	198980 ± 9130
	(850 ± 180)	(270 ± 20)	(12 ± 1)	(630 ± 20)
Wensleydale	8300 ± 950	14720 ± 1310	162 ± 16	52530 ± 6690
	(630 ± 90)	(810 ± 110)	(50 ± 4)	(1270 ± 120)

* Figures in parenthesis represent the exchangeable metals

Plants (Table 4.4)

For all five sites at which both species occur Pb concentrations in M. verna shoots were greater than for T. alpestre. The opposite was true for Zn, Cd, and Ca. In order to compensate for the effect of changes in soil metal availability in making site comparisons, the [shoot]:[soil] concentration ratios, termed relative concentration ratios (RCR) were calculated. These are presented in Figure 4.2. The highest Pb RCR was recorded in M. verna from Tideslow Rake, with three other populations showing higher RCR than any of the five T. alpestre populations sampled. The lowest RCR was found in T. alpestre from Bradford Dale, whilst the lowest ratio for M. verna was recorded from the Whitesike population. The Zn RCR for M. verna from the different populations showed remarkably little variation by comparison with those for T. alpestre, which were more variable and consistently larger.

In spite of the fact that the lowest metal concentrations of all were found in the soils and plants at Whitesike, the highest Cd RCR was found in M. verna from this site and the second highest for T. alpestre. Results also showed that Cd RCR for the latter species were generally greater than for M. verna, suggesting some similarity in behaviour for Cd and Zn. Figures 4.3a, b, c, a1, a2 & c1show the relationships between shoot metal concentrations and exchangeable and total soil metals for all sites. For M. verna, in all sites considered separately, the relationships were significantly positive between soil exchangeable Pb levels and Pb concentrations in the shoot. Consequently the relationships were highly significant (positive) ($p<0.001$) for all samples pooled together. In spite of the fact that in half of the populations no significant correlations were apparent, the overall relationship between total and shoot Pb concentrations of M. verna was

Table 4.4: Concentrations of lead, zinc, cadmium and calcium in aerial plant parts of _M. verna_ and _T. alpestre_, sampled at eight Pennine mine sites ($\mu g.g^{-1}$ dry matter; means ± S.E., n = 8)

	(_M.verna_)				(_T. alpestre_)			
LOCALITY	Pb	Zn	Cd	Ca	Pb	Zn	Cd	Ca
*								
B. Rock	2635 ± 381	3603 ± 444	37 ± 5	13317 ± 1450	662 ± 202	19079 ± 2485	53 ± 7	30195 ± 6536
B. Moor	692 ± 194	528 ± 123	7 ± 2	35686 ± 1955	203 ± 97	13041 ± 1895	164 ± 24	40316 ± 6189
B. Dale	1464 ± 411	1331 ± 128	20 ± 2	15398 ± 1371	166 ± 64	21147 ± 1889	35 ± 5	23253 ± 1562
D. L. Rake	1040 ± 125	377 ± 31	5 ± 1	25386 ± 2119				
Whitesike	1683 ± 641	1645 ± 344	6 ± 2	10196 ± 1169	222 ± 36	17562 ± 2833	9 ± 1	23773 ± 4061
G. Dale	117 ± 16	742 ± 90	21 ± 3	14380 ± 682				
T. S. Rake	765 ± 175	1333 ± 253	12 ± 2	36050 ± 6239				
Wensleydale	104 ± 19	1168 ± 234	15 ± 3	17531 ± 3011	57 ± 11	16216 ± 2330	95 ± 12	35894 ± 4021

* For full names see Table 4.2

Figure 4.2 Relative concentration ratios ([shoot]:[soil], for Pb, Zn & Cd in M. verna and T. alpestre from different sites in the Pennines. Mean values are plotted ± S.E.

M. verna

a - Black Rocks
b - Bonsall Moor
c - Bradford Dale
d - Dirtlow Rake
e - Whitesike
f - Grattondale
g - Tideslow Rake
h - Wensleydale

T. alpestre

a - Black Rocks
b - Bonsall Moor
c - Bradford Dale
e - Whitesike
h - Wensleydale

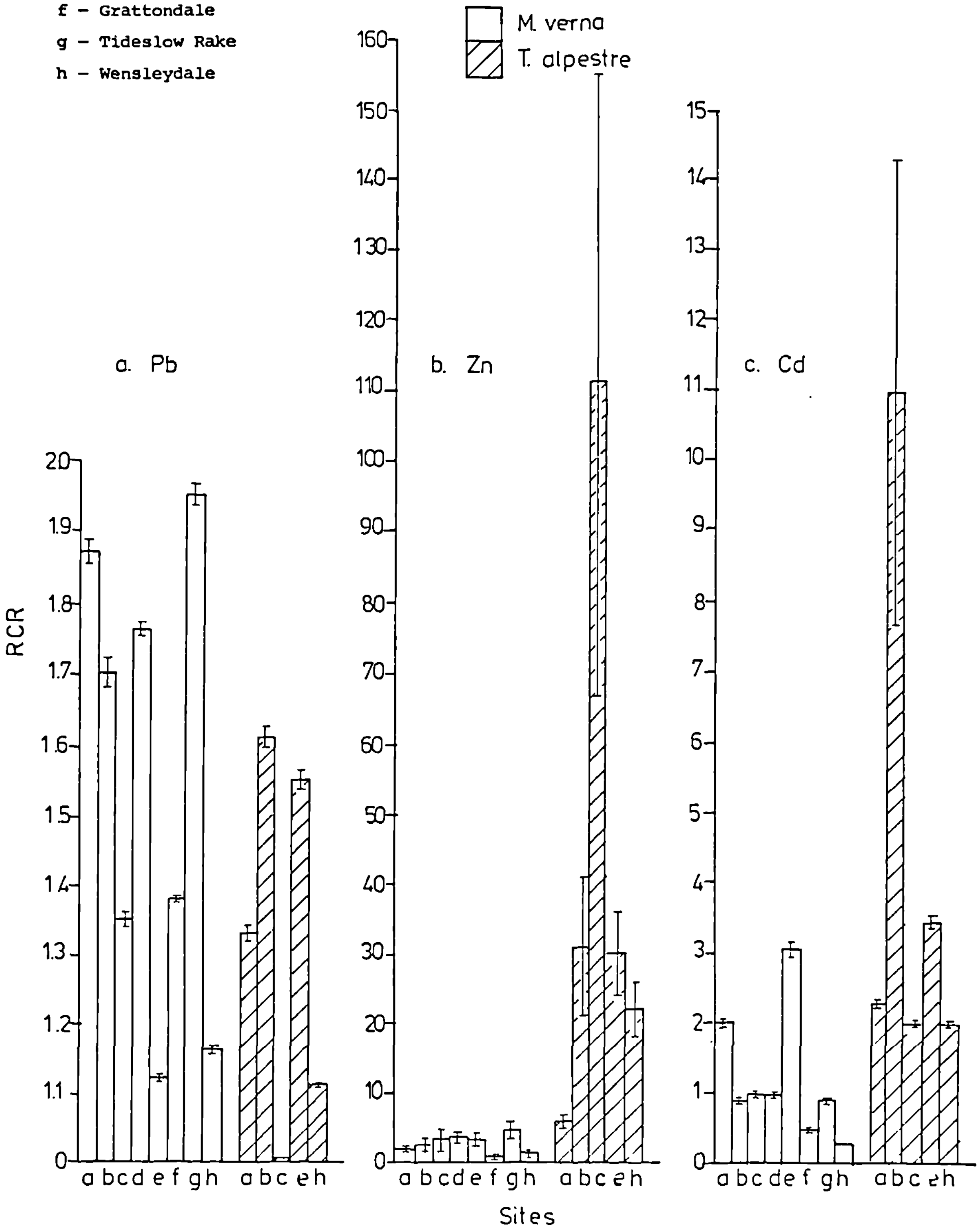


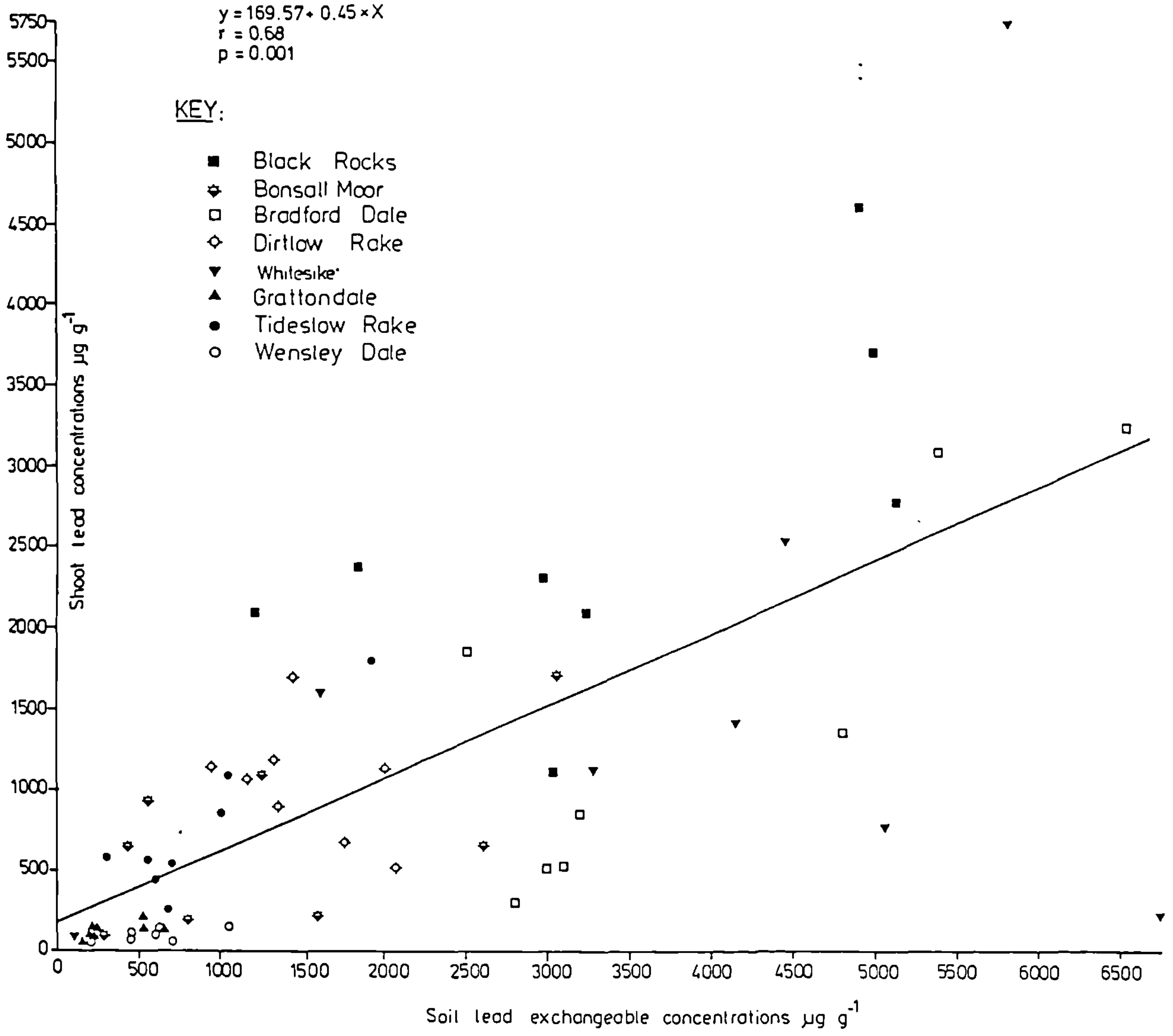
Figure 4.3a, b, c, a_1, b_1 & c_1 The relationships between shoot (*M. verna* and *T. alpestre*) metal (Pb, Zn & Cd) concentrations and exchangeable and total soil metals from eight sites in the Pennines. Linear regressions have been fitted and the equations are shown together with the product-moment correlation coefficients (r) and their p-values.

KEY: *M. verna*

■ Black Rocks
✧ Bonsall Moor
□ Bradford Dale
◇ Dirtlow Rake
▼ Whitesike
▲ Grattondale
● Tideslow Rake
○ Wensley Dale

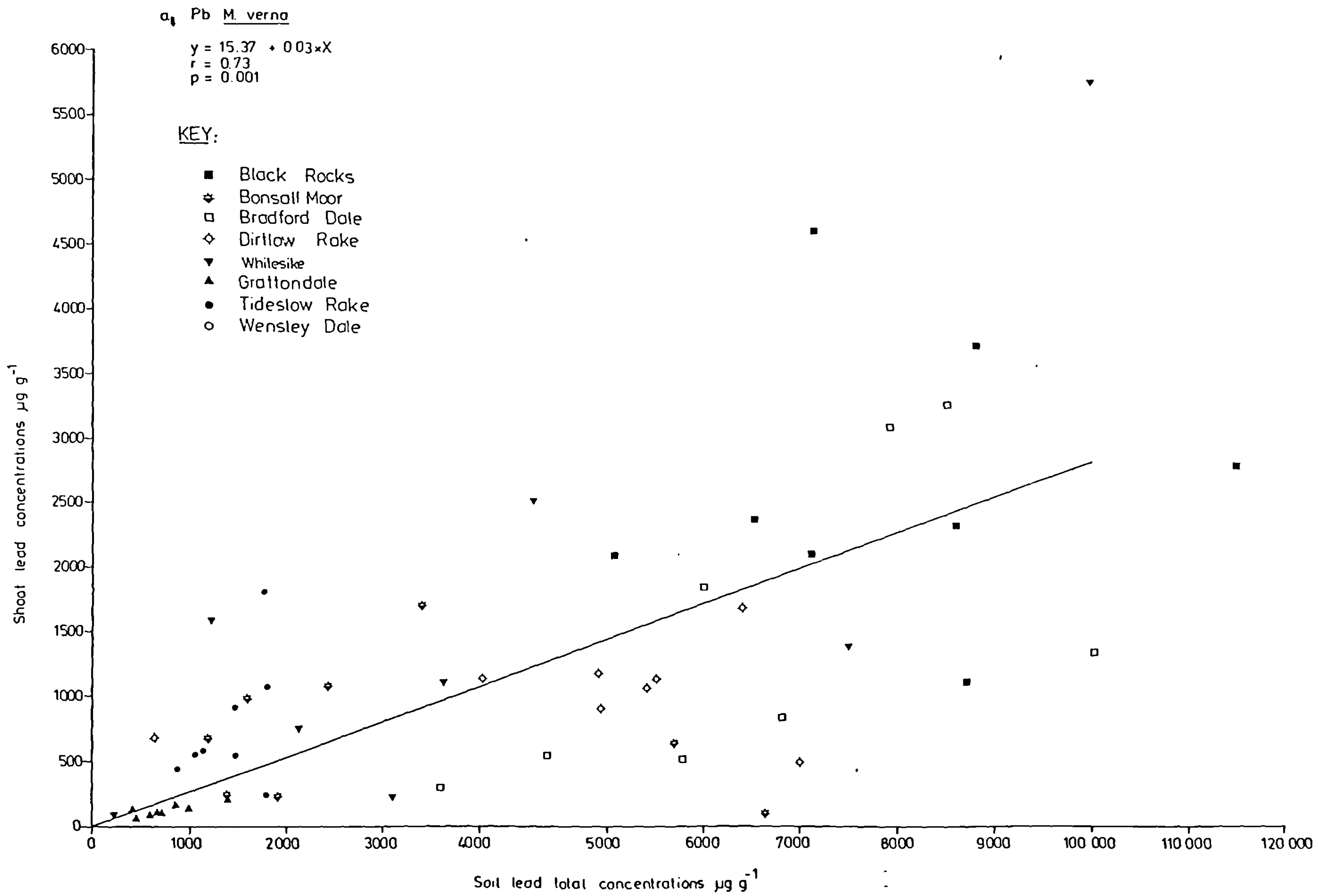
KEY *T. alpestre*

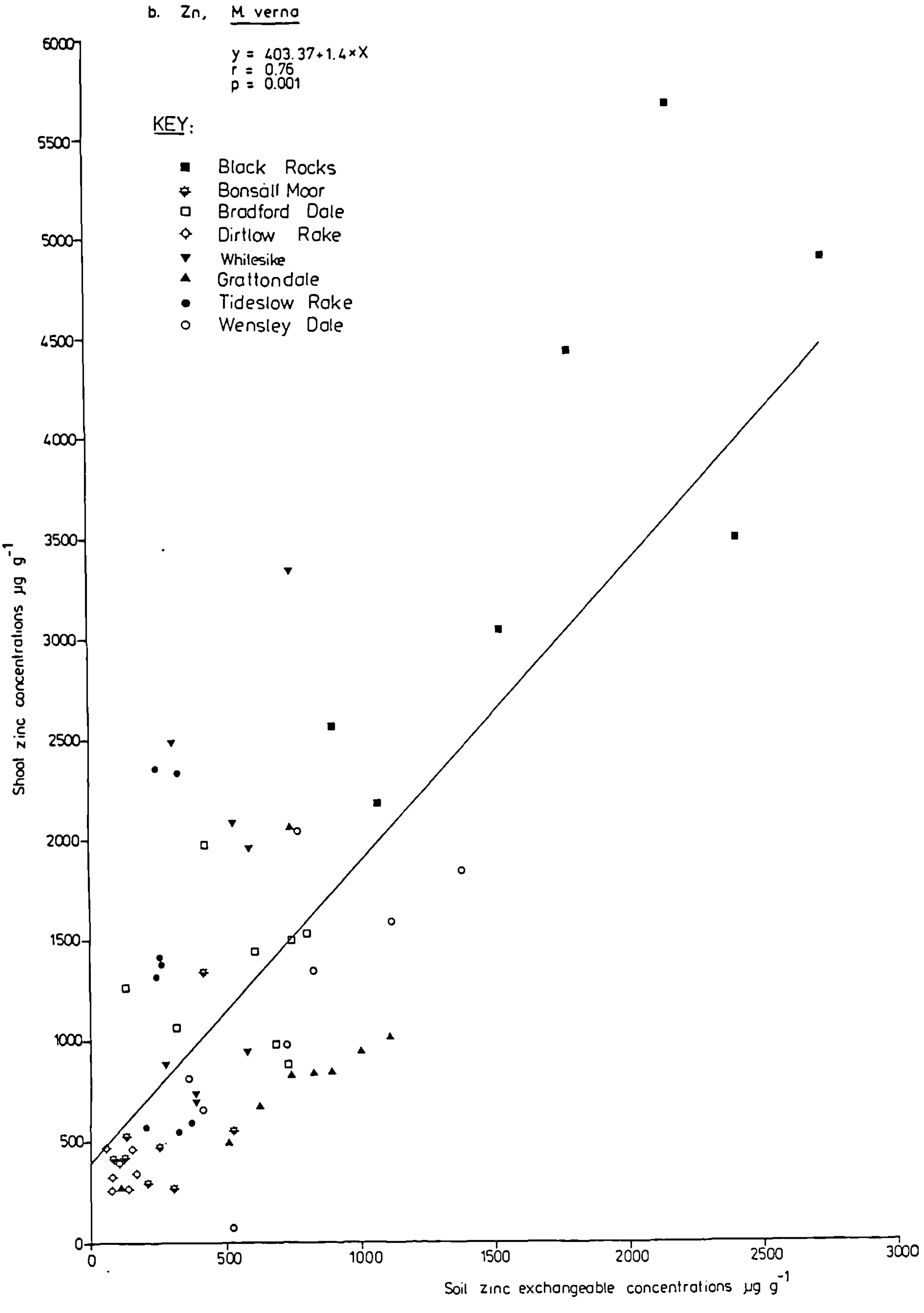
■ Black Rocks
✧ Bonsall Moor
□ Bradford Dale
▼ Whitesike
○ Wensley Dale

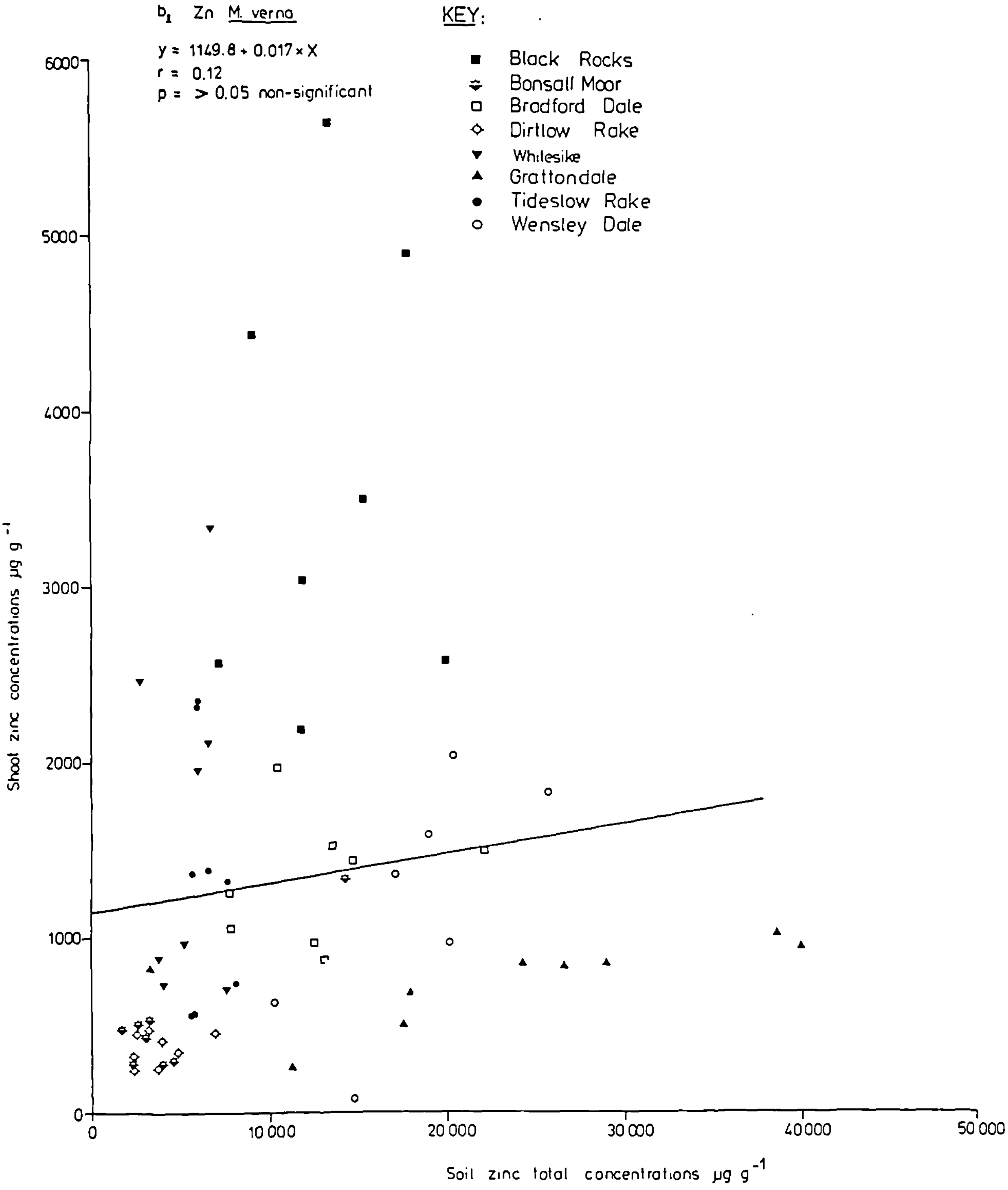


a. Pb, *M. verna*

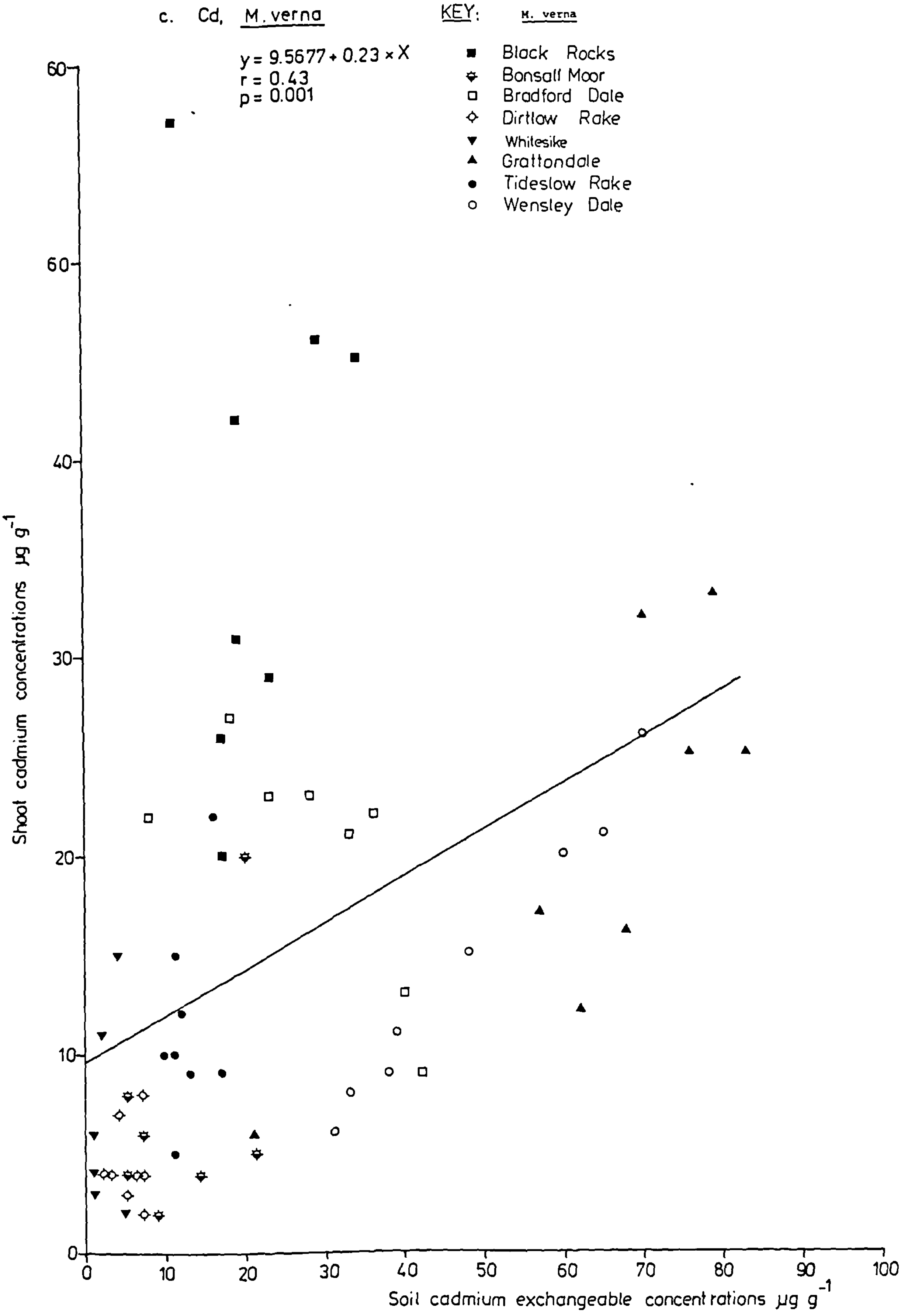
y = 169.57 + 0.45 × X
r = 0.68
p = 0.001
KEY:
■ Black Rocks
Bonsall Moor
□ Bradford Dale
◇ Dirtlow Rake
▼ Whitesike
▲ Grattondale
● Tideslow Rake
○ Wensley Dale
Shoot lead concentrations µg g⁻¹
5750
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
6500
Soil lead exchangeable concentrations µg g⁻¹

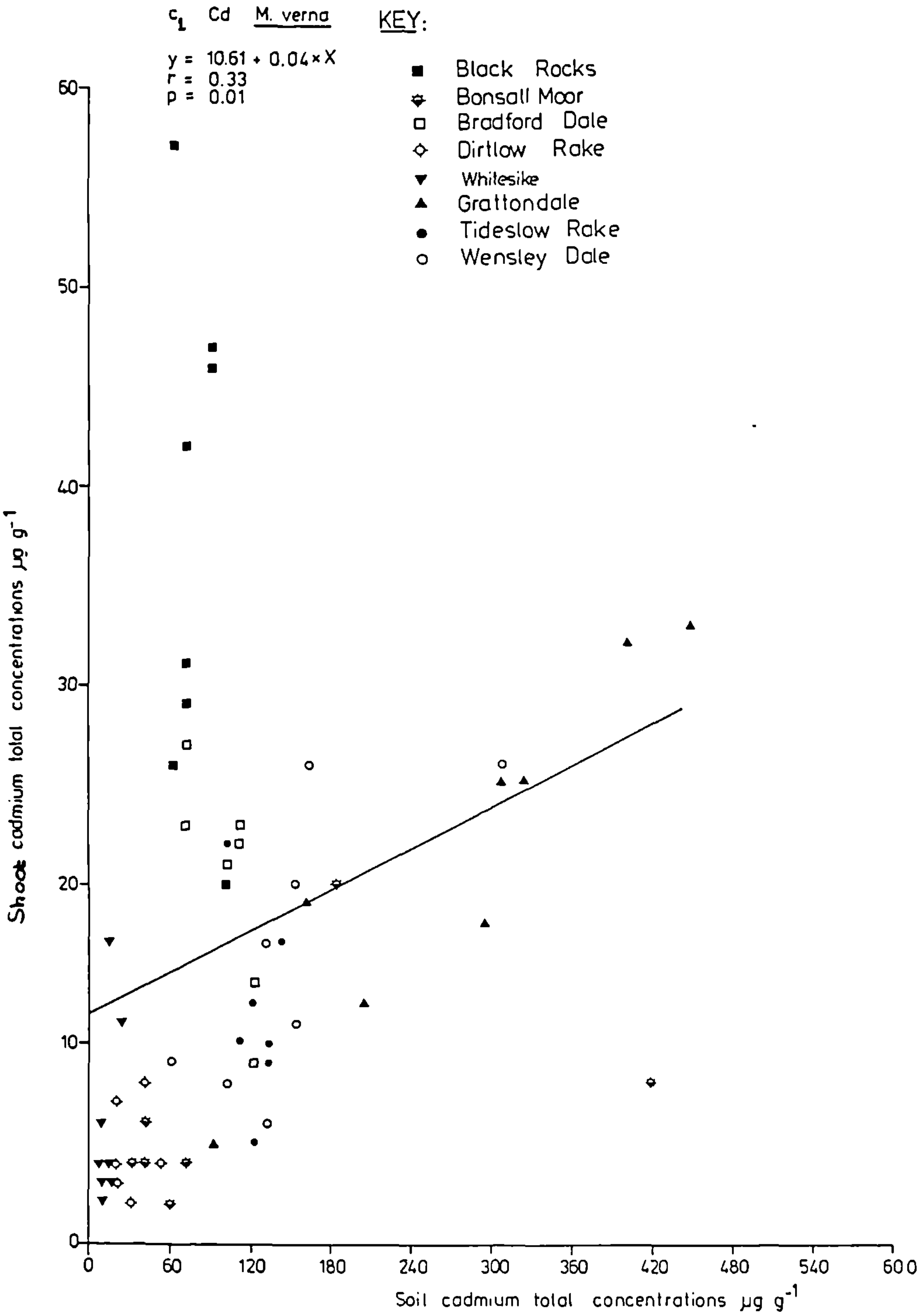

a₁ Pb M. verna
y = 15.37 + 0.03×X
r = 0.73
p = 0.001
KEY:
■ Black Rocks
✡ Bonsall Moor
□ Bradford Dale
◇ Dirtlow Rake
▼ Whitesike
▲ Grattondale
● Tideslow Rake
○ Wensley Dale
Shoot lead concentrations µg g-1
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
100 000
110 000
120 000
Soil lead total concentrations µg g-1

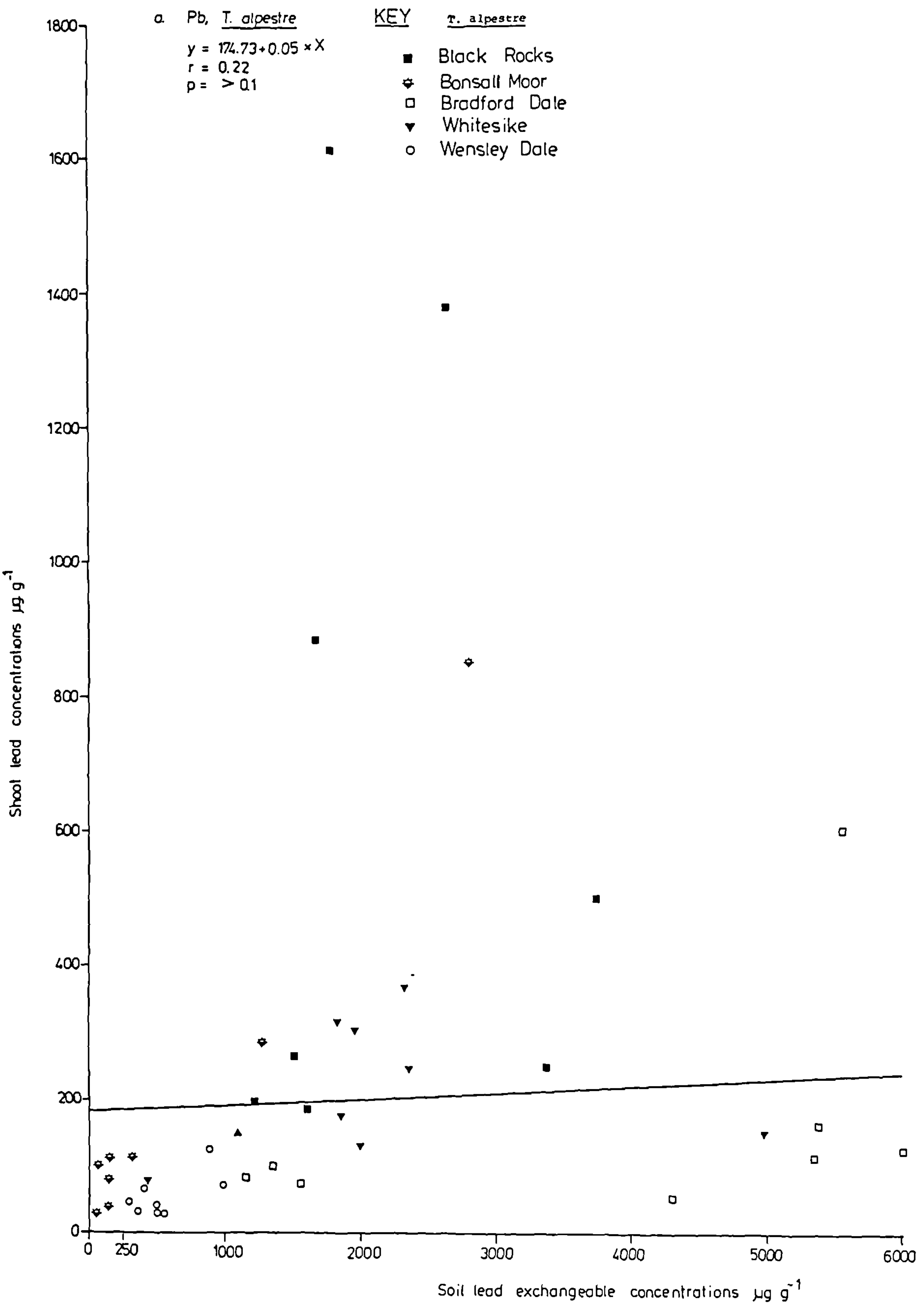

b. Zn, M. verna
y = 403.37+1.4×X
r = 0.76
p = 0.001
KEY:
■ Black Rocks
Bonsall Moor
□ Bradford Dale
◇ Dirtlow Rake
▼ Whitesike
▲ Grattondale
● Tideslow Rake
○ Wensley Dale
Shoot zinc concentrations µg g⁻¹
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
Soil zinc exchangeable concentrations µg g⁻¹
0
500
1000
1500
2000
2500
3000

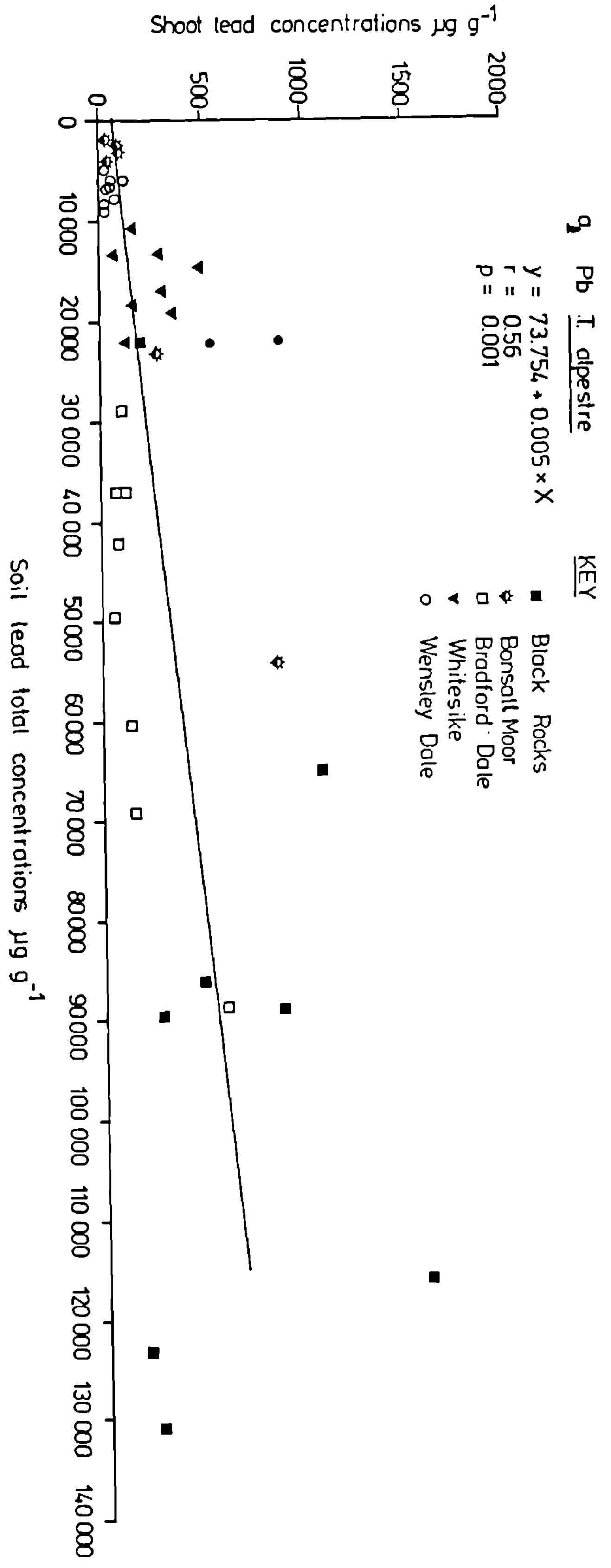


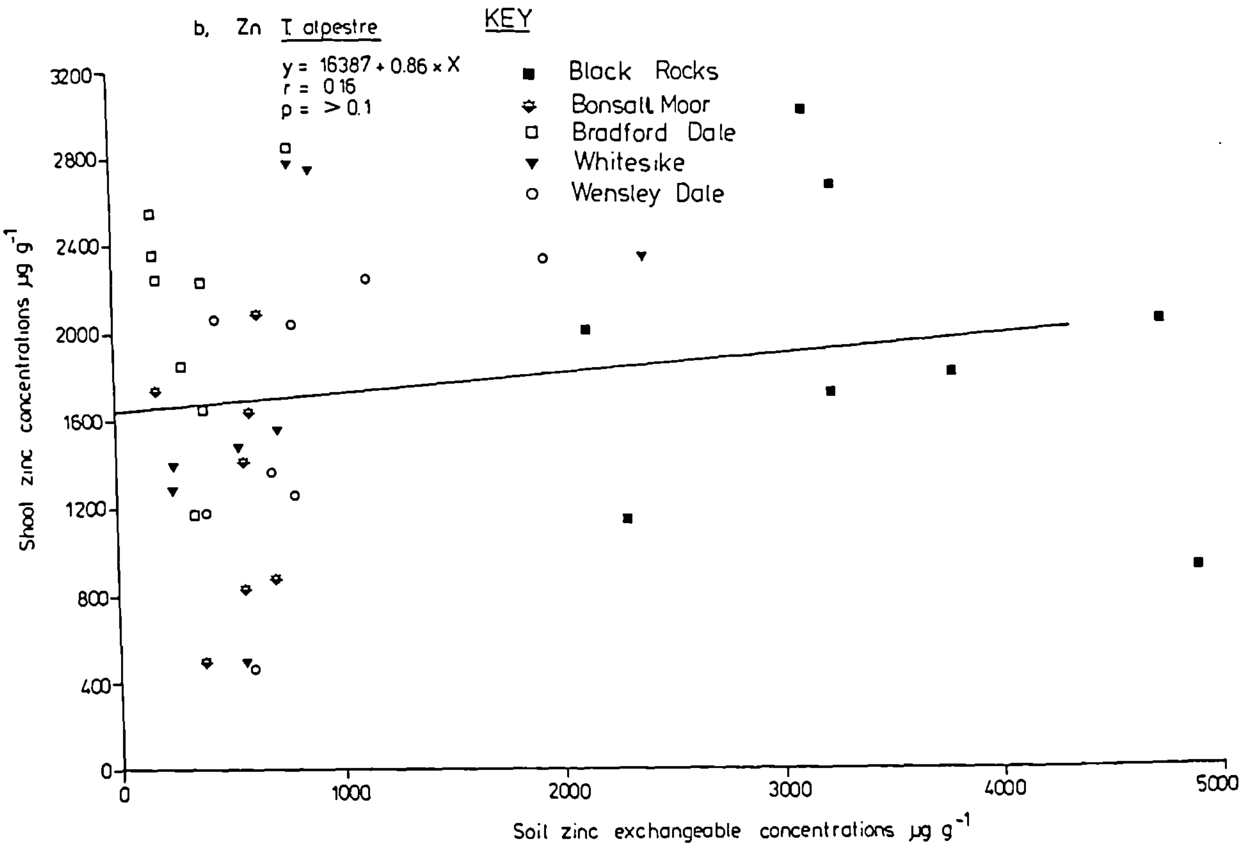
b1 Zn M. verna
y = 1149.8 + 0.017 × X
r = 0.12
p = > 0.05 non-significant
KEY:
■ Black Rocks
Bonsall Moor
□ Bradford Dale
◇ Dirtlow Rake
▼ Whitesike
▲ Grattondale
● Tideslow Rake
○ Wensley Dale
Shoot zinc concentrations µg g -1
6000
5000
4000
3000
2000
1000
0
0
10 000
20 000
30 000
40 000
50 000
Soil zinc total concentrations µg g -1

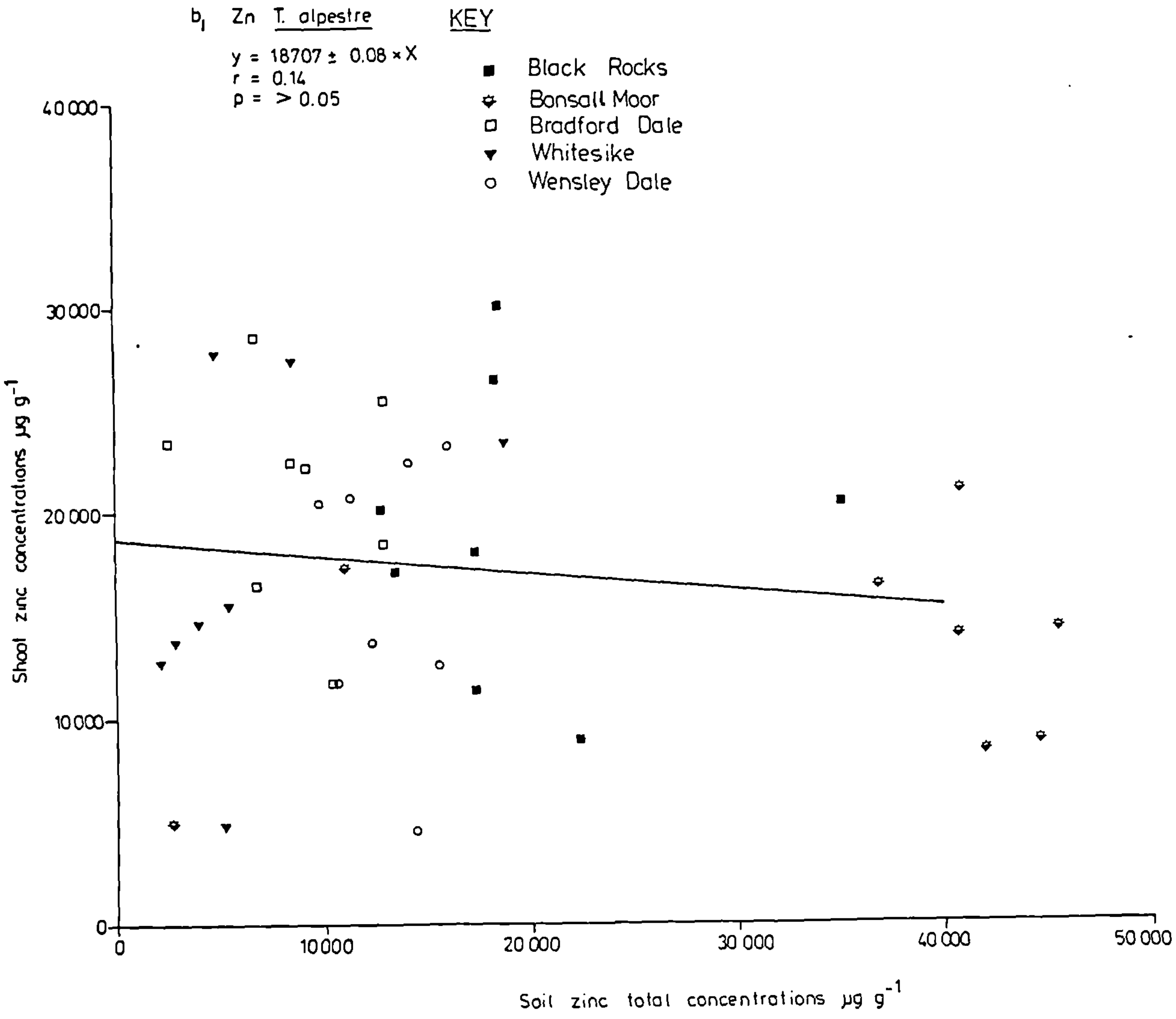

c. Cd, M. verna
y = 9.5677 + 0.23 × X
r = 0.43
p = 0.001
KEY: M. verna
■ Black Rocks
Bonsall Moor
□ Bradford Dale
◇ Dirtlow Rake
▼ Whitesike
▲ Grattondale
● Tideslow Rake
○ Wensley Dale
Shoot cadmium concentrations µg g-1
60
60
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Soil cadmium exchangeable concentrations µg g-1

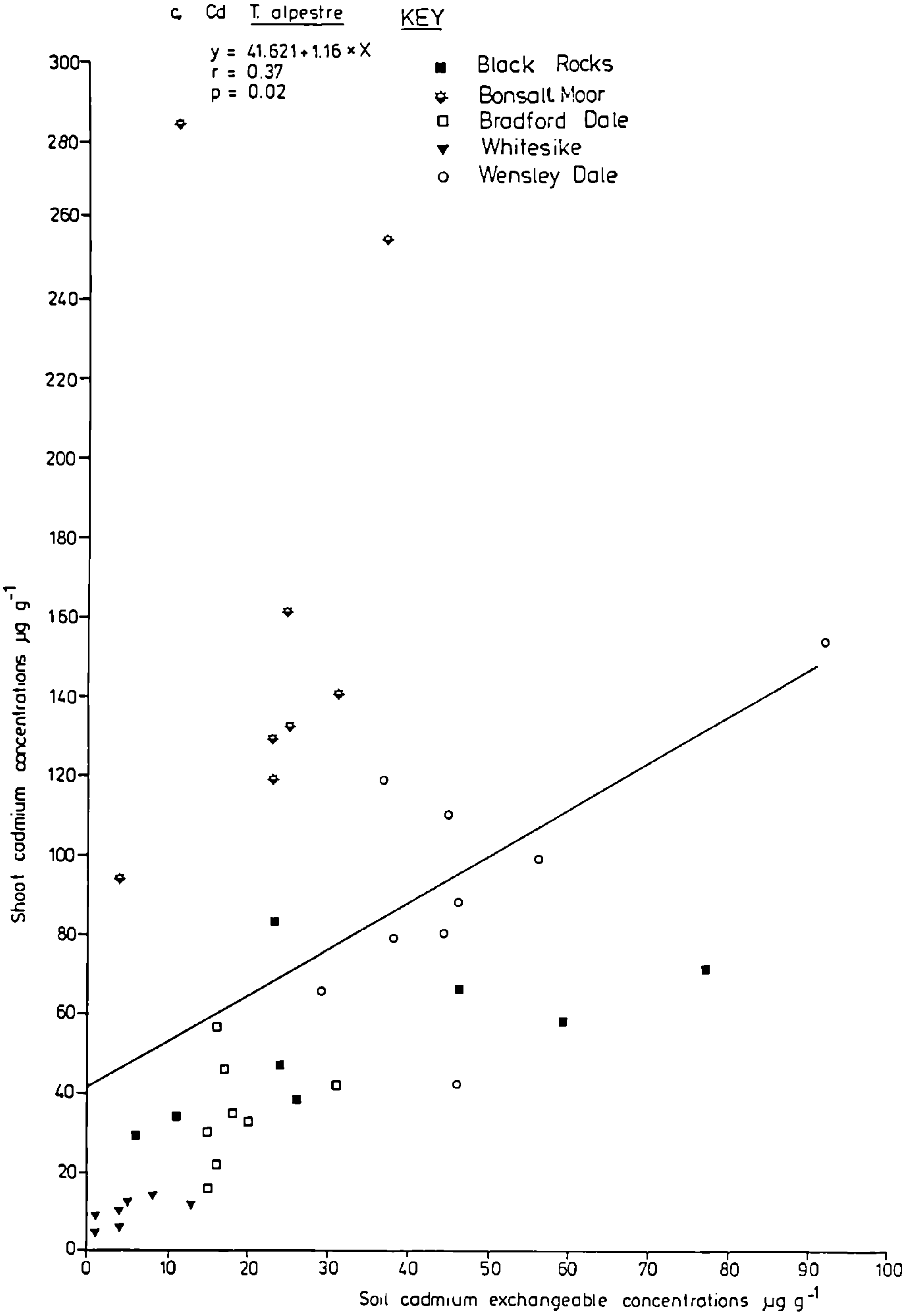

c_1 Cd M. verna
y = 10.61 + 0.04×X
r = 0.33
p = 0.01
KEY:
■ Black Rocks
Bonsall Moor
□ Bradford Dale
◇ Dirtlow Rake
▼ Whitesike
▲ Grattondale
● Tideslow Rake
○ Wensley Dale
Shoot cadmium total concentrations µg g^{-1}
Soil cadmium total concentrations µg g^{-1}
0
10
20
30
40
50
60
0
60
120
180
240
300
360
420
480
540
600

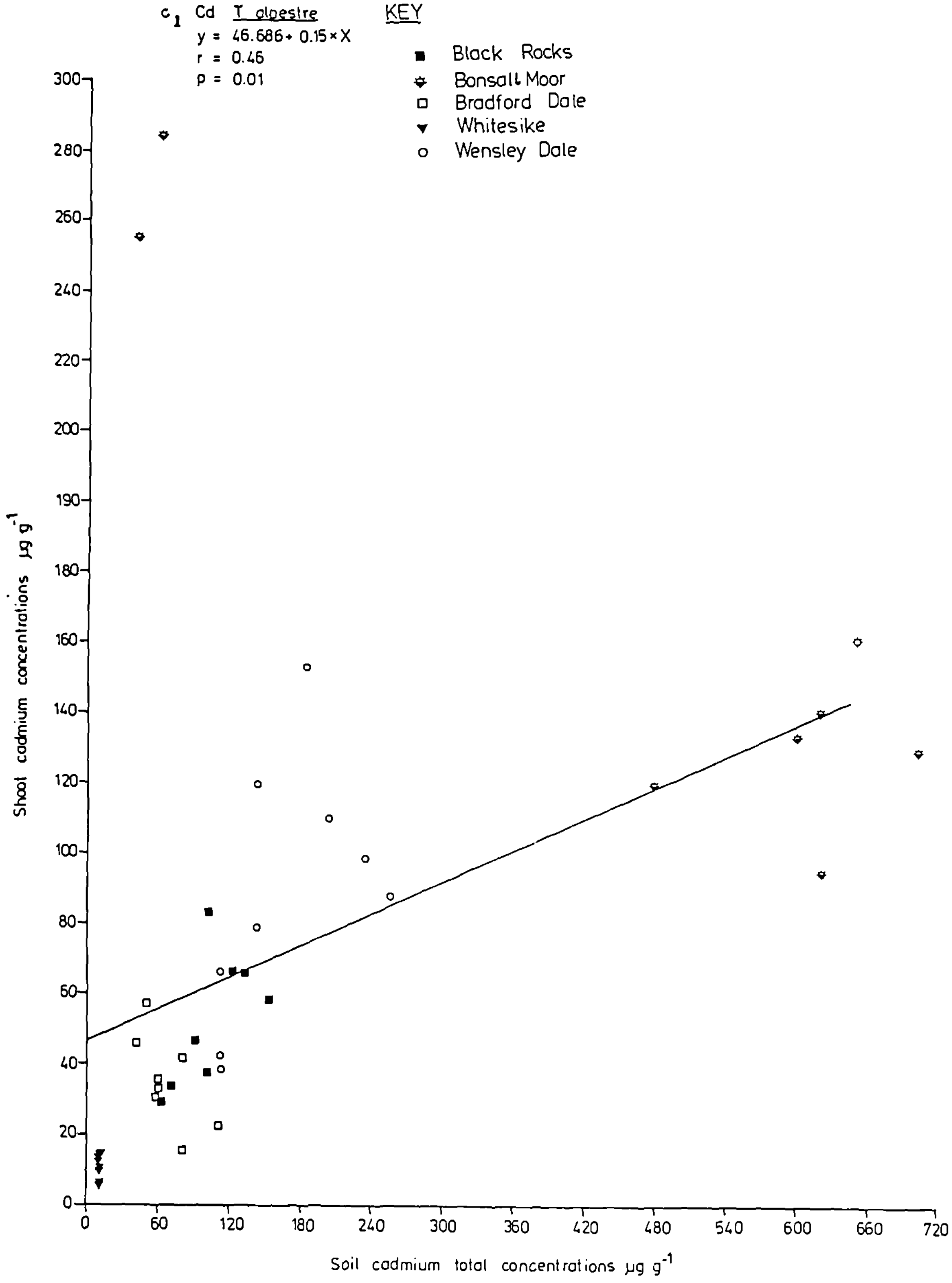

a. Pb, T. alpestre
y = 174.73+0.05 ×X
r = 0.22
p = > 0.1
KEY
T. alpestre
■ Black Rocks
✧ Bonsall Moor
□ Bradford Dale
▼ Whitesike
○ Wensley Dale
Shoot lead concentrations µg g⁻¹
1800
1600
1400
1200
1000
800
600
400
200
0
0
250
1000
2000
3000
4000
5000
6000
Soil lead exchangeable concentrations µg g⁻¹


a Pb T. alpestre
y = 73.754 + 0.005 × X
r = 0.56
p = 0.001
KEY
■ Black Rocks
✡ Bonsall Moor
□ Bradford Dale
▼ Whitesike
o Wensley Dale
Shoot lead concentrations μg g-1
0
500
1000
1500
2000
0
10 000
20 000
30 000
40 000
50 000
60 000
70 000
80 000
90 000
100 000
110 000
120 000
130 000
140 000
Soil lead total concentrations μg g-1


b, Zn T. alpestre
y = 16387 + 0.86 × X
r = 0 16
p = > 0.1
KEY
■ Black Rocks
⬘ Bonsall Moor
□ Bradford Dale
▼ Whitesike
○ Wensley Dale
Shoot zinc concentrations µg g-1
3200
2800
2400
2000
1600
1200
800
400
0
1000
2000
3000
4000
5000
Soil zinc exchangeable concentrations µg g-1


b1 Zn T. alpestre
y = 18707 ± 0.08 × X
r = 0.14
p = > 0.05
KEY
■ Black Rocks
Bonsall Moor
□ Bradford Dale
▼ Whitesike
○ Wensley Dale
Shoot zinc concentrations µg g-1
40 000
30 000
20 000
10 000
0
0
10 000
20 000
30 000
40 000
50 000
Soil zinc total concentrations µg g-1


c. Cd T. alpestre
y = 41.621 + 1.16 × X
r = 0.37
p = 0.02
KEY
■ Black Rocks
✧ Bonsall Moor
□ Bradford Dale
▼ Whitesike
○ Wensley Dale
Shoot cadmium concentrations µg g⁻¹
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
0
10
20
30
40
50
60
70
80
90
100
Soil cadmium exchangeable concentrations µg g⁻¹


c1 Cd T. alpestre
y = 46.686+ 0.15×X
r = 0.46
p = 0.01
KEY
■ Black Rocks
✧ Bonsall Moor
□ Bradford Dale
▼ Whitesike
○ Wensley Dale
Shoot cadmium concentrations µg g-1
Soil cadmium total concentrations µg g-1
0
20
40
60
80
100
120
140
160
180
190
200
220
240
260
280
300
0
60
120
180
240
300
360
420
480
540
600
660
720

significant. In five of the M. verna populations considered separately the relationships between soil exchangeable and Zn concentrations of the shoot were significantly positive and the overall correlation highly significant ($p<0.001$). In contrast, no overall significant results were obtained for total soil Zn and shoot concentrations. In two M. verna populations, negative relationships were found between soil exchangeable and Cd concentrations of the shoot, with four correlations significant at ($p<0.001$) and two non-significant relationships. Consequently, an overall positive correlation was detected. Although for half of the populations the results for total Cd were not significant, the overall relationship was again positive and significant.

In two T. alpestre populations the relationships between soil exchangeable and plant Pb concentrations were significantly positive at ($p<0.001$), but the overall correlation was not significant. However for total Pb the result was positive and significant for all the samples pooled together, although in three populations the individual correlations were not. For exchangeable Zn, only two T. alpestre populations showed positive results and none were detected for total Zn in any of the populations; the overall relationships were not significant in both cases. For Cd, three T. alpestre populations showed positive relationships at $p<0.001$, but the overall result was positive at only $p<0.02$.

For total Cd the overall relationship was positive and significant, at $p<0.01$, although two of the populations showed no clear relationships.

4.3.2 Seasonal variation in the lead, zinc and cadmium concentrations in soils and plants

A comparison of soil samples collected from the rooting zones of M. verna and T. alpestre and from bare (uncolonized) soil at the Dovegang lead mine site at which this study was performed, suggested that there were no significant differences (t-tests) in total and exchangeable metals in these three situations, on any one scoring date. The summarized data are presented in Table 4.5a.

As far as the three metals Pb, Zn and Cd are concerned the data demonstrate the relatively homogeneous nature of the ground within the chosen 100 m^2 sampling area. For each metal there was some change in extractable concentration with time. Exchangeable Pb concentration was at its highest in July and August 1983 and at its lowest level in December. Zn and Cd concentrations were less variable; the highest values for both were in July and October.

Data presented in Table 4.5b show concentration changes in M. verna and T. alpestre shoots over the sampling period for Pb, Zn and Cd. The relative concentration ratios (RCR) were also calculated and presented in Figure 4.4. For Pb in both species there was a rise in RCR in December with lowest values in July for M. verna and in August for T. alpestre. RCR's were in general low throughout the summer for both species. Both species showed the same trend for Pb, and in December M. verna had a higher RCR than T. alpestre. In both plants the changes in Zn-RCR were generally similar to those for Pb, with T. alpestre showing the second highest value in June and M. verna peaking in May. Following low values of RCR in August, the two plants showed a rise during the winter (December sampling). T. alpestre had the higher Zn RCR on every scoring date and also showed the largest changes in Zn RCR. Although the Cd RCR showed no large peak it did show differences

Table 4.5 Metal concentrations in soil and plants from Dovegang lead mine spoil

4.5a Mean, S. E. & range of background total soil heavy metal concentrations in Dovegang lead mine spoil; ($\mu g.g^{-1}$).

Pb	Zn	Cd	Ca	No. of samples
35690 ± 1330	45350 ± 2000	600 ± 30	73550 ± 4900	48
16700 - 56000	10500 - 76900	170 - 1110	25400 - 169800	

Seasonal variation in the exchangeable heavy metal concentrations ($\mu g.g^{-1}$) of soil (Mean, S. E. & range)

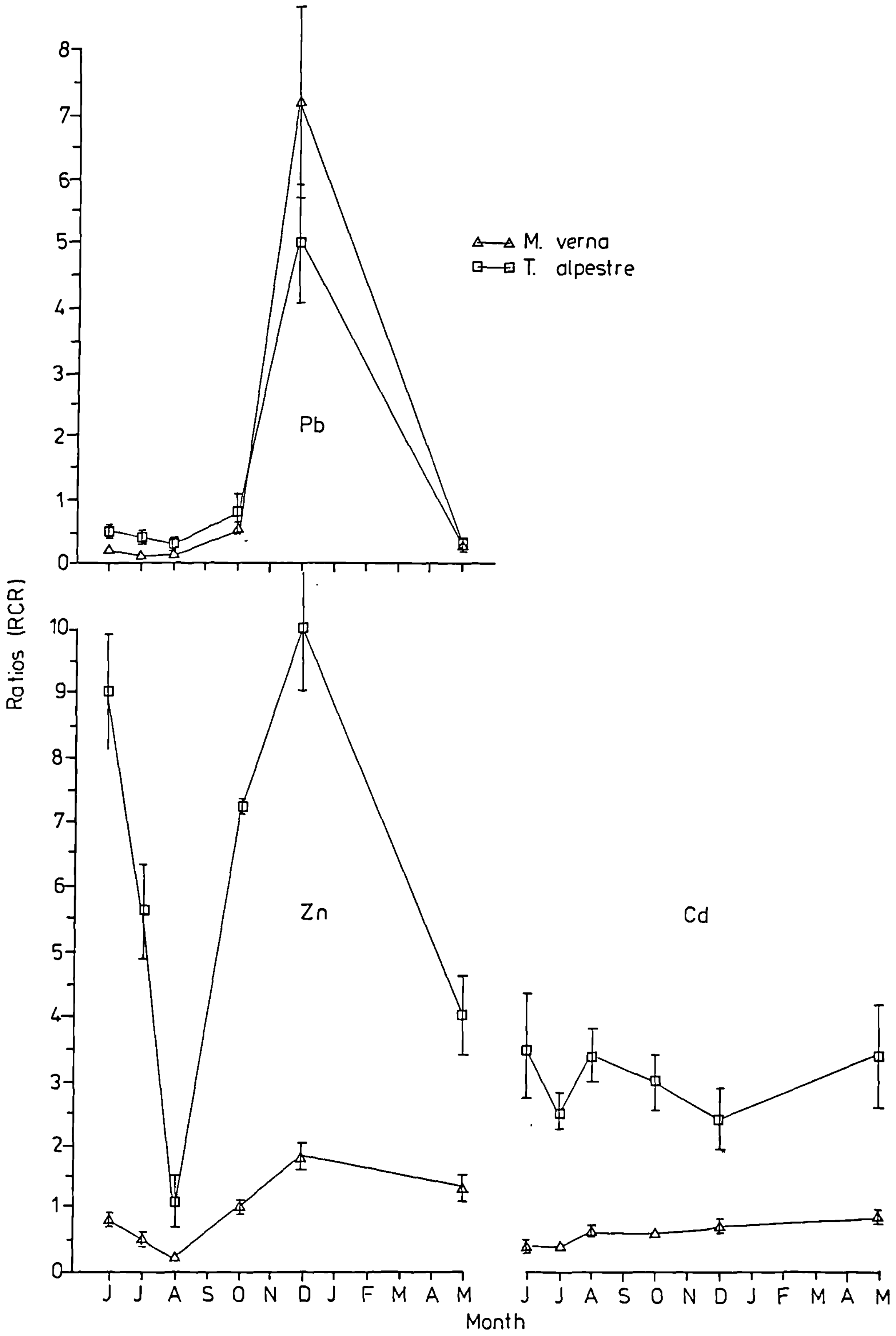
Sampling time	Pb	Zn	Cd	No. of samples
June	1395 ± 74	963 ± 24	37 ± 2	48
	465 - 3340	595 - 1415	20 - 76	
July	2149 ± 88	1347 ± 30	55 ± 2	60
	817 - 4010	749 - 2108	34 - 94	
August	2222 ± 83	1218 ± 23	39 ± 1	40
	1100 - 4040	850 - 1470	29 - 64	
October	874 ± 43	1292 ± 96	52 ± 4	12
	665 - 1100	921 - 2040	34 - 74	
December	139 ± 15	879 ± 45	46 ± 4	12
	67 - 247	659 - 1196	23 - 73	
May	1292 ± 92	934 ± 41	40 ± 2	12
	924 - 2067	664 - 1132	28 - 52	

4.5b Seasonal variation in the heavy metal concentrations in plant dry matter ($\mu g.g^{-1}$, means ± S.E.)

		M. verna		T. alpestre			
Sampling time	Metal	Young parts	Old parts	Rosette	Stem	Fruit	No.of samples
June	Pb	205 ± 47	1463 ± 324	771 ± 133	442 ± 120	211 ± 62	12
	Zn	735 ± 97	2013 ± 284	8494 ± 919	3990 ± 417	3864 ± 329	
	Cd	12 ± 2	34 ± 4	122 ± 24	104 ± 14	144 ± 28	
July	Pb	214 ± 35	796 ± 151	598 ± 61	336 ± 33	32 ± 4	20
	Zn	625 ± 62	1078 ± 95	18 ± 761	3162 ± 433	716 ± 101	
	Cd	22 ± 3	32 ± 3	133 ± 13	80 ± 7	42 ± 3	
August	Pb	324 ± 123	894 ± 51	484 ± 53	580 ± 95	-	20
	Zn	552 ± 297	138 ± 22	1311 ± 478	355 ± 35	-	
	Cd	51 ± 6	27 ± 4	131 ± 15	139 ± 11	-	
October	Pb	5336 ± 155	-	717 ± 233	-	-	12
	Zn	1365 ± 102	-	8358 ± 904	-	-	
	Cd	29 ± 3	-	146 ± 20	-	-	
December	Pb	887 ± 167	-	658 ± 109	-	-	12
	Zn	1470 ± 157	-	8518 ± 631	-	-	
	Cd	28 ± 3	-	100 ± 18	-	-	
May	Pb	330 ± 121	-	417 ± 73	509 ± 222	-	12
	Zn	1169 ± 213	-	3939 ± 462	5113 ± 794	-	
	Cd	33 ± 5	-	128 ± 23	96 ± 14	-	

- = No plant material was analyzed.

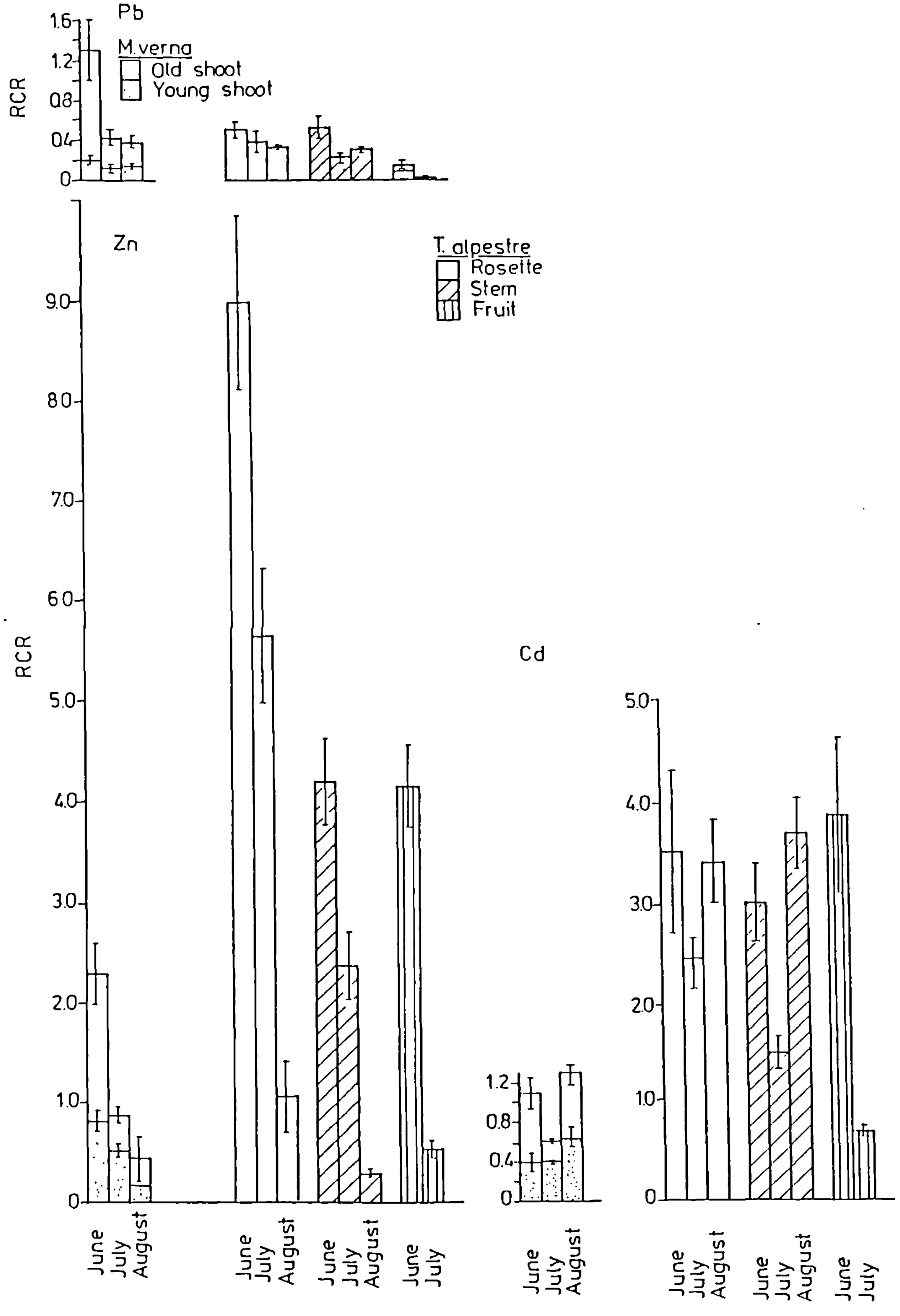
Figure 4.4 Seasonal variation in relative metal concentration ratios (RCR) for M. verna and T. alpestre. (Means ± S.E.)



between the two plants, being higher in T. alpestre than M. verna on all scoring dates. For M. verna the lowest ratios were recorded in June and July followed by slight increases in December and May. in T. alpestre the peaks were recorded during summer with the second lowest Cd RCR in July. The lowest value for this plant was in December, contrary to the trend for Pb and Zn.

Figure 4.5 shows RCR's for the lower and upper shoot parts of M. verna, the rosette, stem and fruit (when present) of T. alpestre for three successive summer months. For Pb there was a general decrease in RCR from June to August, with the highest values in M. verna old shoots and the lowest values in T. alpestre fruit. For Zn the trend was similar to Pb, with highest relative concentration in T. alpestre rosettes, and some variations shown between different parts. As in the case of Pb, lower parts of M. verna were shown to have higher relative concentration than the upper parts. For Cd there was a different trend as the lowest RCR was in July. With higher values in June and August. in the three parts of T. alpestre there were no significant differences during June, as was the case in August for the rosettes and the stems. In general, T. alpestre showed a considerable power of accumulating Zn and Cd in the shoot parts, indicating a significant translocation of metals from root to shoot. This finding was consistent with the analyses presented earlier for plants collected for a range of metalliferous sites.

Figure 4.5 Relative metal concentration ratios (RCR) in different aerial plant parts of *M. verna* and *T. alpestre*. (Means ± S.E.)



4.4 DISCUSSION

4.4.1 Analysis of soils & plants from different sites

It is clear from the results presented that the relationships between total and exchangeable concentrations vary for the three metals in the soils studied. The figures for total and exchangeable concentrations presented are in general very high but in agreement with some of the concentrations quoted in Table 4.1. Differences between various investigations carried out on even the same mine complex are to be expected due to problems of sampling, since abandoned heavy metal mine sites are in most cases extremely variable in distribution and composition of spoil materials (Johnston & Proctor, 1977; Barry & Clark, 1978; Thompson & Proctor, 1983; Shaw, 1984).

Considering the relationships between exchangeable and total concentrations for the three metals, the Cd exchangeable/total ratio was about 3.6 times greater than the Pb or Zn ratio, being about 0.22 (range 0.1–0.33) with mean exchangeable concentrations ranging from 4–68 $\mu g.g^{-1}$ dry soil at the sites sampled. The high exchangeable concentrations of Pb and Zn at most sites suggest extreme toxicity of the soils.

No distinct differences in metal concentration in the soils from the sites supporting M. verna only and those with populations of both species were demonstrated, suggesting that differences in heavy metal status between sites may not be the reason for apparent T. alpestre underdistribution. However, Garcia-Gonzalez & Clark (1985) have suggested that while M. verna occurs on soils with widely-differing concentrations of metals, T. alpestre is restricted to soils having relatively high concentrations. Field evidence does suggest that these two species do occur on naturally metalliferous soils and in the British Isles they seem to be present mostly on abandoned Pb and Zn or

Cu mine workings (Chapter 2). Also, M. verna is more widely distributed than T. alpestre on many of these habitats (Chapter 2) . Both species grow on, and also seem to be almost confined to, these habitats in which one or more toxic metal can be found at abnormally high concentrations, which are toxic to a great number of other species (e.g. Tables 4.1, 4.3). The concentrations of these metals in mine spoil clearly vary from site to site.

At the sites studied, the exchangeable concentrations of Cd were approximately an order of magnitude lower than those for Pb and Zn. Both Zn and Cd are more mobile elements than Pb in soil, particularly at high pH; they are also potentially more toxic (order of toxicity Cd > Zn > Pb). Plants which grow on such soils must therefore be tolerant to the high concentrations of metal in these soils (Bradshaw, McNeilly & Gregory, 1965; Macnair, 1981 and Thurman, 1981); they have evolved physiological mechanisms which enable them to tolerate metal toxicity (see e.g. Wainwright & Woolhouse, 1973; Farago, 1981 and Woolhouse, 1983). Such mechanisms do not generally suppress metal uptake but result in internal detoxification (Baker, 1981). The strategies of survival are thus tolerance and not avoidance of metal toxicity. Plant species differ considerably in their metal uptake characteristics. Further, for any species these could vary for different metals and even between different concentrations of the same metal. The reputation of both species to accumulate metals to high levels in the root and shoot is well documented in the literature. Thus Baumann (1865) reported a Zn concentration of 17.1% in the ash of T. calaminare (T. alpestre) corresponding to a concentration of more than 1% of the dry plant material (Reeves & Brooks, 1983). The observations summarized by Linstow (1924, 1929) and later investigations listed by Ernst (1976), together with those of Denaeyer-De Smet (1970) and Denaeyer-De

Smet & Duvigneaud (1974) are all consistent in reporting accumulation of Zn by T. alpestre to concentrations above 1% on a dry-mass basis. This behaviour has also been referred to more recently by Reeves & Brooks (1983).

The ability of M. verna to accumulate metals to high levels is also well known. Shimwell and Laurie (1972) have shown that this species is an indicator and accumulator of Pb and Zn in the north of England. Ernst (1975) has reported that amongst twelve species investigated, M. verna was the second highest accumulator after T. alpestre (more than 8000 $\mu g.g^{-1}$ Zn in the leaf dry matter). Ernst also found that amongst these species the highest Cu concentration (more than 100 $\mu g.g^{-1}$) was in M. verna followed by T. alpestre (about 80 $\mu g.g^{-1}$). Both T. alpestre and M. verna were the highest in Pb concentration after A. maritima. Kelepertsis & Andrulakis (1983) have reported up to 8.9% Zn in ashed plant shoot material collected from various localities in Macedonia, N. Greece. In the 8 populations of M. verna and the 5 populations of T. alpestre sampled in the present study, although each of the different sites was characterized by its own different concentrations of Pb, Zn and Cd in the shoots of both species, it was possible to define three arbitrary groups with 'higher' 'medium' and 'lower' metal concentrations. The data also suggested differences between the species in their control of metal uptake. For example, in 5 sites in which both plants occurred together, evidently on soil of similar metal status, the concentrations of Pb, Zn or Cd found in the shoot of one species were different in the other, with higher Pb concentrations in M. verna and higher concentrations of Zn and Cd in T. alpestre. This aspect was investigated further under controlled-environment conditions and is reported in the following chapter.

By comparing the present data with the figures in Table 4.1, Pb and Zn concentrations measured in the shoot of each plant were consistent with some earlier reports from the literature. Cd concentrations in the shoot of both species were an order of magnitude lower than those for Zn and Pb, with the concentrations of both Pb and Cd being noticeably lower than those for Zn in the shoot of T. alpestre. Because of the effect of variation in metal availability on the shoot metal concentrations, the RCR's were considered more suitable indices of performance for comparative purposes, in characterizing the relationships between the plant and soil. Variation in metal availability cannot wholly account for the observed differences between shoot metal concentrations of plants from various populations. Similarity between all populations of M. verna has been reflected by the very close RCR's for Zn; this was shown by the RCR's for Pb and Cd between some but not all populations of both species studied. The data presented in Figure 4.3 showed that there is a tendency for the concentrations of Pb, Zn and Cd in the shoot of M. verna to increase with increases in the exchangeable concentrations of soil Pb, Zn and Cd. All these relationships were significant at the $p<0.001$ level. However similar tests for T. alpestre resulted in non-significant relationships between Pb and Zn in the shoot and in the soil, but for Cd the relationship was significant at the $p = 0.02$ level.

There was no strong evidence to suggest that a threshold concentration of Pb, Zn or Cd in the soil had to be exceeded before concentrations in the shoot of both species increased. This could indicate the strong ability of both species to accumulate metals from low or high metal concentrations in the soil, although no truly 'low' metal soils were employed in the present study. Similar results for Zn in M. verna shoots were found by Barry & Clark (1978).

Problems of interpreting the relationship between the concentrations of metals in plant tissue and soil, involving more than one population are well known (see e.g. Ernst, 1965, 1968; Alloway & Davies, 1971; Shimwell & Laurie, 1972; Baker, 1974; Johnston & Proctor, 1977). Problems can also arise even within the same population (Jowett, 1964; Gregory & Bradshaw, 1965; McNeilly & Bradshaw, 1968; Simon & Lefebvre, 1977 and Barry & Clark, 1978). Such problems could be due to variations in plant response and in the amounts of available metals, along with the different physiological and phenological states of the plant (seasonal effects). These obstacles make it difficult to assess satisfactorily the overall relationship between the concentrations in the plant tissue and soil.

4.4.2 Seasonal variation of metal concentrations

The results presented in Table 4.5a, show that there were some variations in soil exchangeable metals during the year. These can probably be related to the climatic influences such as rainfall and temperature with some limited emphasis on the former (Figure 4.1) . The extraction by 1M-ammonium acetate gave a measure of the water-soluble metals as well as those held on the exchange complex of the soils. The major effect of rainfall will be in altering the equilibria between metals in the soil solution and those held on the exchange complex. The volume of soil solution may rise after episodes of rainfall and there may be some solubilization of mineral materials resulting in an increase in the total number of ions in solution, while the concentration of any ion may actually decrease as a result of dilution effects. Conversely, the drying out of the soil during periods of drought frequently experienced in loose and porous mine spoil, results in a reduction in the volume of soil solution and hence the concentrating of soluble salts. As the insoluble nature of many heavy metal salts is well known (Shaw, Rorison & Baker, 1984), rainfall may not have a significant effect on the concentrations if the soil solution and solid phase are in equilibrium. However the month which has the highest rainfall has also the lowest temperature (December). Rainfall can also have a leaching effect, particularly in free-draining mine spoil, which could explain the lowest level of extractable Pb being in December in the present study although the effect was not shown to the same degree by Zn or Cd. The main effects of low temperatures will be on the weathering of minerals. Snow and frost action in the winter months fragment the soil mineral particles into smaller ones, which makes the metals more susceptible to being solubilized when the temperature rises. Exchangeable Pb concentration showed an increase from December to May, with Zn showing the same trend but not to the same extent. Cd concentrations were generally similar most of the year, with a peak in July, following a slight increase in rainfall coinciding with an increase in temperature. Similar results were found by Shaw (1984). However, Bauer & Lindsay (1965) and Giordano & Mortvedt (1978, cited by Giordano & Mortvedt, 1980) showed no differences in extractable Zn at different soil temperatures.

The data on seasonal changes of metal concentrations in the plants which are presented in Table 4.5b and Figure 4.4 show some changes in the shoot concentrations of both species. For a plant like M. verna growing on metalliferous soil, contamination by soil metals will be likely on and between the lower parts of the plant. Varying degrees of sample contamination at different stages of the year may affect the interpretation of seasonal data. Shaw (1984) indicated the difficulty in ensuring that no soil particles are trapped between fine leaves and between them and the stem. Thus the upper parts with more green shoot and leaves are likely to be less contaminated. Further, evidence in the literature suggests that in some species the metal concentrations in the dead and green leaves together are higher than in the green leaves alone. In order to minimize these problems, the green parts (the upper and younger shoots) were used to investigate the seasonal changes of metal concentrations in the plant shoot. For *T. alpestre*, as the rosette of basal leaves persists throughout the year, it is appropriate to use these leaves for seasonal sampling. With regard to dead leaves on *T. alpestre* rosettes, no separate record was made of their metal concentrations. Plants of the Dovegang population are very small and the fine dead leaves which are on the base of the rosettes are more exposed to the soil surface and could easily be contaminated by soil particles. Stem and fruit parts of *T. alpestre* were also analyzed for Pb, Zn and Cd in three successive summer months.

As demonstrated in the first part of this chapter the two species, although both metallophytes, showed some differences in their uptake characteristics with generally higher concentrations of metals accumulating in the shoot of *T. alpestre* indicating more translocation from root to shoot. Both species, as presented in Figure 4.4, have the same trend in the changes of Pb and Zn RCR, with the peak values in December when plants are essentially dormant. Such relative increases in metal concentration during winter can best be related to some or all of the following effects; (1) the dilution due to the increased dry matter production in spring and summer (Rains, 1971; Crump & Barlow, 1982),(2) since metals are accumulated in the leaves during the growing season, mature leaves have higher metal concentrations than young leaves which are still expanding (Guha & Mitchell, 1966; Mitchell & Reith, 1966), (3) loss of dry matter by respiration, leaching and translocation may result in an apparent increase in metal concentrations of the shoot. Mitchell & Reith (1966) found a 100-fold increase in Pb levels in pasture species, from a minimum value in the spring to a winter maximum, (4) the effects of aerial deposition. Martin & Coughtrey (1982) suggested that seasonal variations in Cd at a contaminated site may be a result of aerial deposition rather than plant physiological effects. Conversely, Mitchell & Reith (1966) discounted aerial Pb contamination in accounting for the rise of Pb concentration in Lolium perenne plants during winter at supposedly uncontaminated sites, and speculated that the increase was due to continued translocation from root to shoot while the plants were dormant. The balance of evidence seems to be pointed to the effects of increased biomass dilution as the more likely factor responsible for the observed seasonal trends of plant metal content in the case of Cd concentration in T. alpestre.

It is most probable that the metal content of the root is at a minimum during winter when the plant is dormant and temperatures are low (Shaw, 1984). Although this is a point of dispute (Jones & Jarvis, 1981; Tinker, 1981) results obtained in the present study seem to agree with results of Shaw (1984). Matthews & Thornton (1982) found that root Pb and Cd concentrations were decreased in winter relative to those in shoot and attributed this to remobilization rather than increased uptake. The effect of reduction in temperature may also count for reduced ability of growing roots to restrict uptake and hence movement of metals into the shoot, particularly when the supply of essential elements is limited (Jones, Jarvis & Cowling, 1973). In the present study it was observed that the increase in the concentration of Zn in the plant shoot of M. verna or T. alpestre or both, followed the pattern of rainfall. However the interaction between the different factors made it difficult to simplify the explanation for seasonal changes in metal concentration.

The changes of metal concentrations in other parts of M. verna (the old shoot) and T. alpestre (stem and fruit) were also investigated. Metal concentrations in these parts were determined during the summer and compared with those of the young shoots and the rosettes (Figure 4.5). Such comparison has revealed some other aspects of the relationship between the different parts of the plant and metal concentrations and transport. Immobile or less mobile ions have a tendency to accumulate in the older parts (leaves) (Guha & Mitchell, 1966; Mitchell & Reith, 1966; Tinker, 1981; Crump & Barlow, 1982; Shaw, 1984). The findings of all the studies cited agree with the data reported in the present study for the upper and lower parts of M. verna. A similar situation emerged for Zn in T. alpestre. Here, although the concentrations of Zn were higher in the rosette than those in the stem or fruit, the latter parts contributed a high percentage of the total content in the plant. These parts also have high concentrations of Pb and Cd and soon senesce and are shed. This could be an effective strategy for detoxifing the plant, i.e. an 'external' metal tolerance mechanism (Reilly & Stone, 1971; Howard-Williams, 1972, Rascio, 1977).

It can be recognized from the above discussion that although there are some differences between the two species, they also seem to be identical in some of their properties, such as the similarity in the trend of seasonal variation of their metal concentrations and the high concentrations of these metals accumulated in the tissues of both plants.

4.5 SUMMARY

The work presented in this chapter can be summarized as follows:

1. The above-ground biomass of Minuartia verna and Thlaspi alpestre together with soil samples taken from the rooting zone were sampled as follows: (a) From eight heavy-metal-contaminated sites in the Pennines, UK at the end of the growing season in 1982. (b) Over a one year period from a plot of 100 m^2 at Dovegang lead mine, Derbyshire. Measurements of Pb, Zn and Cd concentrations in plants and soil were made at suitable intervals throughout the year.

2. There were some differences in the plant and soil metal concentrations between the different sites with some soils reflecting extreme concentrations of one or more metal.

3. The concentrations of Pb in M. verna shoots were greater than in T. alpestre at most sites. Conversely, Zn & Cd concentrations were always higher in T. alpestre.

4. The relationships between the extractable metal concentrations in soil and M. verna shoots were significantly positive at the $p<0.001$ level for Pb, Zn and Cd, but for T. alpestre there was no significant correlation for either Pb or Zn, the correlation for Cd was significant at the $p = 0.02$ level. Correlations between total metal concentrations in soil and those in plant dry matter rarely proved significant.

5. There were no clear differences in metal concentrations at those sites lacking T. alpestre, when compared with those at which both species grow in association.

6. From the seasonal study it emerged that the exchangeable concentrations of Pb and Zn were at their lowest level in December. Zn and Cd were less variable than Pb but there was a trend for highest concentration in summer for all metals.

7. In both species there was a peak in the shoot Pb and Zn concentration in the winter, with similar trends in both plants. In contrast, the trend for Cd was for the highest concentration at the beginning and at the end of summer in the shoot of *T. alpestre*, but Cd concentration was almost constant in *M. verna* shoots.

8. From both studies, the variation in the amounts of the soil-available metals could not account for the plant metal contents, in different sites and/or at different stages of the season.

9. Although the two species are absolute metallophytes they differ in metal-uptake characteristics and the amounts of metal concentrated in the shoot.

10. The older parts of *M. verna* show higher Pb, Zn and Cd concentrations than younger parts. Similar results were seen for Zn in *T. alpestre*.

CHAPTER 5

RESPONSES OF MINUARTIA VERNA AND THLASPI ALPESTRE TO HEAVY METALS SUPPLIED UNDER CONTROLLED ENVIRONMENT CONDITIONS

5.1 INTRODUCTION

The analyses of plants of M. verna and T. alpestre collected from field sites in the South and North Pennines reported in Chapter 4 suggested some differences in metal uptake characteristics both at the species and population level. Differences in heavy metal tolerance between the two species could, at least in part, account for the observed field distribution patterns. Such a theory might go some way to explain the restricted distribution of T. alpestre relative to that of M. verna if it could be demonstrated that T. alpestre is confined to sites of very high metal status and it is consistently more metal-tolerant than M. verna. Inter-population differences in tolerance would also indicate whether or not tolerance is a constitutional property of the species (at least within the range of sites investigated in this study), or to what extent isolated populations could be recognized as locally-adapted races, selected in response to differing selection pressures resulting from variations in heavy metal toxicity prevailing at the sites colonized. Simple biogeochemical investigations can do little more than reveal large-scale differences in metal uptake and accumulation between species. Consequently, a series of experiments were devised to explore the response of M. verna and T. alpestre to the main heavy metal contaminants present at the field sites investigated (lead, zinc and cadmium), supplied under controlled experimental conditions. Such an approach allows both inter-specific and inter-population variations in response to be assessed and can also reveal aspects of the physiological mechanisms of metal tolerance involved. Responses to a range of other potentially toxic metals were also examined in order to gain some information about the specificity of metal tolerances in both species.

The comparative experiments reported in this chapter employed both soil and solution culture techniques to assess the responses of the two species in a range of growth characteristics, including seed germination, root growth, biomass yield and heavy metal uptake and distribution. The standard experimental design thus included metal treatments which could be compared with controls which had no metal addition. In this way both inhibitory and stimulatory effects of added metals could be detected. The series of experiments were devised to investigate both short - and long-term responses to heavy metals under controlled environment conditions, supplied both singly and in appropriate factorial combinations.

5.2 METHODS OF MEASUREMENT OF HEAVY METAL TOLERANCE

The most frequently used method for assessing tolerance to heavy metals is that originally developed by Wilkins (1957), subsequently modified by other workers (e.g. Jowett, 1958; Craig, 1977; Nicholls & McNeilly, 1979). This method, commonly referred to as the 'root elongation technique', employs measurements of relative inhibition of root elongation of seedlings or mature plants when grown in solutions with and without heavy metal ions to calculate an index of metal tolerance. It was developed almost entirely using rooted tillers of various grass species to allow a rapid short-term screening of large numbers of individuals, so that information can readily be gained both at the population and genotype level.

Wilkins (1978) and Brown (1983) have fully reviewed the various forms of the test employed and their relative merits and problems in the assessment of adaptation of plants to edaphic factors. In essence, there are two basic types of method widely used by other workers. The first, the so-called 'sequential method', requires consecutive measurements of root growth, initially in the absence, and then in the presence of the added metal ion. This method, which can readily be applied to individual plants, was that originally proposed by Wilkins (1957) and is clearly useful in determining indices at the level of the genotype and providing estimates of intra-population variability. The second, the 'parallel method', employs batches of replicated (often cloned) plant material grown simultaneously in control and treatment solutions for the same period of time. A mean index of tolerance can then be derived from a simple root fraction which is frequently expressed as percentage value. Whilst this method does not yield so much definite information about variation at the level of the individual, it is one which appears to have been more widely used by other workers in view of its rapidity and simplicity, only one set of root measurements being required if starting material is similar. Within-treatment variability can also be assessed from derived standard errors using a form of the 'delta method' (Kendall & Stuart, 1977) on numerator and denominator variances. A 'parallel-type' method was thus employed in the tolerance tests reported in this chapter. Indices of metal tolerance (IT) are in all cases expressed as percentage values.

The rationale and validity of such simple short-term assessments of metal tolerance based purely on root growth have been questioned by some workers (e.g. Baker, 1978; 1981). Similarly, there have been criticisms of the use of only one metal concentration for screening tolerance. Accordingly, Craig (1977) used probit analysis and Nicholls & McNeilly (1979) a regression approach on root growth responses over a range of metal concentration. Similar doubts have been raised about the use of single-salt background solutions in metal tolerance studies. Wilkins' original work (1957) and many of the earlier studies reported by Bradshaw & McNeilly (1981), used a background solution of calcium nitrate, as it was found that tolerance to most heavy metal ions was generally enhanced in the presence of calcium. This simple system clearly avoided potential problems of precipitation of metal ions by anions such as phosphate and sulphate which would normally be supplied in a full nutrient culture. The argument for this approach was that it was highly unlikely that plants would become nutrient-deficient during the short duration of most experiments. This may well be true for vegetative material such as grass tillers where internal reserves may be relatively large, but could still represent a problem for small seedlings (such as those of M. verna where seed reserves are minimal. For this reason, some workers have elected to use a full or modified (low or zero phosphate and sulphate) basal solution in metal tolerance studies (Davies & Snaydon, 1973; Coughtrey & Martin, 1977; Brown, 1983; Shaw, 1984 and Baker et al., 1986). A further refinement has been employed by Johnston & Proctor (1981) who devised a background solution which mirrored the composition of the soil solution. For the present work, it was felt desirable to use a full nutrient solution but at low concentration so that both measurements of tolerance and more long-term measures of plant performance and metal uptake could be assessed in the same solution. 0.1-strength Rorison solution (Hewitt, 1966) appeared convenient in this respect (Appendix 3).

Whilst there is a vast background of literature relating to the use of the root elongation method to assess metal tolerance in populations of grasses growing in metal-contaminated sites, there have been few reports of its use with dicotyledonous plants. However, Halliday (1960); Baker (1970) and Shaw (1984) were able to make measurements of tolerance with rooted cuttings and seedlings of *M.verna*.

5.3 GENERAL MATERIALS AND METHODS

5.3.1 Seed collections

Seed collections were made each year since 1980 mainly from populations at the metalliferous mine sites listed in Table 5.1. Seeds of all these populations were used in one or more experiments throughout this study.

Table 5.1 Sites of seed collections

Site	Species	Grid. ref.
1. Black Rocks (Cromford, Derbyshire)	*M.verna*	SK 293558
	T. alpestre	
2. Bonsall Moor (near Matlock, Derbyshire)	*T. alpestre*	SK 237600
3. Bradford Dale (near Youlgreave, Derbyshire)	*M. verna*	SK 202637
	T. alpestre	
4. Clough Wood (Darley Dale, Derbyshire)	*T. alpestre*	SK 258618
5. Dirtlow Rake (near Castleton, Derbyshire)	*M. verna*	SK 155821
6. Grattondale (near Elton, Derbyshire)	*M. verna*	SK 207607
7. Tideslow Rake (near Tideswell, Derbyshire	*M. verna*	SK 159778
8. Wensleydale (near Winster, Derbyshire)	*T. alpestre*	SK 265603
9. Whitesike (near Garrigill, Cumbria)	*T. alpestre*	NY 751425
10.Eller Beck (near Woodhall, N. Yorkshire)	*T. alpestre*	SD 984900

5.3.2 Growth room environment and general techniques of culture

All the experiments reported in this chapter but one, were carried out in a controlled-environment room with the following growth conditions: Day length of 16 hours at a light intensity of 10,000 lux with 20-21°C day and 15-17°C night; relative humidity of 60-70%. Such growth rooms have been described by Rorison (1964). The second experiment reported was conducted in the glasshouse at Tapton Experimental Gardens. Supplementary light was supplied for 16 hours per day by 100 watt Phillips mercury lamps; the daytime temperature was 25°C approximately and the night-time was 18°C. In all but two experiments, solution culture was used; in these (Experiments II and VIII) soil culture was used.

In the experiments designed to measure the tolerance of seedlings to heavy metals such as Pb, Zn, Cd, etc, these metals were usually supplied at concentrations which in conventional nutrient solutions would be expected to result in their partial or complete precipitation (Shaw, 1984). Because of some possible problems, such as metal leaching or accumulation in sand culture, this medium seemed inappropriate, and so solution culture was considered the most suitable medium for both short - and long-term tests. Root growth is often abnormal in solution culture but it was considered unlikely that this would invalidate the results since the effect is apparently general among species grown in this way (Hodgson, 1972).

A wide range of nutrient solutions has been used by other workers for culturing plants hydroponically (e.g. Hewitt, 1966). Solutions have generally been devised for crop plants which are fast-growing and have greater nutrient requirements than the majority of native species, in particular plants growing on lead mine spoils where nutrient status may be very low. Rorison nutrient solution (Hewitt, 1966) was chosen for all the present work as it is a dilute but balanced solution, and appropriate for such species. It was considered that a ten-fold dilution of this solution could still be of sufficient strength to grow plants satisfactorily with metal interaction reduced to a minimum particularly Pb with PO4 Shaw (1984). It was also considered that using a circum-neutral pH would be appropriate in view of the prevailing field conditions. The composition of this solution is given in Appendix 3.

"Analar" chemicals were used wherever possible in the preparation of the stock nutrient solutions. Heavy metal treatments were prepared by addition of appropriate volumes of stock solutions of metal salts to the basal nutrient solution. Pb was added as $Pb(NO_3)_2$, Zn as $Zn\ SO_4.7H_2O$ and Cd as $CdCl_2.2.5\ H_2O$.

5.3.3 Culture vessels

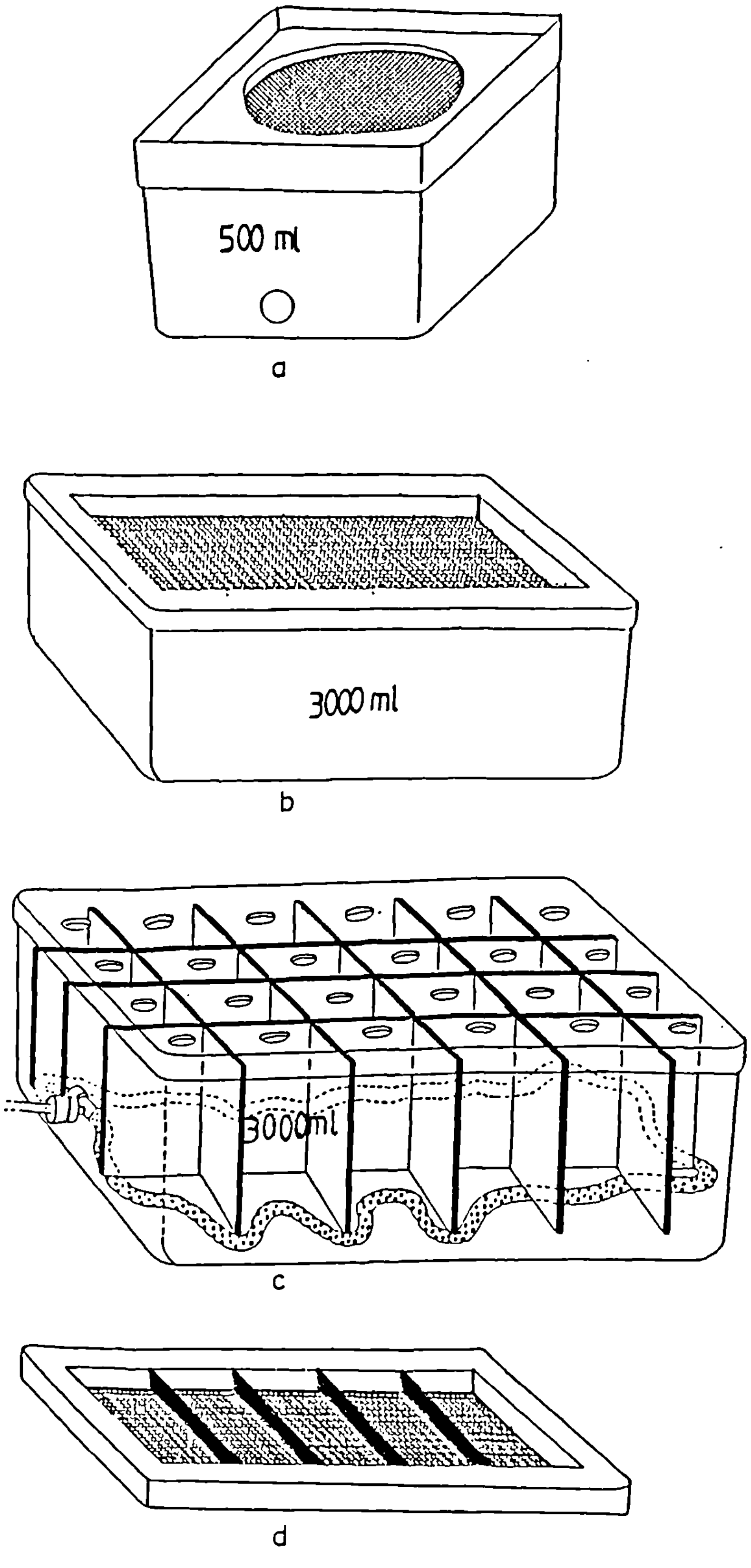
Two sizes of polystyrene containers were used for solution culture work, 500 ml and 3000 ml capacity. All containers were painted on the outside with black bitumastic paint to limit algal growth. The smaller size was used for most of the short-term experiments with young seedlings and the larger was used in the solution culture experiment involving older seedlings. In Expt. III wider containers were used in order to provide enough space for simultaneous screening of 5 populations of each species. The different types of vessels employed are illustrated in Figure 5.1. For soil experiments 3.5" diameter plastic plant pots were used.

5.3.4 Methods of seed germination and subsequent plant growth in tolerance testing and metal uptake experiments

In the germination experiments seeds were germinated directly on the metal treatments. In all metal tolerance experiments seeds were germinated on floating nylon-mesh rafts on 0.1-strength Rorison solution and then transferred to metal treatments as soon as radicles emerged.

Most experiments were carried out using seedlings in their first days after germination. At such a stage, seedlings were reasonably uniform and sufficient in number for a large-scale experiment.

Figure 5.1 Vessels employed in the solution culture experiments.



In solution culture experiments, two main methods of support for the seedlings were used. In the first method, used for very young seedlings, rafts were constructed from "Styrofoam" and plastic mesh, covered with a layer of black alkathene beads, approximately 1.5 cm in depth. This system held the plants in an erect position, with the root at an appropriate level in relation to the position of the solution surface. It avoided problems of both water-logging and desiccation (Hodgson, 1972) and allowed seedlings to be withdrawn without damage (McGrath, 1979). For larger seedlings, holes of appropriate dimensions were made in the painted plastic lid of the culture vessel, at a suitable distance from each other. Each vessel was divided into compartments in order to prevent the root system of different plants becoming entangled. The first system was used mainly for the measurement of indices of metal tolerance and the second in metal uptake experiments.

Solutions were aerated continuously and the pH was adjusted to 6.5 by using N/5 H2SO4 or NaOH. All solutions were changed every two days in order to maintain low levels of metal addition and avoid problems of pH change. After a suitable period of treatment application (generally 2 weeks), plants were harvested and the length of the longest root (tolerance expts.) and dry weight and metal concentrations of root and shoot (uptake expts.) were recorded. Chemical analysis of plant material employed the methods described previously in Chapter 4. All harvest data were subjected to full statistical analysis by analysis of variance (GENSTAT statistical program, 1980 version was used).

Two types of soil culture were used according to the nature of the experiment. In the first type, naturally-contaminated soil was employed, collected from the field (Expt. II). In the other soil experiment (Expt. VIII), an artificially-contaminated soil was made from JIPC + added metal salts. This experiment was maintained in the growth room under the environmental conditions described earlier in section 5.3.2.

5.4 EXPERIMENTAL OBJECTIVES, DESIGN AND PROCEDURE

In this section the experimental objectives, design and procedure are reported for all the experiments in this chapter, according to the sequence in Table 5.2.

Table 5.2 Experiments performed on the responses of *M. verna* and *T. alpestre* to heavy metal treatments

5.4.1	**Germination experiments**
I	Germination responses of seeds to zinc treatment.
II	Germination responses of seeds to metalliferous soil from different sites.
5.4.2	**Metal tolerance experiments**
III	Response of young seedlings to lead, zinc and cadmium treatments.
IV	Response of seedlings to lead, zinc and cadmium supplied in factorial combination.
V & VI	Response of seedlings to a range of heavy metals (12 metals).
VII	Response of five different populations of *T. alpestre* to treatment with twelve heavy metals.
5.4.3	**Metal uptake experiments**
VIII	Heavy metal (lead, zinc and cadmium) uptake by *M. verna* and *T.alpestre* from soil culture.

IX Heavy metal (Pb, Zn, Cd, Al, Ag, Co, Cr, Cu, Fe, Mn, Mo & Ni) uptake by five different populations of T. alpestre (The uptake part of Expt. VII).

5.4.1 **Germination experiments**

Expt. I The germination responses of M. verna and T. alpestre to Zn treatment.

Objectives

Heavy metal tolerance or toxicity can be expressed at all stages of plant growth, but as selection operates as early as seed germination when the radicle comes into contact with soil solution containing toxic metal ions, germination is clearly a critical stage for investigation. If a seed can germinate in the presence of high concentrations of metal ions then it must be regarded as being tolerant and able to establish on a metalliferous substrate. Experiment I was designed to investigate the effects of a range of Zn concentrations on the germination of seed samples of M. verna and T. alpestre. This metal was selected in view of its widespread occurrence and abundance at the majority of sites colonized by these two species (Table 4.3), its universal phytotoxicity, and its likely mobility even in circumneutral soils.

Design

The design of the experiment was:

One population of T. alpestre (Bradford Dale) + two populations of M. verna (Bradford Dale & Tideslow Rake) x 5 concentrations of Zn x 5 replicates = 75 units (petri dishes), 50 seeds in each unit.

Procedure

1250 seeds of each population were counted and randomly divided into 25 samples (50 seeds each). Each sample was then placed in regular spacing on Whatman no. 1 filter papers in 9 cm plastic petri dishes. To each of the 5 petri dishes for each population, 3 ml of ZnSO4.7H20 solution of known concentrations (0, 1, 5, 10 and 50 $\mu g.ml^{-1}$ of Zn) were added. The petri dishes were placed under a continuously-illuminated light bank, in a fully-randomized design. 1 ml of distilled water was added to each dish every day to minimize the effect of evaporation on Zn concentrations. After 5 days, the first germination score was made and subsequently a daily record was kept. Germination was recognized as the emergence of the radicle from the seed coat.

Expt. II The germination responses of M. verna and T. alpestre seed to metalliferous soil from two different sites.

Objectives

The aim of this experiment was to study the effect of naturally-contaminated metalliferous soils from two different mine localities on the germination rate of both species. Seeds were germinated on both their natural substrate and reciprocally.

The experiment included one soil from a site where both species grow together, the other being from a site where only M. verna is recorded. The responses were compared with those observed on an uncontaminated soil (JIPC).

Design

The design of the experiment was:

3 populations (one population of T. alpestre (Bradford Dale) and two populations of M. verna (Bradford Dale & Tideslow Rake) x 3 soils x 5 replicates = 45 units with 25 seeds in each unit.

Procedure

Bulk soil samples were collected from Bradford Dale and Tideslow Rake. The soils were sieved in the field with a 1 cm sieve to remove coarse stone and root fragments. After sieving, each soil was mixed thoroughly and then stored in polythene bags at 5^oC. Next morning the soil was mixed again and equally divided into 3.5" pots (382 g/pot). The third soil (JIPC), used as a control, was divided in the same manner. All pots were placed in the glasshouse at Tapton Experimental Garden under the conditions described earlier (see 5.3.2). The soil in all pots was then sub-irrigated with water until field capacity was reached.

Seeds of M. verna and T. alpestre were sown onto the moist soil surface at known positions. Each pot was then covered with a plastic petri dish to keep the surface soil moist during the first experimental stages. After approximately 9 days the first seeds had germinated and a record was made over the next 21 days. The experiment was monitored for a further three weeks to ensure that every germinated seed was recorded. However, no more seeds germinated after the first 21 days scoring.

5.4.2 Metal tolerance experiments

Exp. III The response of young seedlings to Pb, Zn and Cd treatment.

Objectives

The aim of this experiment was to study the response of different populations of each species to Pb, Zn and Cd treatments. In Chapter 4, plant and soil material was sampled from 8 different sites (Table 4.2) and analyzed for these three metals, so it was considered to be appropriate to use seeds collected from these sites in a laboratory experiment in order to ascertain whether or not species and populations showed differences in their response to metal treatments.

Design

Experimental design was as follows:

2 species x 5 populations x 3 metals x 4 concentrations + control x 2 replicates = 260 units (15 plants in each unit).

Procedure

Seeds from 5 different populations of each species (sites number 1, 2, 3, 5, 6, 7, 8 and 9; Table 5.1) were scattered on one layer of capillary-mat on rafts constructed to facilitate simultaneous screening of 5 populations of both species. The vessels used were 3 litre capacity plastic containers. The experiment was set up in the growth room under the conditions described in section 5.3.2. The culture solution used was 0.1-strength Rorison solution with 0.6, 2, 6, and 10 $\mu g.ml^{-1}$ Pb added or 8, 16, 33 and 50 $\mu g.ml^{-1}$ Zn or 0.5, 1.5, 4 and 6 $\mu g.ml^{-1}$ Cd.

At first, the seeds were left to germinate, then seedlings thinned down to 15 uniform individuals and then the treatments applied. After 18 days growth, a destructive harvest was made and the length of the longest root was recorded for each individual.

Expt. IV Response to Pb, Zn and Cd supplied in factorial combination.

Objectives

This experiment was a logical extension of the previous one which examined species and population responses to metals supplied individually. This experimental situation is rarely reflected in the natural environment as the three principal metals frequently occur together, albeit at varying concentrations, in the soils colonized by the two species (see Table 4.3). The experiment aimed to examine the possible significance of additive and synergistic effects by investigating tolerance to Pb, Zn and Cd supplied in factorial combination. In order to confine such a potentially large experiment to what was practically feasible, only one metal level for each element was used and only one population for each species.

Design

The experimental design was:

2 species x 3 metals in factorial combination x 2 replicates = 32 units (20 plants in each unit).

Procedure

Seeds of T. alpestre (Bonsall Moor) and M. verna (Grattondale) were sown on rafts floating on 0.1-strength Rorison solution in 500 ml containers (Figure 5.1a). After the seeds had germinated, 20 uniform individuals were selected. Treatments were then applied using one concentration of Pb or Zn or Cd in the following factorial arrangement (Pb, Zn, Cd, PbZn, PbCd, ZnCd, PbZnCd and control. The concentrations used were 10 $\mu g.ml^{-1}$ Pb, 8 $\mu g.ml^{-1}$ Zn and 1 $\mu g.ml^{-1}$ Cd. These concentrations were selected as the previous experiment suggested that they brought about an approximately similar and intermediate degree of root growth inhibition. After 18 days growth, the plants were harvested and the root lengths recorded.

Expt. V Response of M. verna and T. alpestre to a range of heavy metals.

Objectives

Although Pb, Zn and Cd are considered to be the prime heavy metal contaminants of the mine soils supporting populations of M. verna and T. alpestre used in earlier experiments, they are not the only metals which may be present at elevated concentrations. Other more common but nevertheless potentially toxic elements such as Al, Fe and Mn may also be present in abundance together with other transition metals often associated with Pb/Zn mineralization, such as Cu, Co, Cr, Mo and Ni. Species and populations may thus show tolerances to some or even all of these other metals depending on the extent to which multiple tolerances have evolved or co-tolerances exist. A constitutional tolerance within a species resulting from a non-specific detoxification system could confer tolerance to a whole range of metallic elements to which it may be exposed in mineralized soils. This and the following experiment examined the response of both species to a range of 12 heavy metals in order to ascertain to what extent other metal tolerances could be demonstrated. A major difficulty in this type of experiment is the concentration ranges of metals used. As little information is available from the literature, particularly in relation to the background solution employed in the tolerance tests, ranges were decided somewhat arbitrarily, based largely on the known order of phytotoxicity of these elements.

Design

A randomized block design was arranged as follows:

2 species, *M. verna* (Tideslow Rake) and *T. alpestre* (Bradford Dale) x 12 metals x 3 concentrations + control x 2 replicates = 148 units (5 plants per unit).

Procedure

The same procedure used in Expt. III was followed using a range of 9 other metals in addition to Pb, Zn and Cd, as follows: 10, 15 & 20 $\mu g.ml^{-1}$ for Pb as $Pb(No_3)_2$; 50, 100 & 250 $\mu g.ml^{-1}$ for Zn as $ZnSO_4.7H_2O$; 2.5, 5 & 10 $\mu g.ml^{-1}$ for Cd as $CdC\ell_2.2.5H_2O$; 5, 20 & 40 $\mu g.ml^{-1}$ for Aℓ as $A\ell_2(SO_4)_3.16H_2O$; 2, 5 & 10 $\mu g.ml^{-1}$ for Ag as $AgNO_3$; 2, 5 & 9 $\mu g.ml^{-1}$ for Co as $Co(NO_3)_2.6H_2O$; 1, 3 & 5 $\mu g.ml^{-1}$ for Cr as $K_2Cr_2O_7$; 10, 20 & 50 $\mu g.ml^{-1}$ for Cu as $Cu SO_4.5H_2O$; 10, 20, 35 $\mu g.ml^{-1}$ for Fe as $FeSO_4.7H_2O$; 10, 20 & 35 $\mu g.ml^{-1}$ for Mn as $MnSO_4.4H_2O$; 15, 30 & 60 $\mu g.ml^{-1}$ for Mo as $Na_2MoO_4.2H_2O$ and 3, 8 & 15 $\mu g.ml^{-1}$ for Ni as $NiSO_4$. Seedlings were thinned down to 5 uniform individuals before the treatments were started with the pH being adjusted to 6.5. After about 10 days of growth in the treatments, the plants were harvested and the root lengths recorded and the indices of tolerance (IT) calculated.

Expt. VI Response of M. verna (Tideslow Rake) and T. alpestre (Bradford Dale) seedlings to a single concentration of a range of heavy metals.

Objectives

The aim of this experiment was discussed in the previous section. The experiment was essentially a repeat of the previous one, using the same range of metals, supplied only at one concentration but at a lower pH of 5.5. This was deemed necessary in view of the likely precipitation of some of the range of added metals at high pH, they being generally mobile in more acid soils.

Design

2 species x 12 metals x one concentration + control x two replicates = 52 units (6 plants per unit).

Procedure

The single concentrations of metals used were 20 $\mu g.ml^{-1}$ Pb, 50 $\mu g.ml^{-1}$ Zn, 10 $\mu g.ml^{-1}$ Cd, 20 $\mu g.ml^{-1}$ Al, 2 $\mu g.ml^{-1}$ Ag, 9 $\mu g.ml^{-1}$ Cr, 5 $\mu g.ml^{-1}$ Cu, 10 $\mu g.ml^{-1}$ Fe, 20 $\mu g.ml^{-1}$ Mn, 15 $\mu g.ml^{-1}$ Mo and 3 $\mu g.ml^{-1}$ Ni. pH was adjusted to 5.5 in all cultures. Plants were harvested after 14 days growth in the treatment solutions and root lengths recorded.

Expt. VII Response of 5 different populations of T. alpestre to treatment with 12 heavy metals.

Objectives

The previous two experiments were designed to provide some information on possible differences in species response to a broad range of heavy metals. The final experiment in this series examined the extent of inter-population variations in these responses, using five population samples of *T. alpestre* in a comparative experiment. The scope of this experiment extended beyond simple tolerance tests by allowing plants to grow on in culture to a stage at which sufficient dry matter had been produced to enable chemical analysis of plant parts, to provide additional information on metal uptake and accumulation (reported in the final section of this chapter).

Design

One species x 5 populations x 12 metals x one concentration + control x 2 replicates = 130 units (12 plants per unit).

Procedure

In this experiment, *T. alpestre* seeds from 5 different populations (1, 3, 4, 9 and 10 Table 5.1) were sown on rafts (Figure 5.1d) constructed to allow simultaneous screening of 5 populations and floated on 0.1-strength Rorison solution in 3000 ml capacity vessels.

After seeds had germinated, appropriate treatments were applied with 12 seedlings representing each population in each replicate. The same metals listed in Expts. V & VI were used at the following concentrations: 20 $\mu g.ml^{-1}$ Pb, 25 $\mu g.ml^{-1}$ Zn, 5 $\mu g.ml^{-1}$ Cd, 20 $\mu g.ml^{-1}$ Al, 0.5 $\mu g.ml^{-1}$ Ag, 5 $\mu g.ml^{-1}$ Co, 1 $\mu g.ml^{-1}$ Cr, 1 $\mu g.ml^{-1}$ Cu, 6 $\mu g.ml^{-1}$ Fe, 10 $\mu g.ml^{-1}$ Mn, 10 $\mu g.ml^{-1}$ Mo and 2 $\mu g.ml^{-1}$ Ni. The pH was adjusted to 5.5 in order to make the metals more freely available in solution but with no adverse effects to the plants.

After 21 days of growth in the treatments, the plants were harvested and a record was made of the root length and also root and shoot oven-dry weight yields. Plants were bulked in random pairs in order to have sufficient dry matter for chemical analysis since seedlings were very small in some of the treatments. Due to the small dry weight and time resource limitations, the metal analyses of seedlings were carried out on bulked material (2 replicates each consisting of 6 whole 'duplicate' plants for each population except for Whitesike, where the root systems were analyzed separately from the shoot). Agreement between these 'replicate' samples was generally very good. The analyses were kindly carried out by Dr. R. D. Reeves,Department of Chemistry & Biochemistry, Massey University, Palmerston North, New Zealand, using Inductively Coupled Plasma emission spectrometry, (ICP).

5.4.3 Metal uptake experiments

Expt. VIII Heavy metal (Pb, Zn & Cd) uptake by M. verna and T. alpestre (Bradford Dale) from soil culture.

Objectives

Metal uptake by the species in the field was considered in Chapter 4. In order to evaluate further these results it was considered appropriate to perform metal uptake experiments under controlled conditions. It was also thought that using an uncontaminated soil with added heavy metals would simulate field conditions under a constant climatic regime. More than one metal concentration was produced in order to allow a study of the relationships between plant and soil metal concentrations.

Design

2 species x 3 metals x 5 metal concentrations + control x 3 replicates = 96 units (25 seeds per unit).

Procedure

300 g JIPC sieved to <4.0 mm was placed in 3.5" plant pots. Stock solutions were made for Pb ($Pb(NO3)2$), Zn ($ZnSO4.7H2O$) and Cd ($CdCl2.2.5H2O$). Three replicates were prepared for each final metal concentration: 50, 500, 1500, 2500 & 3500 $\mu g.g^{-1}$ Pb and 10, 50, 200, 500 & 1500 $\mu g.g^{-1}$ Zn and 2, 5, 10, 12 & 15 $\mu g.g^{-1}$ Cd. The soil in each pot was mixed thoroughly with the appropriate volume of stock metal solution to homogenize the mixture inside the pots. Subsequently pots were stood in plastic saucers and sub-irrigated with distilled water until field capacity was reached. 25 seeds of each species, <u>M. verna</u> and <u>T. alpestre</u> (Bradford Dale) were placed on the surface of the soil in each pot.

All pots were randomized within the experimental area after each pot had been covered with a plastic lid to prevent the surface soil drying out during the early stages of germination. The pots were moved around within the experimental area approximately every 4 days to minimize the centre and edge effects. Germination was monitored and seedling mortality recorded. One destructive harvest of shoot and root dry matter was made after 11 weeks growth. Plant material was washed thoroughly and rinsed in several changes of distilled water. Although the root system was handled with great care, it proved impossible to remove the entire system from the soil matrix. Plant material was oven-dried at 80°C for 24 hours and, as it proved very difficult to separate the roots of one plant from another, the shoots and roots within each pot were bulked separately, weighed and analyzed for Pb or Zn or Cd using the same techniques described in Chapter 4.

Expt. IX Metal (Pb, Zn, Cd, Al, Ag, Co, Cr, Cu, Fe, Mo, Mn, Ni) uptake by five different populations of T. alpestre.

The objectives, design and procedure of this Experiment are all reported in Expt. VII.

5.5 RESULTS AND DISCUSSION

5.5.1 Germination responses

Results of the two germination response tests (Expts. I & II) are summarized in Figure 5.2 a,b,c and Figure 5.3 a,b,c respectively. For both populations of M. verna and also the single population of T. alpestre, there were no significant differences in final percentage germination between all five concentrations of Zn used in Expt.I, with the latter species showing a considerably higher final percentage germination (98-100%), compared to 50-60% for the former species.

High seed viability and rapid germination of T. alpestre is typical of this species (Grime et al., 1981). Riley (1956) reported 94% germination in his seed samples. Ernst (1965) found a greater percentage and rate of germination with M. verna, T. alpestre and three other plant species from zinc-contaminated sites when germinated in 50 $\mu g.ml^{-1}$ Zn as compared to the control solution (1 $\mu g.ml^{-1}$ Zn). When the present data were subjected to two-way ANOVA, no significant effect of Zn concentration was found on either the rate or the final percentage germination achieved by either species. The results largely agree with those obtained by Ernst (1965) but in Ernst's experiment an effect of increasing Zn concentration above 50 $\mu g.ml^{-1}$ was demonstrated. Ernst

Figure 5.2 Germination response of *M. verna* and *T. alpestre* seed to Zn treatments.

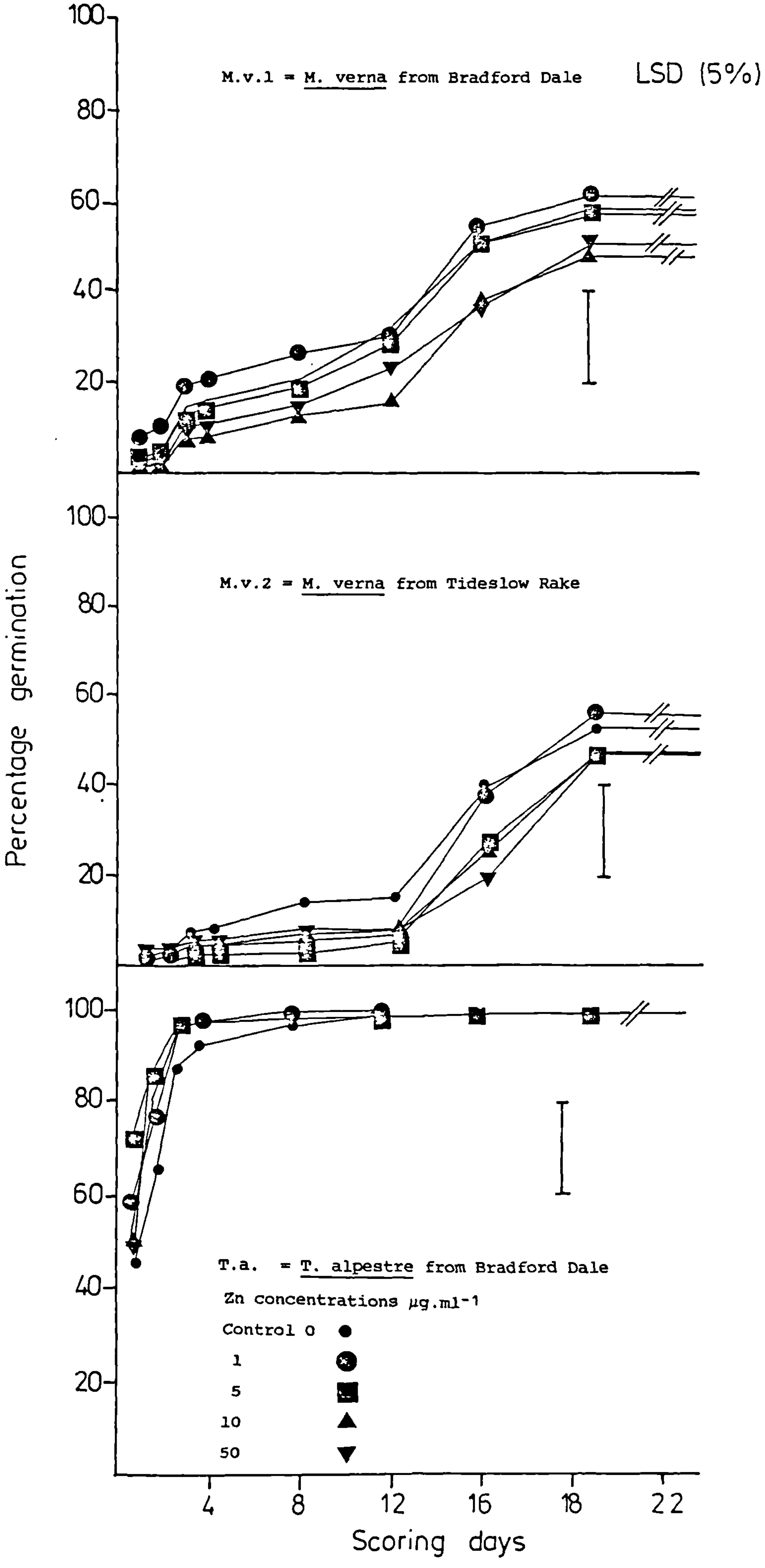
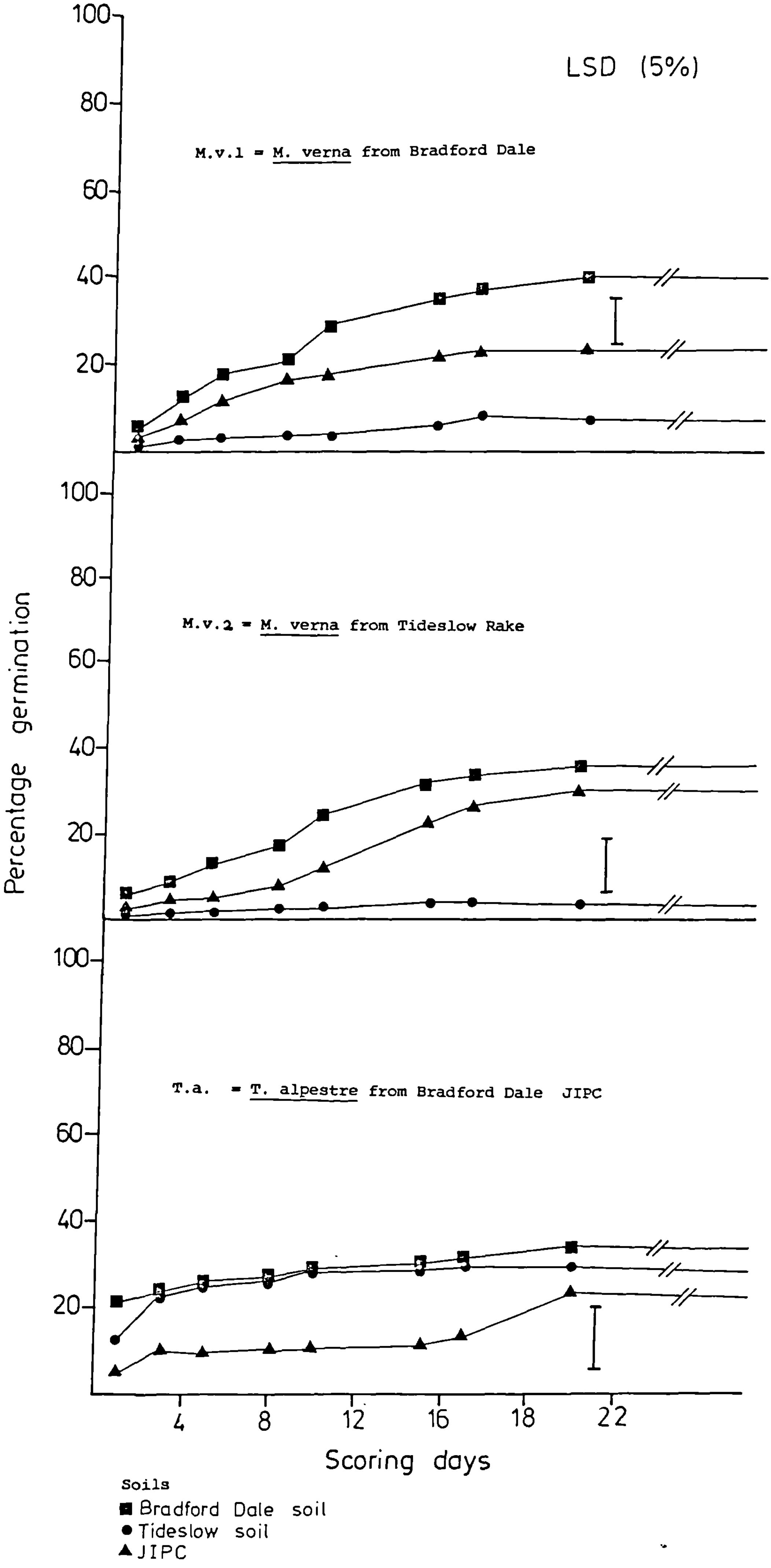


Figure 5.3 Germination response of *M. verna* and *T. alpestre* seed to metal-contaminated soils.



(1965) also reported that in relation to the control, the percentage germination of *M. verna* was decreased to less than 50% at 500 µg.ml^{-1} Zn and to 7.5% at 10,000 µg.ml^{-1} Zn, whereas the percentage for *T. alpestre* was 88.1% at the former concentration and 21.1% at the latter. Such high Zn concentrations however do seem physiologically unrealistic. The effect of increasing toxicity on seed germination percentage was also reported by Walley, Khan & Bradshaw (1974) for 'Cu soil' and 'Zn soil' in commercial seed samples of the grass *Agrostis tenuis*; for 'Cu soil' in the grasses *Arrhenatherum elatius* and *Festuca rubra* (Karataglis, 1980) and for Cr, Al, Cu and Zn in *Cynodon dactylon* (Wu, Huff & Johnson, 1981). Baker *et al*. (1983) reported that 50 µg.ml Cu delayed and reduced germination of two species of *Silene*, while Co had less effect. However Baker (1974) found no significant Zn treatment or population effects with reference to germination of *Silene maritima* seeds from tolerant and non-tolerant populations. He concluded that the initial stages of seed germination were basically physical processes unaffected by zinc treatment; radicle elongation and subsequent seedling development and survival were however a function of zinc treatment.

Observations by Wu, Huff & Johnson (1981) also showed that seed germination responded to metal toxicity with less sensitivity than root growth. Furthermore, Wong & Bradshaw (1982), using solution culture, measured the effects of Al, Cd, Cr, Cu, Fe, Hg, Na, Ni, Pb and Zn on the germination and growth of *Lolium perenne*, and no clear relationships were found between seed germination and primary effects of metals on root extension. They concluded that to manifest the effect of heavy metals on germination, a much higher concentration than that needed to inhibit root elongation is required. Finally, Shaw (1984) found that the conditions on contaminated soil had no significant effect either on the rate of germination or on the final percentage achieved by the majority of the species she screened. Nevertheless the same author reported that two of the species, Dactylis glomerata and Trisetum flavescens, did respond to the toxic soil with a decrease in germination percentage.

The second experiment in this series (Expt. II) was designed to investigate possible germination responses of the two species on contaminated and uncontaminated soils, similar to Shaw's soil experiments. The results of this screening are shown in Fig. 5.3 a,b, & c. Both species germinated faster and produced a higher final percentage germination on Bradford Dale soil when compared to the responses on Tideslow Rake and control (JIPC) soils. For the Bradford Dale population of M. verna these differences were highly significant at most scoring dates. Anomalously, both populations of M. verna germinated poorly on Tideslow soil, suggesting that factors other than metal toxicity could be responsible. However, T. alpestre behaved rather more predictably, producing significantly greater germination on the two metalliferous soils by comparison with the JIPC, although the final percentage germination on all soils was low and did not differ significantly. The experiment thus produced some unexpected results in that stimulatory effects of Bradford Dale soil were suggested in both species and both populations (native and non-native) of M. verna. The poor germination of T. alpestre on soils contrasts markedly with its germination behaviour in Expt. I, suggesting that factors other than the chemical nature of substrate may be important.

Evidence in the literature that contaminated soils affect seed germination is inconclusive. Allen & Sheppard (1971) found no difference in the germination of Cu-tolerant races of Mimulus guttatus on normal soil and there are other similar reports for other species, e.g. Agrostis tenuis (Walley et al., 1974; Karataglis, 1980), Betula papyrifera (Cox, 1979) and Achillea millefolium, Agrostis capillaris, Anthoxanthum odoratum and Briza media (Shaw, 1984).

These reports and observations recorded in the literature in addition to what has been found in the present work, lead to the general conclusion that of the effect of heavy metals on seed germination may depend on all or some of the following factors: the species, the metal used, metal concentration, substrate conditions and the method of testing. However, metallophyte species such as M. verna and T. alpestre may require very high metal concentrations before showing any response as suggested by the work of Ernst (1965). Comparisons between the two species shows that T. alpestre can have the higher rate and final percentage germination. The 'normal' pattern of seed germination (as observed in Expt. I) appears to differ markedly between the two species. In T. alpestre germination was rapid and virtually complete (95%+) in 3-4 days; in M. verna germination was slower and incomplete (50-60%) even after 21 days. Some periodicity of germination in this species was thus suggested. Both species grow in stressed environments where substrate problems, notably superficial drought, may affect and control seed germination in the field (as suggested in Expt. II). The differing germination characteristics of the two species can be viewed in the light of these problems. Rapid responses to favourable conditions for seed germination and high germination capacity could be viewed as one strategy for successful seedling establishment on mine spoils and similar substrates subjected to periodic drought. Periodicity in germination affected through the maintenance of a seed bank in the soil, could also be viewed as a successful insurance policy against drought and other environmental stresses. The significance of these observations are considered in greater detail in Chapter 6, where seedling establishment of both species was monitored under field conditions.

5.5.2 Metal tolerance

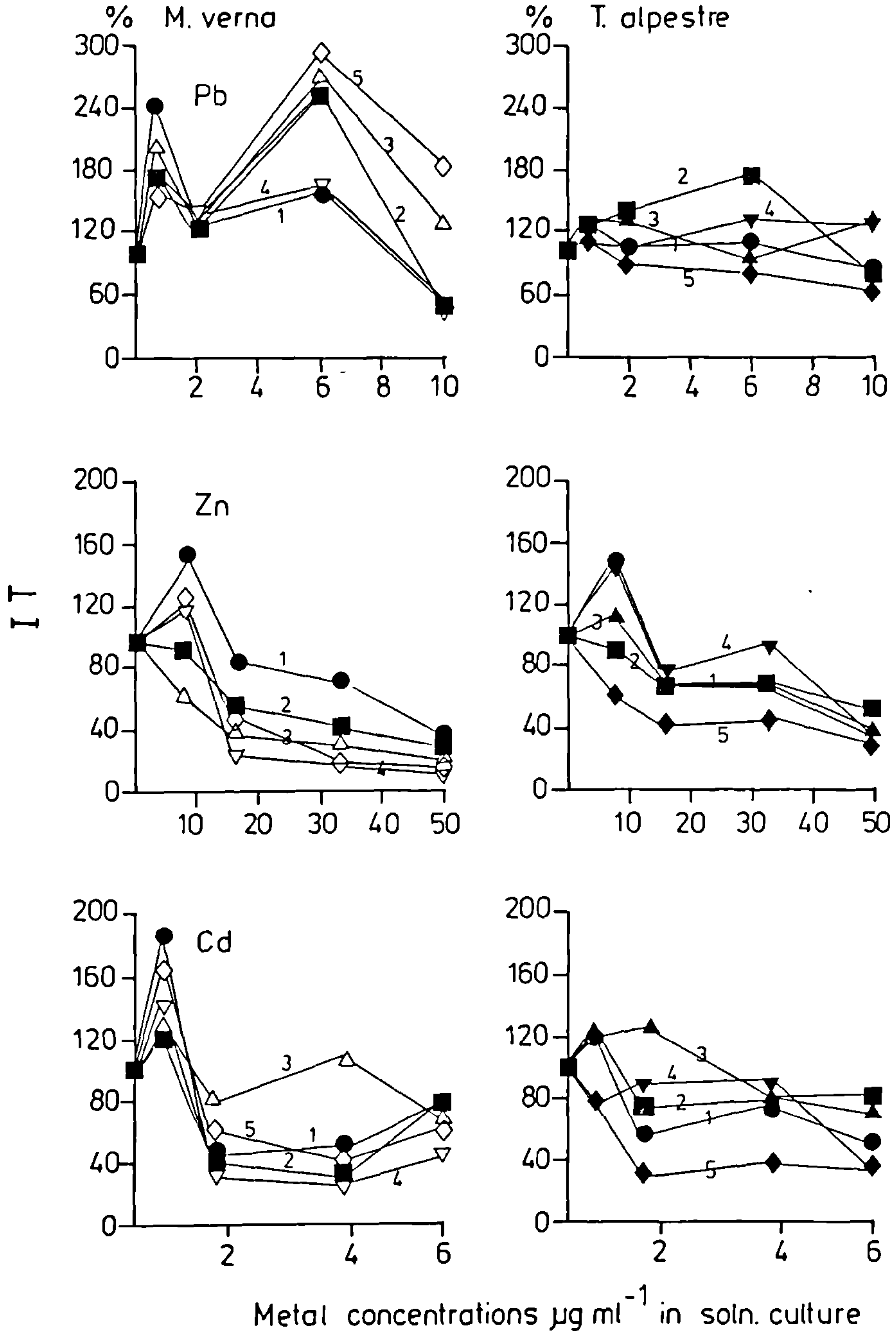
5.5.2.1 Seedling responses

The results of Expts. III-VII are presented in Figures 5.4, 5.5 & 5.6 to illustrate for each species the effects of Pb, Zn, Cd, Al, Ag, Co, Cr, Cu, Fe, Mn, Mo and Ni on root elongation. Additionally, Figure 5.8 summarizes the effects of these metals on the root length of 5 populations of T. alpestre. The significance levels related to the effects of metal concentrations, differences in populations or species response derived from the ANOVA tables are given in Appendix 4.1. In all results of IT tests, ANOVA showed a significant difference in the response of M. verna and T. alpestre. In those experiments involving more than one population, the differences between these were significant at $p<0.05$. In general terms, the results seem to suggest some significant inter - and intra-specific differences in heavy metal responses.

5.5.2.2 Effects of single metals

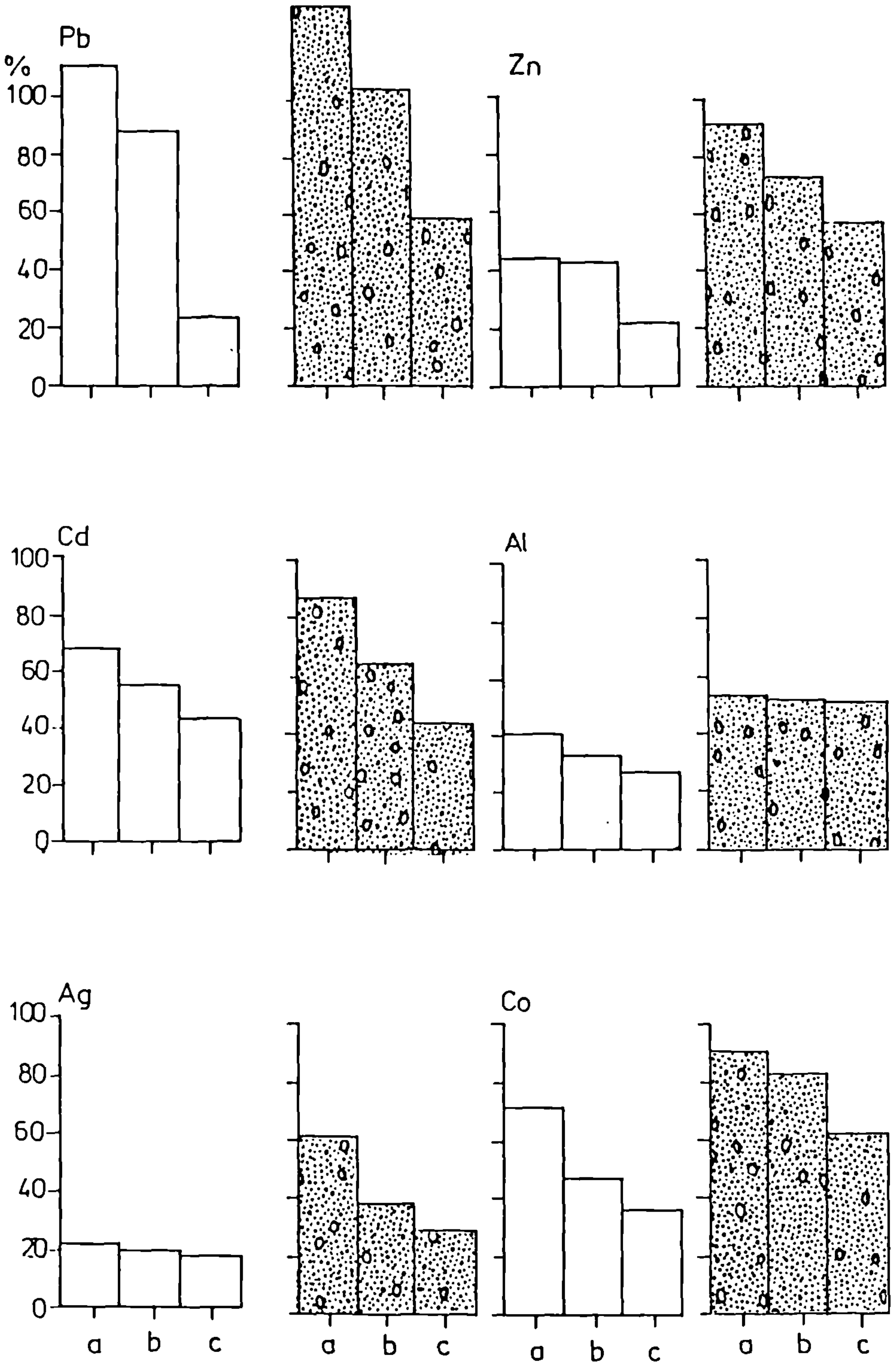
The elevated concentrations of Pb, Zn & Cd in the lead-mine spoils which have been studied are evident from Chapter 4. However both total and extractable concentrations did vary substantially from site to site (Table 4.3). It thus remained to ascertain to what extent such variations were reflected in plant response. Experiment III was designed to screen for Pb, Zn and Cd tolerance in 5 populations of M. verna and T. alpestre (Table 5.1).

Figure 5.4 Root elongation response (IT) of five different populations of M. verna and T. alpestre to heavy metals (Pb, Zn & Cd) treatments in solution culture.



Populations of M. verna	Populations of T. alpestre		Metals & concentrations $\mu g.ml^{-1}$ Pb	Zn	Cd
1–Black Rocks	Black Rocks	●	0.6	8	0.5
2–Bradford Dale	Bradford Dale	■	2.0	16	1.5
3–Dirtlow Rake △	Bonsall Moor	▲	6.0	33	4.0
4–Grattondale ▽	Wensleydale	▼	10.0	50	6.0
5–Tideslow Rake ◇	Whitesike	◆			

Figure 5.5 Root elongation response (IT) of *M. verna* and *T. alpestre* to a range of heavy metals treatments in solution culture.



Metals and concentrations µg.ml^{-1}

	Pb	Zn	Cd	Al	Ag	Co	Cr	Cu	Fe	Mn	Mo	Ni
a -	10	50	2.5	5	2	2	1	10	10	10	15	3
b -	15	100	5	20	5	5	3	20	20	20	30	8
c -	20	250	10	40	10	9	5	5	35	35	60	15

Species

M. verna □

T. alpestre ▨

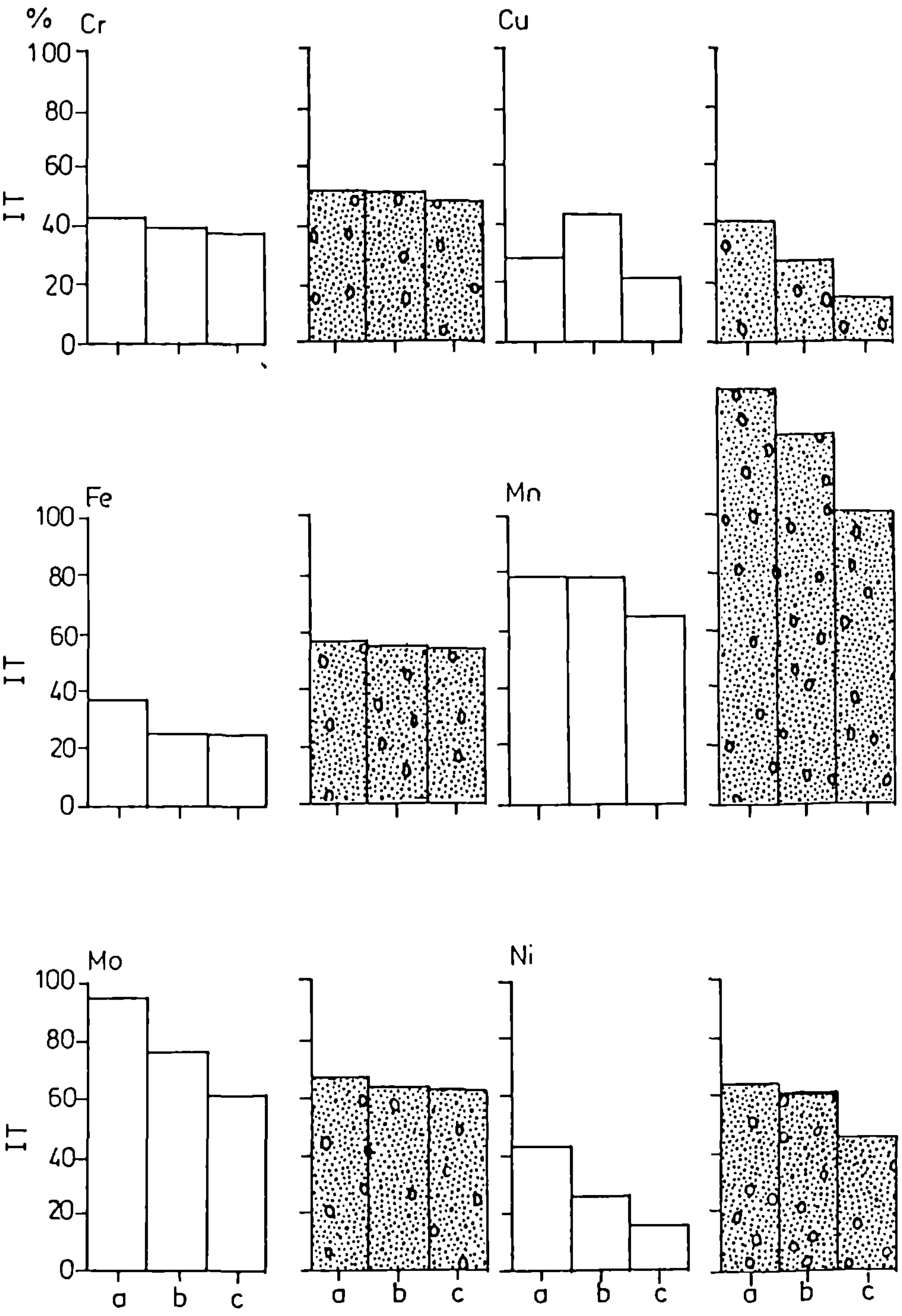
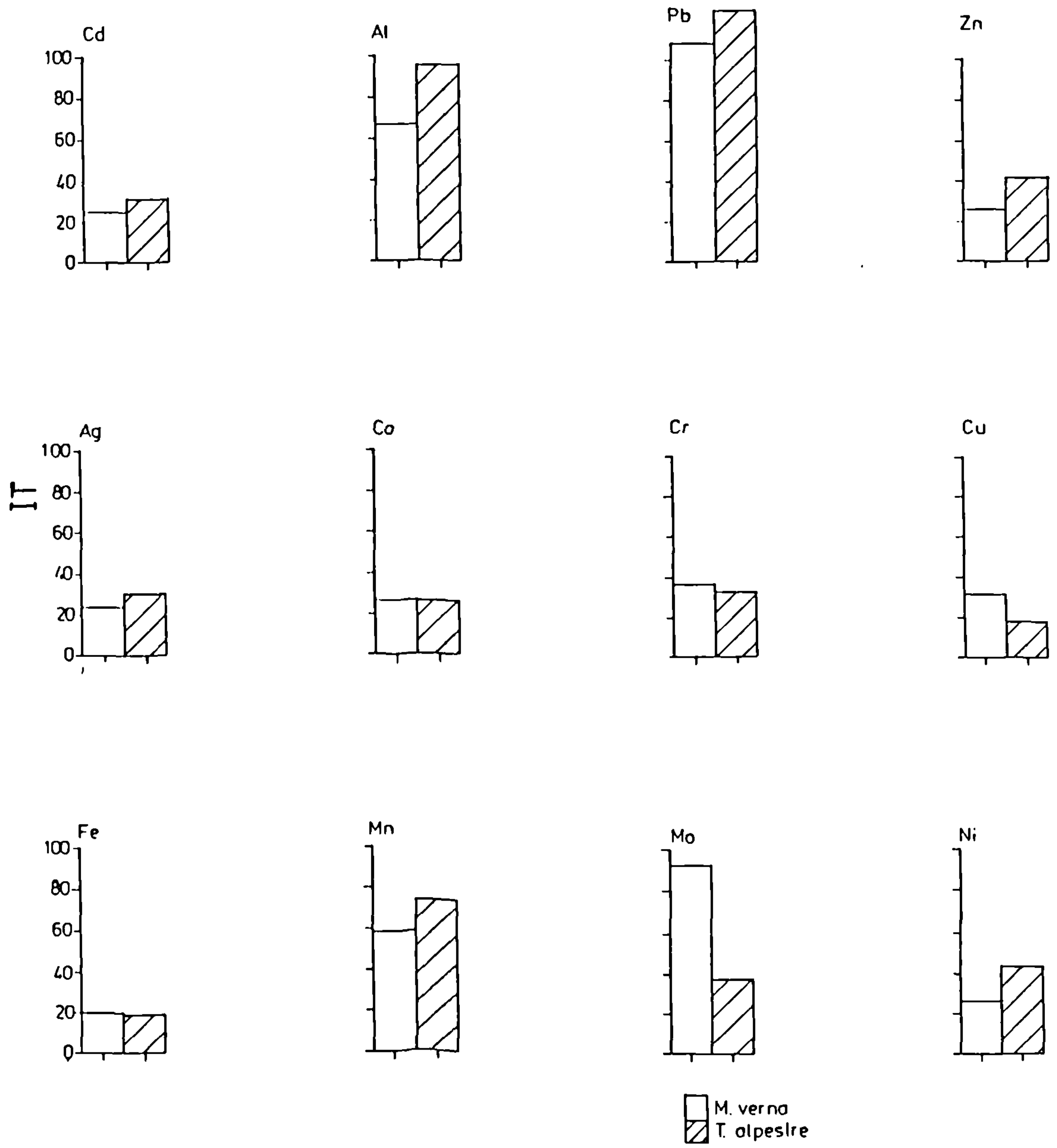

%
Cr
Cu
Fe
Mn
Mo
Ni
IT
100
80
60
40
20
0
a
b
c

Figure 5. 6 Root elongation response (IT) of *M. verna* and *T. alpestre* to a range of heavy metals treatments in solution culture.



Metals and concentrations $\mu g.ml^{-1}$

Pb	Zn	Cd	Al	Ag	Co	Cr	Cu	Fe	Mn	Mo	Ni
20	50	10	20	2	1	1	5	10	20	15	3

In tolerance experiments one of the principal objectives should be to use a range of metal concentrations in solution, which could be related to those levels the plants are likely to experience in the soils from which they were sampled. This raises the problems of interpreting the relationship between 'available' metals in the soil and in the solution culture. The concentrations employed were thus determined empirically. The results of tolerance tests summarized in Figure 5.4 showed the effects of Pb, Zn & Cd and their different concentrations on the root elongation of both species studied. In general the order of toxicity was Cd > Zn > Pb. These findings are in agreement with results reported by Shaw (1984) but for Lolium perenne, Wong & Bradshaw (1982) quote the order of toxicity for these three metals as Pb > Cd > Zn.

Within the lead treatments, all populations of M. verna and most of T. alpestre populations responded positively when low concentrations were added, thus a stimulatory effect of Pb on the root elongation was demonstrated in the 0.6, 2 and 6 $\mu g.ml^{-1}$ treatments. The same effect was observed in two populations of each species at 10 $\mu g.ml^{-1}$ Pb. Although there was a positive effect of Pb on both species particularly at the three lower concentrations, the root growth of M. verna was stimulated to a greater extent than that of T. alpestre. At the highest lead concentration the decrease in the root growth of M. verna from two populations (Black Rocks & Bradford Dale) was almost 50% whereas in T. alpestre from the same sites IT's of about 80% were recorded. However at the 10 $\mu g.ml^{-1}$ Pb level, two populations of M. verna (Dirtlow and Tideslow Rake) and two of T. alpestre (Bonsall Moor and Wensleydale) still showed stimulatory responses and IT's in excess of 120%. One could postulate that when root growth of M. verna can be stimulated by low concentrations of Pb more than T. alpestre, the latter species is more tolerant of the higher Pb concentrations. Thus the concentrations of Pb added caused stimulations and reductions in the root growth despite the likely problem of precipitation.

Within zinc treatments, root growth of M. verna from four different populations was stimulated by the addition of 8 $\mu g.ml^{-1}$ Zn whereas this was stimulated in T. alpestre in three populations. By contrasting both the stimulatory and inhibitory effects of Zn with those of Pb, the results seem to suggest that Zn has less of the former and more of the latter effects at the ranges of metal concentration employed. The consistent decrease in root growth of M. verna from all populations with increasing Zn concentration is not apparent to the same degree in the T. alpestre populations. Although there was a noticeable decrease in IT at 16 $\mu g.ml^{-1}$ Zn, no differences have been detected in the response of three populations of T. alpestre grown at this and the 33 $\mu g.ml^{-1}$ Zn, concentration. At the highest concentration (50 $\mu g.ml^{-1}$), the decrease in the IT's of M. verna was more in most populations than any population of T. alpestre. Thus the results seem to support the idea of the special relationship between the latter species and Zn. In M. verna from Black Rocks, the high IT to Zn (Figure 5.4) corresponded with the high Zn concentration in the soil from the same site (Table 4.3).

In the lowest Cd treatment (0.5 $\mu g.ml^{-1}$), root growth was stimulated in all M. verna populations and in two populations of T. alpestre. In higher Cd treatments, varying degrees of root growth inhibition were reflected in the IT values of almost all populations of both species. Overall significant differences between species, populations, Cd treatments and the interaction terms, were revealed in the full ANOVA tables (Appendix 4.1). In most populations of both plants IT was higher than 50% even in the highest Cd concentrations which might suggest that both species are highly tolerant to Cd, a metal known to produce inhibitory growth responses at concentrations less than 1 $\mu g.ml^{-1}$ even in Cd-tolerant Holcus lanatus (Coughtrey & Martin, 1977).

The relationships between IT and the exchangeable and total soil metal (Pb, Zn & Cd) concentrations at the sites from which the studied populations originated were tested and the results presented in Table 5.3. No significant positive correlations were found for Pb in both M. verna and T. alpestre. However there was one significant negative correlation ($r = -0.93$, $p < 0.01$) between the IT of T. alpestre plants (10 $\mu g.ml^{-1}$ Pb treatment) and the exchangeable Pb concentrations in the soil. For Zn, there was only one significant positive correlation ($r = 0.87$, $p < 0.05$); this was between IT of M. verna populations (33 $\mu g.ml^{-1}$ Zn treatment) and the exchangeable Zn in the soil. There were no significant positive correlations found between IT of T. alpestre and the exchangeable or total Zn concentrations in the soil. However there were positive trends in the relationships; these were clearer between IT and the exchangeable Zn concentrations in the soil.

In all Cd treatments, no significant positive correlation was found. A significant negative correlation ($r = -0.82$, $p < 0.05$) was found between IT of M. verna (1.5 $\mu g.ml^{-1}$ Cd treatment) and the exchangeable Cd concentrations in the soil. For T. alpestre two significant positive correlations ($r = 0.83$, $p < 0.05$: 4 $\mu g.ml^{-1}$ Cd treatment) and $r = 0.97$, $p < 0.01$: 1.5 $\mu g.ml^{-1}$ Cd) between the IT and the exchangeable and total Cd concentration in the soil respectively, were demonstrated.

Relationships between index of tolerance and metal concentrations in the soil have been suggested before by other workers Gregory & Bradshaw, 1965; Antonovics, Bradshaw & Turner, 1971; Wilkins, 1978;

Table 5.3 The relationships between metal tolerance and exchangeable and total metal (Pb, Zn and Cd) concentrations in the soil and indices of tolerance in five populations of M. verna and T. alpestre. Product-moment correlation coefficients (r) are shown, together with their significance values (n = 5)

M. verna		Exchangeable	T. alpestre	
Pb treatments μg.ml^{-1}				
	r	p	r	p
0.6	= -0.13	NS	= 0.65	NS
2.0	= 0.13	NS	= 0.54	NS
6.0	= 0.10	NS	= 0.31	NS
10.0	= -0.49	NS	= -0.93	<0.01
Zn treatments μg.ml^{-1}				
8	= 0.77	NS	= 0.62	NS
16	= 0.77	NS	= 0.16	NS
33	= 0.87	0.05	= -0.054	NS
50	= 0.67	NS	= -0.35	NS
Cd treatments μg.ml^{-1}				
0.5	= -0.09	NS	= -0.15	NS
1.5	= -0.82	0.05	= 0.36	NS
4.0	= -0.64	NS	= 0.83	<0.05
6.0	= -0.61	NS	= -0.27	NS
		Total Concentrations		
Pb treatments μg.ml^{-1}				
0.6	= -0.019	NS	= -0.66	NS
2.0	= -0.33	NS	= 0.081	NS
6.0	= -0.15	NS	= 0.25	NS
10.0	= -0.33	NS	= -0.61	NS
Zn treatments μg.ml^{-1}				
8	= 0.54	NS	= 0.73	NS
16	= 0.15	NS	= 0.71	NS
33	= 0.048	NS	= 0.57	NS
50	= -0.13	NS	= 0.73	NS
Cd treatments μg.ml^{-1}				
0.5	= -0.20	NS	= 0.50	NS
1.5	= -0.69	NS	= 0.97	<0.01
4.0	= -0.66	NS	= 0.64	NS
6.0	= -0.80	NS	= 0.59	NS

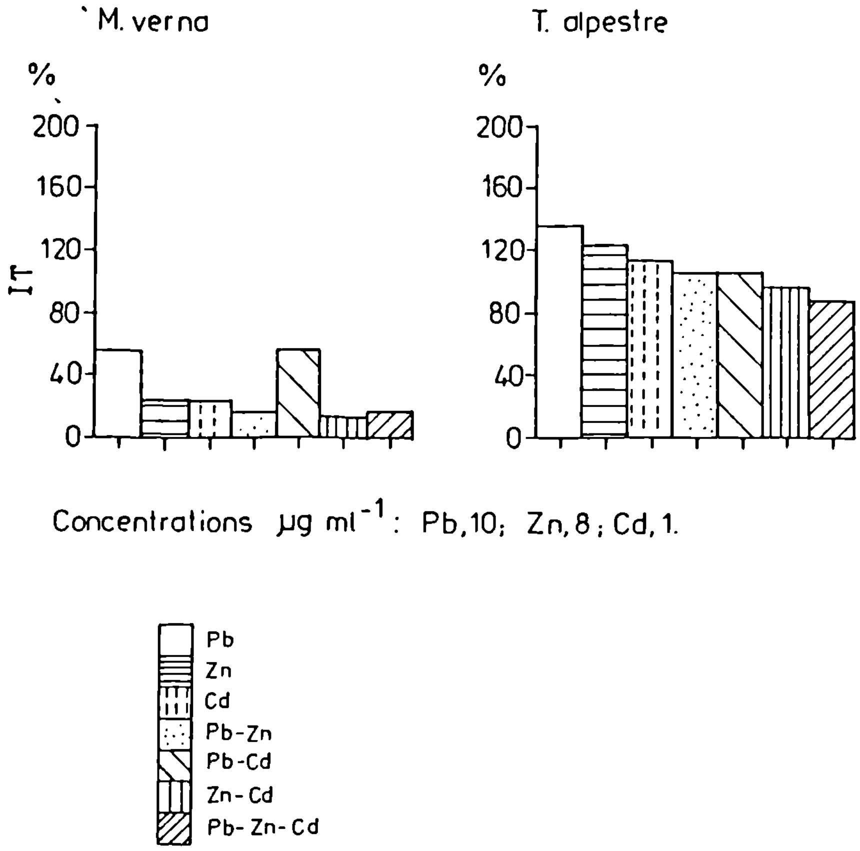
Atkins, Trueman, Clark & Bradshaw, 1982 and Ingrouille & Smirnoff, (1986). The last-mentioned authors reported a significant positive correlation ($r = 0.97$, $p < 0.01$) between IT (derived from dry weight measurements) of four populations of T. alpestre and the exchangeable Zn concentrations in the soil. In the present study, although no similar relationship for this plant was demonstrated unequivocally the trend was in agreement with the correlation reported by Ingrouille & Smirnoff (1986). However it must be stressed that the IT's in the present work and those of these authors cannot be compared directly because of the differing experimental conditions employed.

The present results show that metal tolerance differs between populations and treatments, especially for M. verna which shows more variability at the highest metal treatments. That significant positive correlations can be demonstrated between IT of both species and Cd concentrations in the soil, and the positive trends for Zn suggest that the degree of metal tolerance in the populations of M. verna and T. alpestre studied can be related to the concentration in their soil of origin. However the facts that such correlations are far from perfect and that no clear relationships could be shown for Pb, suggest that metal tolerances in M.verna and T. alpestre (as suggested by the root elongation technique) are complex physiological traits not necessarily developed in direct response to local conditions of metal concentration and availability.

5.5.2.3 Effect of metals supplied in factorial combination (Expt. IV)

The results of this experiment are presented in Figure 5.7. Factorial ANOVA for root-length data showed very highly significant effects (0.1%) for species, Zn, Cd and the interaction between species

Figure 5.7 Root elongation response (IT) of *M. verna* and *T. alpestre* to single and combined treatments of Pb, Zn and Cd in solution culture.



and Zn, Cd, Pb-Zn, Pb-Cd, Zn-Cd and Pb-Zn-Cd treatments. No significant differences were shown for the following: Pb, Pb-Zn, Pb-Cd and Pb-Zn-Cd, or the interaction between species and Pb.

In the discussion of the previous experiment it was suggested that the toxic effect was in the order Cd > Zn > Pb. in the present experiment the order seems to be almost the same. Thus the data for IT values in both experiments correspond with each other in the main with only one anomalous result, in which the 8 $\mu g.ml^{-1}$ Zn treatment decreased root growth of *M. verna* to almost 25%, whereas in the previous experiment, the same concentration produced a stimulatory response. For *T. alpestre* there was a significant stimulation of root growth by singly added metals, the highest being in Pb treatment. This result also seems to confirm the results of the previous experiment.

Single metal treatments were used to compare their effect with the effect of the combined treatments to reveal additive or synergistic effects. In the factorial treatments, the combinations of PbZn, ZnCd and PbZnCd have the most drastic effect of all on root growth of *M. verna*, with a trend of a decreasing effect on root growth of *T. alpestre* by the last two combinations, indicating a positive interaction between metals in these treatments. Root growth of *M. verna* was almost equal in Pb and PbCd treatments, which suggests some amelioration of Cd toxicity by Pb. However for all other combined treatments in *M. verna* and for *T. alpestre*, IT values are less than in any of the single metal treatments, suggesting additive effects on the root growth. The effects of both individual and combination treatments were more pronounced for *M. verna* root growth than for *T. alpestre*, so that IT's for *M. verna* were in most treatments less than 40%, whereas the lowest IT for *T. alpestre* was more than 80%.

There have been numerous studies of the effects of Pb, Zn and Cd on the growth of different plant species, but very few of these studies have concentrated on the effects of these metals in combination. Most of these investigations however have been on crop plants, and few have employed a factorial design in tolerance experiments. Such a design has been employed by Coughtrey & Martin (1978; 1979) in their investigations of the interactions of Pb, Zn and Cd on tolerance and uptake in two populations of Holcus lanatus L. Other experimental studies suggesting possible synergistic effects of Pb, Zn & Cd have been described by Alloway (1969), Carlson & Bazzaz (1977), Dijkshoorn, Lampe & Kowsoleea (1975), Hassett, Miller & Koeppe (1976). Miller, Hassett & Koeppe (1977) found a positive interaction of Pb and Cd in their effect on growth and metal uptake in Zea mays L. The results presented here together with most of the previous observations reported suggest that T. alpestre is more tolerant to Pb, Zn and Cd both singly and when supplied in combination. The results also suggested that the effects of metal interactions were clearer in M. verna.

5.5.2.4 Tolerance to other heavy metals

As the three metals Pb, Zn and Cd are the major contaminants of lead-mine habitats, most experimental work was concentrated on these three. The occurrence of other heavy metals (e.g. Co, Cu, Fe, Mn & Ni) in mineralized substrates colonized by M. verna and T. alpestre has recently been reported by Garcia-Gonzalez & Clark (1985). These metals and Pb, Zn, Cd Al, Ag, Cr, & Mo were used in Expts. V, VI & VII. Kelepertsis & Andrulakis 1983 and Kelepertsis, Andrulakis & Reeves, 1985 have reported Zn, Pb, Cu, Mn, Fe, As, Co, Cr, Mn & Ni in M. verna and the soil in which this plant grows in N. Greece.

Analysis of soil samples from lead mine sites at which seeds have been collected (Table 5.3) indicated the presence of most of these metals, some of them at elevated concentrations.

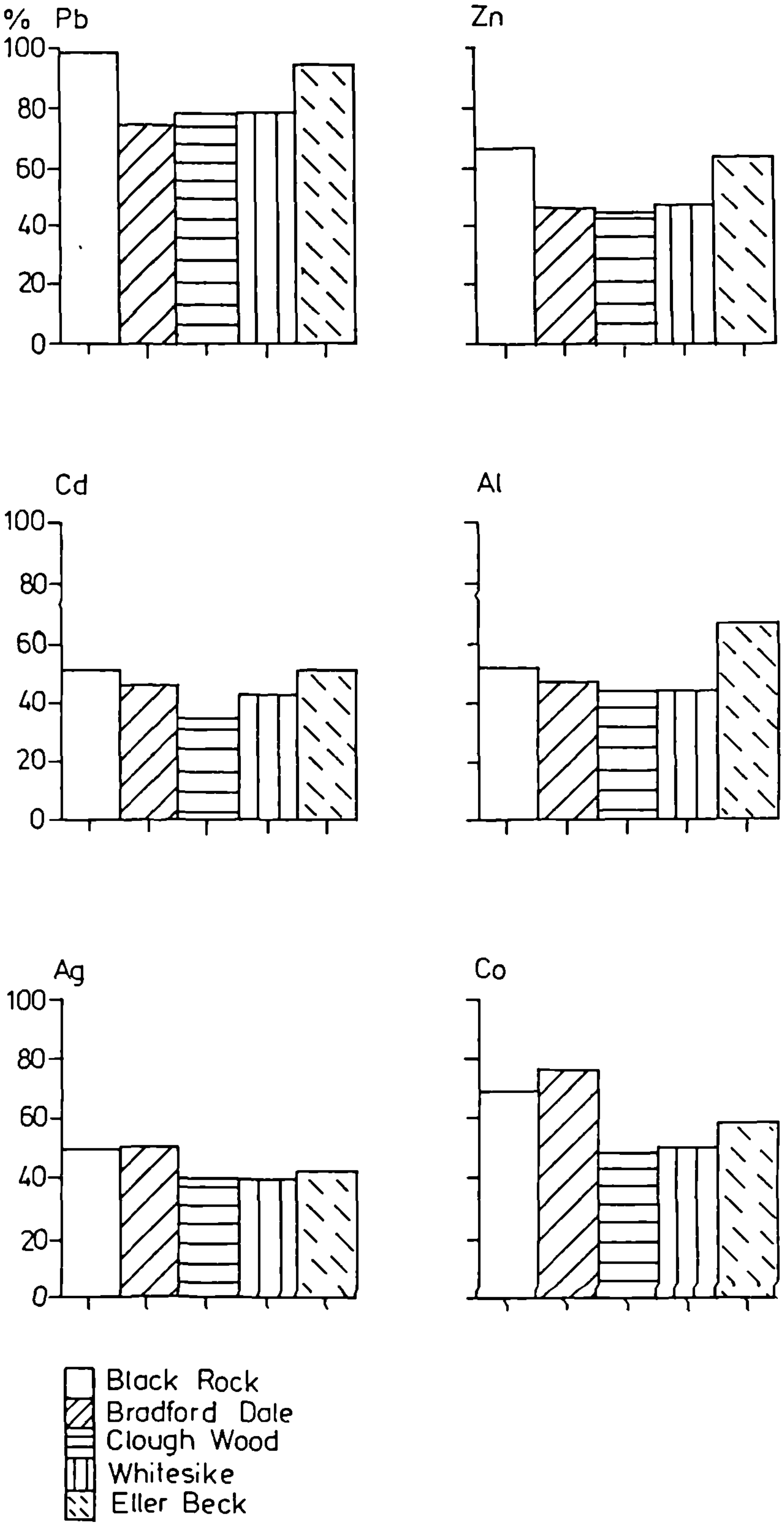
In the first experiment of these trials (Expt. V) ANOVA (Appendix 4.1) showed that there were highly significant differences at 0.1% for species response and the interaction between species and Mn, Pb, Cu, Ag, Mo, Cd & Zn, and at 1% for Co and at 5% for Ni, Cr, Fe & Al. Results presented in Figure 5.5 show that for all metals except Cu and Mo, <u>T. alpestre</u> seems to be more tolerant than <u>M. verna</u>. In general, IT's of both species decreased with the increase in the concentrations of metals, the increasing inhibitory responses of some elements (e.g. Cd, Co & Ni) being more pronounced than others (e.g. Fe & Al). The metal concentrations used are within the range for most metals considered to be toxic to the majority of plant species. The root growth achieved by both species, particularly <u>T. alpestre</u>, could be an indication of their high degree of tolerance to a wide range of metals. The effect of the metal treatments on root growth of the two species ranged from a considerable decrease (87%) in <u>T. alpestre</u> root growth at 50 $\mu g.ml^{-1}$ Cu treatment to an increase of almost 150% for the same species in 10 $\mu g.ml^{-1}$ Mn.

Expt. VI, used one low concentration of each metal, a lower pH in the basal nutrient solution (5.5) and an increased number of days in the metal treatments. In general, the effects of treatments on both species corresponded with those observed in the previous experiment. Some differences in the effect of some of the metals were however observed, possibly due to the lower pH used in this experiment. The root growth of both species in the control treatment was two times greater in this experiment than it was in Expt. V.

The ANOVA Tables (Appendix 4.1) for the root length showed that there was a significant difference at 0.1% between species, metals and their interaction. Results of this experiment are presented in Figure 5.6. Greater inhibition of root growth has been demonstrated by most metals used, when compared with earlier experiments, probably due to enhanced metal toxicities at a lower pH. Nevertheless, 20 $\mu g.ml^{-1}$ Pb stimulated root growth of both species. Following the same trend, the inhibition of root growth in the 20 $\mu g.ml^{-1}$ Al treatment has been decreased by about 45% when compared to the effect of same concentration in the previous experiment. The decrease in pH appears not to have any effect on the relationship between the root growth of M. verna and Ag or Mo. The results for Cu and Mo confirm the observations from the earlier experiment. Although 5 $\mu g.ml^{-1}$ Cu was used instead of 10 $\mu g.ml^{-1}$ Cu, due to the drastic effect of this metal on both species (specifically on T. alpestre), the effect of Cu remained very strong. Although all these metals regarded as toxic at the concentrations used, results suggested that some are more toxic than others at least where root extension of M. verna and T. alpestre is concerned.

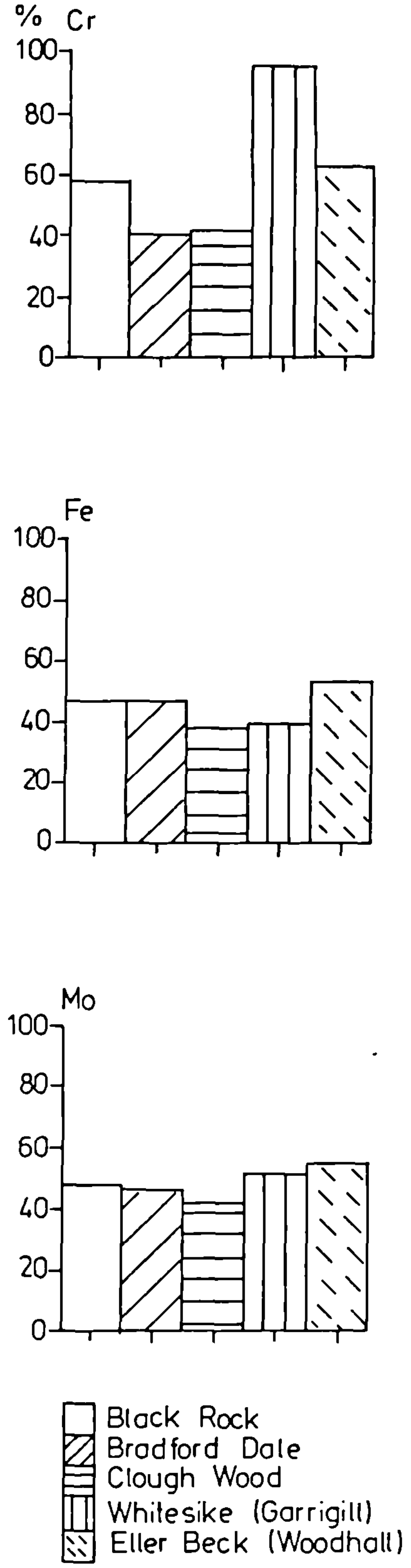
The final experiment in this series (Expt. VII) extended the previous two in a study of inter-population response to the 12 metals, using five populations of T. alpestre. IT's derived from root elongation are presented in Figure 5.8. Data for root elongation were analyzed by ANOVA. Treatment means and ANOVA tables are presented in Appendices 4 and 4.1 respectively. There were significant differences between populations at 0.1% level for all metals but Co. In general, the effect of all metals on T. alpestre from different populations in the present experiment followed the same trend as in the previous one.

Figure 5.8 Root elongation responses (IT) of five different populations of *T. alpestre* to a range of heavy metal treatments in solution culture.



Metals and concentrations $\mu g.ml^{-1}$

Pb	Zn	Cd	Al	Ag	Co	Cr	Cu	Fe	Mn	Mo	Ni
20	25	5	20	0.5	5	1	1	6	10	10	2


%
Cr
Cu
Fe
Mn
Mo
Ni
100
80
60
40
20
0
Black Rock
Bradford Dale
Clough Wood
Whitesike (Garrigill)
Eller Beck (Woodhall)

Soil samples from all the sites used in this experiment were analyzed for all the metals excepting Ag (see Table 5.4). The concentrations of most metals were shown to be higher than their background presence in the earth's crust. Garcia-Gonzalez & Clark (1985) have reported that Pb, Zn, Cd, Co, Cu, Fe, Mn & Ni are in abnormally high concentrations in the soil of six different habitats of M. verna and T. alpestre, with a tendency for the latter species to be present where the higher metal concentrations are found. They also suggested that consequently there is less variation in concentrations of metals between habitats of T. alpestre, whereas M. verna occurs on soils with widely differing levels of metals. However such a suggestion is not necessarily a valid generalization as the evidence could be circumstantial. In the present study the analysis of soils from different lead mines where both or only one species occur (Table 4.3) does not support this generalization.

An attempt to detect relationships between IT (Figure 5.8) and total metal concentration in the soil (Table 5.4), did not reveal any significant positive correlations for any of the 12 metals. One significant negative correlation was however found between IT and Mo concentrations in the soil, ($r = -0.94$, $p < 0.01$).

5.5.3 Metal uptake experiments

5.5.3.1 Heavy metal (Pb, Zn & Cd) uptake by M. verna and T. alpestre from soil culture. Expt. VIII.

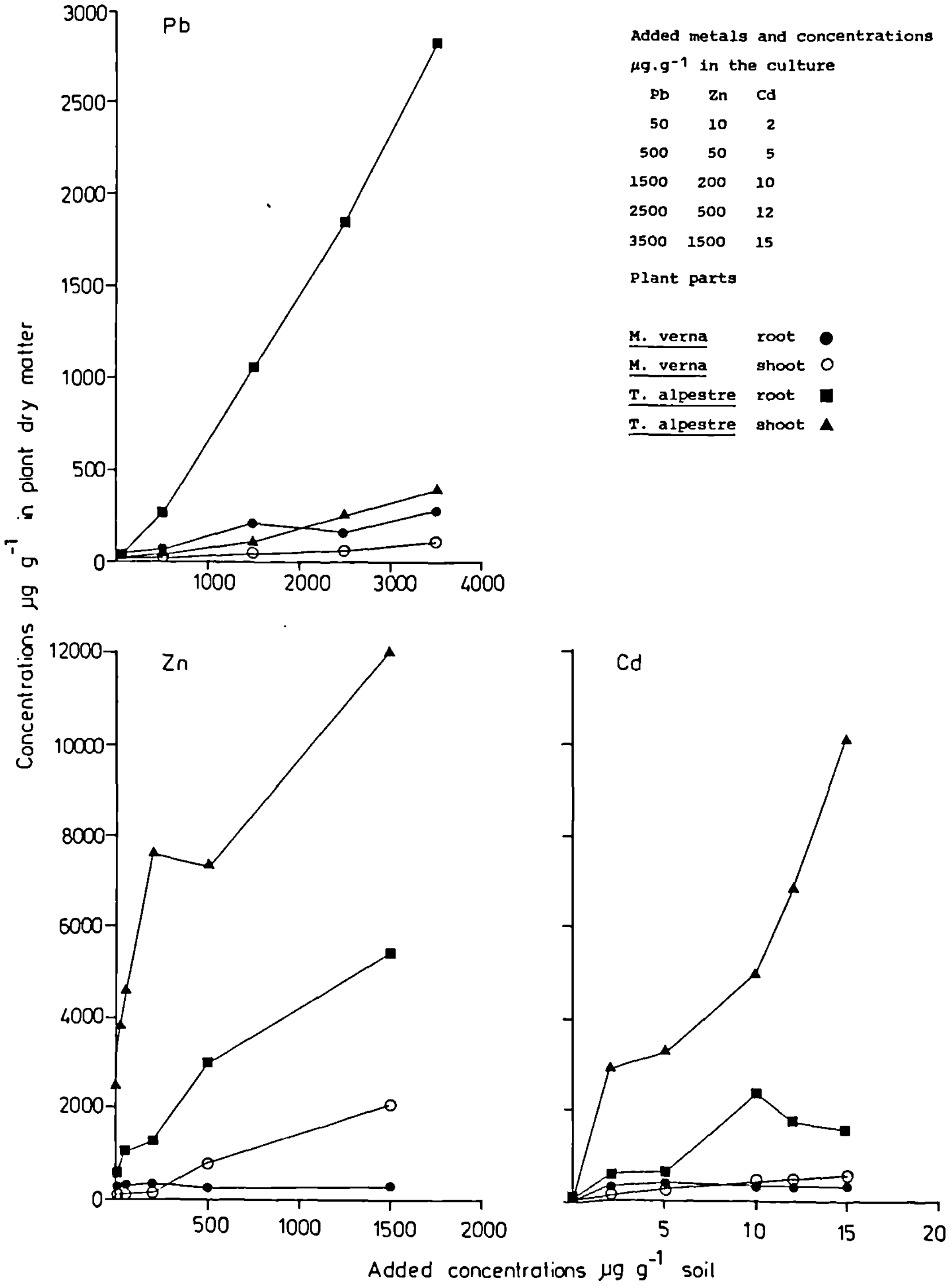
The trends in uptake of Pb, Zn & Cd by plants of both species grown for 11 weeks in metal-amended soil culture are shown in Figure 5.9. All yield and metal concentration data were analyzed by one - or two-way ANOVA as appropriate for the design of this experiment.

TABLE 5.4 TOTAL METAL CONCENTRATIONS ($\mu g.g^{-1}$ DRY SOIL) FOR ELEVEN METALS IN COMPOSITE SOIL SAMPLES FROM THE SITES OF THE POPULATIONS STUDIED

SITE	PB	ZN	CD	AL	CO	CR	CU	FE	MN	MO	NI
B.ROCK	39200	38000	348	17800	21	35	357	30100	695	24	74
B.DALE	53400	15300	143	8910	12	29	378	104000	473	34	105
C.WOOD	20400	22900	270	25400	34	50	281	44100	907	44	148
ELL.B.	28100	18100	132	19000	11	35	445	12200	483	<12	55
WHIT.S.	57000	7480	31	5500	50	129	336	38400	2760	14	141
Range in normal soils *	2-20	1-40	0.03- 10	10-200	0.05-4	10-200	0.1-3	50- 1000	5-500	0.01- 0.2	5-500

* From Allen et al. (1974)

Figure 5.9 Pb, Zn & Cd concentrations ($\mu g.g^{-1}$ plant dry matter) in M. verna and T. alpestre, grown in soil culture.



Added metals and concentrations $\mu g.g^{-1}$ in the culture

Pb	Zn	Cd
50	10	2
500	50	5
1500	200	10
2500	500	12
3500	1500	15

Plant parts

M. verna	root	●
M. verna	shoot	○
T. alpestre	root	■
T. alpestre	shoot	▲

ANOVA tables are presented in Appendices 4.1 . For plant weight, ANOVA revealed no significant differences in plant dry weight for either species in any of the Zn treatments. However root weight of M. verna was significantly reduced by the higher Zn amendments. In the Pb treatments, shoot and total dry weight of both species were significantly different (in the controls) at the 5% level but there were no significant differences in root weight. The effects of Cd treatments on root, shoot and total dry weight of M. verna were all significant at the 5% level; for T. alpestre these effects were significant only for root weight ($p < 0.001$).

In both species, the effects on metal uptake by an increase in substrate metal concentrations were more significant than on plant dry weight yield. The relationships between plant and soil metal concentrations are presented in Table 5.5. All relationships but two (M. verna root x soil Zn & Cd concentrations) were significantly positive. For Zn in the root of M. verna, the results suggest a constant relationship between root and soil concentrations. A similar result has been reported for Ni in Nothofagus fusca (Timperley, Brooks & Peterson, 1970). For Cd, although the general trend of the data indicated Cd concentration increase in the root of this plant, r was not significant, possibly because of transport to the shoot increased with the increase in the added metal, effectively decreasing the root-metal concentrations.

For metal uptake by shoots, the two-way ANOVA tables (Appendix 4.1) suggested significant treatment main effects for species and metals and their interaction, but for root metal uptake, treatment effects were significant for species only.

TABLE 5.5 THE RELATIONSHIP BETWEEN METAL (Pb, Zn & Cd) CONCENTRATIONS IN THE PLANT TISSUE AND IN THE SOIL

		M. verna		*T. alpestre*	
Metal	Plant Part	r	p	r	p
Pb					
	Shoot	0.98	<0.001	0.976	<0.001
	Root	0.92	=0.01	0.997	<0.001
Zn					
	Shoot	0.99	<0.001	0.92	<0.01
	Root	0.018	NS	0.98	<0.001
Cd					
	Shoot	0.995	<0.001	0.96	<0.01
	Root	0.51	NS	0.83	<0.05

Concentrations of heavy metals in plants from non-metalliferous sites are, in general, considerably lower than those in most plants, such as M. verna and T. alpestre, growing on metal-rich soils. It is clear that such metallophyte species have a greater ability to accumulate heavy metals than many other species; this characteristic has been reported in the literature. The metal concentrations reported in the present results are of a similar magnitude to those for field-collected plants (Chapter 4).

In all cases, metal concentrations in the roots and shoots of T. alpestre were significantly greater than in those of M. verna from the equivalent treatments. The magnitude of these differences generally increased with increasing metal amendment. Responses of the two species to Zn and Cd were broadly similar and differed from those to Pb. Thus for Pb, shoot concentrations of this metal were low and of similar magnitude in both species. The ability of the root of T. alpestre to accumulate Pb was however outstanding, when compared to root concentrations in M. verna, which were similar to shoot values. By contrast, for Zn and Cd, metal concentrations in the shoot of T. alpestre always exceeded those of the root; the same was true for Zn in M. verna in the higher soil treatments, but significant differences for Cd could not be demonstrated. The differences in species response were also shown by the values for Pb, Zn & Cd [shoot]:[root] ratios (Table 5.6). These were generally higher for Zn and Cd than Pb. The generally higher tissue concentrations of both Zn & Cd could reflect the greater mobility of both metals and for Zn, it could also possibly be connected with its essentiality, (Shaw, 1984). Low shoot concentrations of Pb suggest immobilization in the root or a general low uptake. The higher Pb concentrations in roots than shoot for both species is in agreement with the general observation that metals accumulate far more in the roots than in the shoots, possibly due to the restriction of metal transport from root to shoot, particularly in tolerant plants (Baker, 1981).

Frequently, shoots of tolerant plants are shown to contain lower concentrations of metals than non-tolerant counterparts (Wu & Antonovics, 1975; Maschmeyer & Quinn, 1976; Baker 1978a,b; Coughtrey & Martin, 1978 and Shaw, 1984). There are also many reports in the literature of similar or higher metal concentrations in the roots of tolerant plants (e.g. Turner & Marshall, 1972; Peterson, 1975; Maschmeyer & Quinn, 1976; Wu _et al_., 1975a; Baker, 1978a,b; Coughtrey & Martin, 1978; Hoiland & Oftedal, 1980; McGrath _et al_., 1980; Brookes, Collins & Thurman, 1981; Macnair, 1981a). Higher concentrations of Zn & Cd in the shoot than the root as encountered in the present study have also been demonstrated in other tolerant species and ecotypes (Garland & Wilkins, 1981; Shaw, 1984). However there is much evidence in the literature to suggest that plants of toxic metalliferous soils cannot prevent metal uptake but only restrict it and thus accumulate metals in their tissues to varying degrees (Peterson, 1971; 1975; Baker, 1981). The last author has thus concluded that, the strategies of survival are in general tolerance and not avoidance of metal toxicity. The same author has shown that among twelve species, _M. verna_ and _T. alpestre_ together with _Armeria maritima_ emerge as species with considerable powers of metal concentrations in their shoot (see also Table 4.1).

In order to find differences between species in metal uptake and distribution (and thus implicate possible different tolerance mechanisms), plants should be grown under the same standardized conditions, as in the present work. However it should be emphasized that comparisons between reports in the literature using various methods should be made with caution. Any results obtained on metal uptake and distribution are affected by the interaction of various soil factors e.g. pH, the form and concentration of the metal present, and duration of the treatments. Organic matter content and the cation exchange capacity can also influence metal uptake.

Some information on species response to soil metal concentration has been discussed in Chapter 4 for aerial plant parts collected from the field. Positive relationships between the increase in soil metal concentrations for Pb, Zn & Cd and shoot concentrations of M. verna were suggested, and also for Cd in T. alpestre. In the present more rigorous work, some positive soil-plant relationships were suggested (Table 5.5). Similar linear relationships between plant and soil metal concentrations were shown at high threshold levels of soil Zn by Barry & Clark (1978) who showed that for Festuca ovina, Agrostis tenuis and M. verna growing on mine spoil, the relation between shoot Zn and that in the soil was generally a linear one. Also, Kelepertsis, Andrulakis & Reeves (1985) have reported a very highly significant correlation ($p < 0.001$) between shoot and soil Pb, Zn & Cu concentrations in field samples of M. verna from N. Greece.

Baker (1981) has suggested three basic strategies of plant response to toxic soils, accumulators, excluders and indicators, based on the relationship between metal concentrations in shoot, root and soil. He classified T. alpestre and M. verna as accumulators, as Zn [shoot]:[root] ratios were generally >1. In the present work [shoot]:[root] ratios (Table 5.6) have reflected differences between metals and species. In the Pb treatments, ratios for both species in all concentrations were <1 with a tendency for values for T. alpestre to be less than those for M. verna, thus indicating a higher degree of restriction of metal transport from root to shoot by M. verna, and therefore suggesting an exclusion strategy. By contrast, ratios of

TABLE 5.6 [SHOOT]:[ROOT] - METAL CONCENTRATION RATIOS (R)
AND THEIR STANDARD ERRORS FOR PLANTS OF M.V. & T.A.
GROWN IN SOIL CULTURE AMENDED WITH METALS (EXPT.VIII)

		Added metal concentrations in the soil culture																	
		Pb $\mu g.g^{-1}$ soil						Zn $\mu g.g^{-1}$.						Cd $\mu g.g^{-1}$.					
		Control	50	500	1500	2500	3500	CONTROL	10	50	200	500	1500	CONTROL	2	5	10	12	15
M. verna	(R)	0.75	0.375	0.25	0.195	0.402	0.342	0.208	0.152	0.265	0.405	4.167	9.031	0.30	0.149	0.576	1.233	1.608	1.918
	S.E.	0	0.16	0.07	0.07	0.07	0.03	0.02	0.01	0.07	0.14	0.40	1.30	0.18	0.021	0.16	0.15	0.73	0.28
T.alpestre	(R)	0.20	0.385	0.093	0.077	0.127	0.137	8.677	8.941	4.592	6.109	2.454	2.225	1.292	5.487	6.074	2.10	3.974	6.634
	S.E.	0	0.05	0.03	0.009	0.03	0.03	1.29	0.0	3.1	1.18	0.81	0.26	0.51	1.53	2.22	0.42	1.6	0.42

[shoot]:[root] in T. alpestre for Zn and Cd indicated an accumulation strategy. In M. verna the plant showed an exclusion behaviour when grown in added Zn up to 200 $\mu g.g^{-1}$ and in Cd up to 5 $\mu g.g^{-1}$, then behaved as an accumulator at the higher concentrations. Ratios for Zn in T. alpestre also changed at the higher treatments, thus indicating the possibility of change in the response-type with the increase in the substrate concentrations. Although the data considered by Baker (1981) were for plant material collected from the field, the results obtained in the present work seem to be partly in agreement with what the author has proposed. Baker (1981) also suggested that a species may act as an accumulator, an indicator and an excluder over different ranges of metal concentrations in the soil. Shaw (1984) reported that, of the 21 species she studied, M. verna was amongst the lowest in [shoot]:[root] ratios for Pb and Zn when the plant was grown in a soil containing 40-50,000 $\mu g.g^{-1}$ total Pb and about 3700 $\mu g.g^{-1}$ total Zn. The same author postulated that it is also possible that a plant might show different uptake strategies during different stages of its life-history. Slow-growing perennials such as M. verna might exclude metals in the establishment phase whilst mature plants accumulate. Although this might be the case, the present observations have indicated that plants of the same species and age can have different strategies for different metals and concentration ranges.

5.5.3.2 Heavy metal uptake by five populations of T. alpestre from solution culture. Expt. IX. (The uptake part of Expt. VII).

Results of this part of the experiment are presented as follows:

Figures 5.10 & 5.11 give a graphical representation of the whole plant metal concentration for all populations, and the root and shoot of plants of the Whitesike population. Table 5.7, shows [shoot]:[root] metal concentration ratios for the Whitesike population.

Figure 5.10 illustrates the general similarity in behaviour of the five populations, where only with two metals a clear difference was shown (for concentration of Zn in plant of Black Rocks population and those of Fe in plants of Bradford Dale). These high concentrations of Zn and Fe correspond with the high concentrations in the soil (Table 5.4). The considerable enrichment of most metals in the plant dry matter is also apparent.

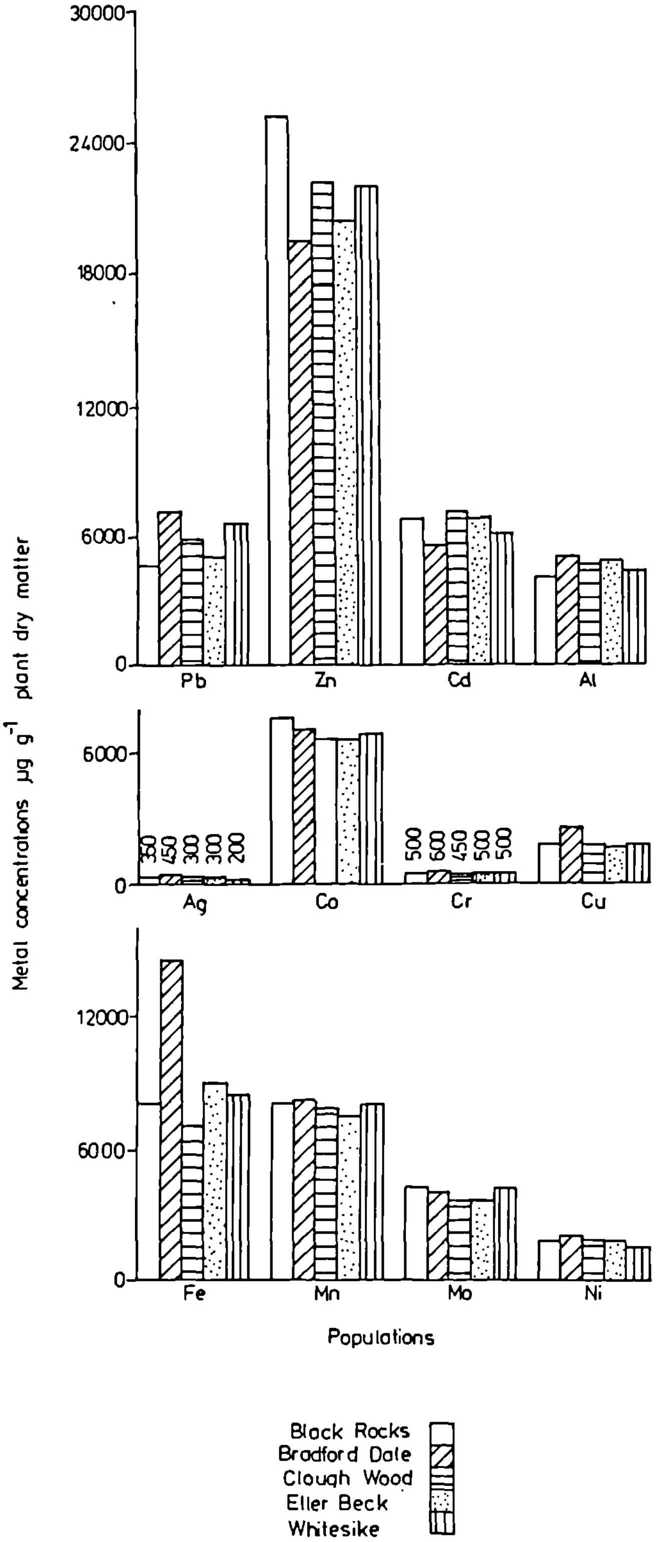
Metal concentrations presented in Figure 5.11 show that for all metals but four (Ni, Co, Mn and Zn), the concentrations in the root system were higher than in the shoot parts. The accumulation behaviour of the species is indicated by the high ratios (>1) found for these four metals, (Table 5.7). However, there are wide variations in the extent to which the absorbed metals are transported from the root system into the remainder of the plant. It was possible to categorize the metals according to the partition ratios into three groups: (i) metals with the highest ratios Ni, Co, Mn and Zn, (ii) metals with intermediate ratios (low ratios) Cd, Mo and Ag, (iii) and a group with very restricted transport Cr, Cu, Al, Fe and Pb. The very small ratios found for metals of the last group suggest that the species could also be an efficient excluder.

TABLE 5.7 [SHOOT]:[ROOT] - METAL CONCENTRATION RATIOS, IN T.ALPESTRE

FROM WHITESIKE (EXPT. IX.)

Metal	Pb	Zn	Cd	Al	Ag	Co	Cr	Cu	Fe	Mn	Mo	Ni
Ratio	0.009	**1.01**	0.51	0.013	0.22	**2.68**	0.080	0.029	0.011	**2.11**	0.35	**2.70**

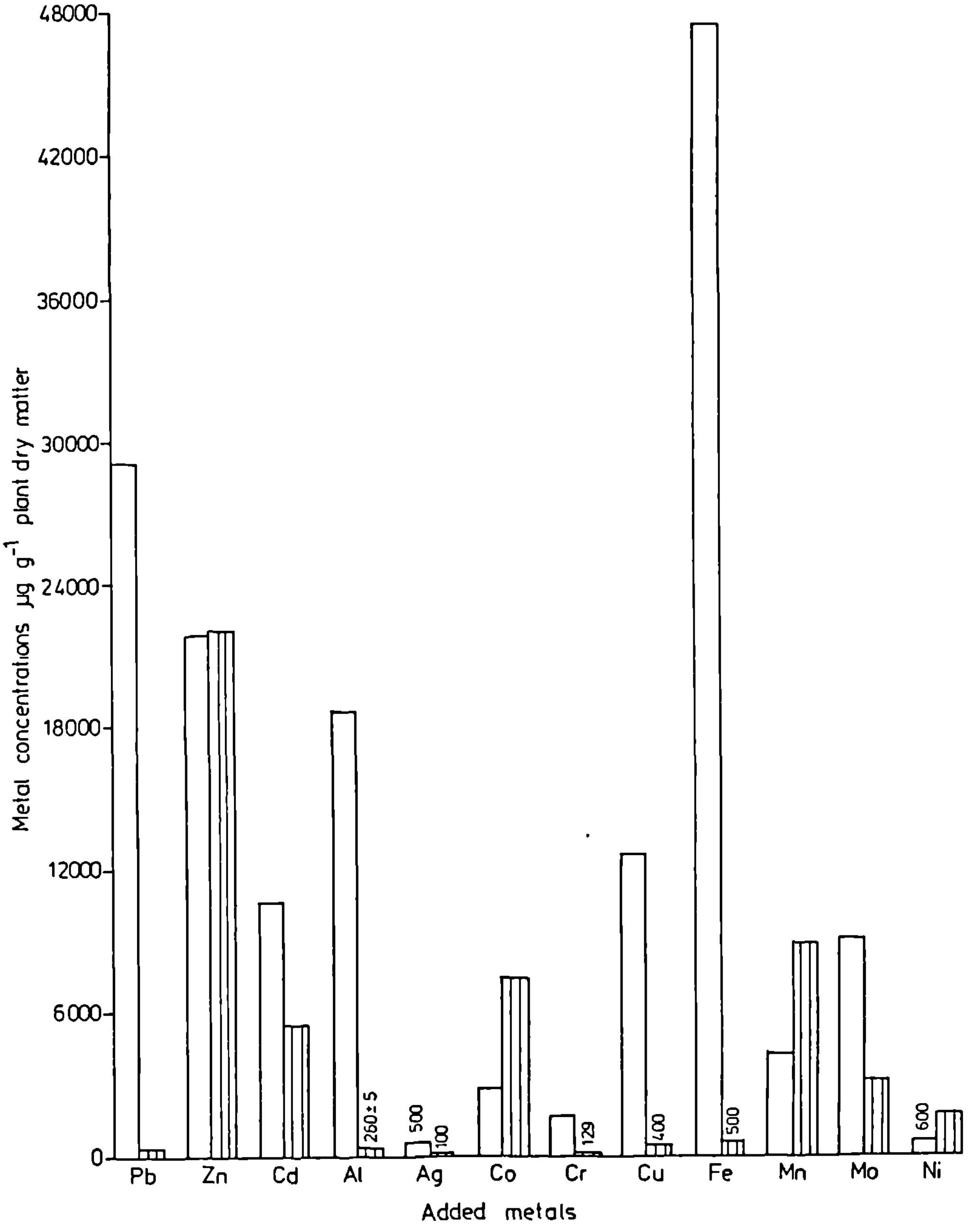
Figure 5.10 Metal concentrations ($\mu g.g^{-1}$ plant dry matter) in *T. alpestre* from five different populations, grown in solution culture



Added metals and concentrations $\mu g.ml^{-1}$ in the culture

Pb	Zn	Cd	Al	Ag	Co	Cr	Cu	Fe	Mn	Mo	Ni
20	25	5	20	0.5	5	1	1	6	10	10	2

Figure 5.11 Metal concentrations ($\mu g.g^{-1}$ plant dry matter) in *T. alpestre* from Whitesike (Garrigill).



Added metals and concentrations ($\mu g.ml^{-1}$) in the solution culture

Pb	Zn	Cd	Al	Ag	Co	Cr	Cu	Fe	Mn	Mo	Ni
20	25	5	20	0.5	5	1	1	6	10	10	2

Shoot ▥
Root ☐

The relationships between IT (from root length, root -, shoot - and total plant-weight) and the metal concentrations in plant tissue may reflect the possible enhancement to uptake by metal tolerance (Gregory, 1965), since the mechanisms of tolerance often involve complexing of metals by cell constituents than their exclusion from the plant (Baker, 1981). Two significant positive correlations ($r = 0.91$, $p < 0.02$ and $r = 0.93$, $p < 0.01$) were found between IT (from root weight) and Cu and Fe concentrations in the plant tissue respectively, and positive but not significant trends for IT (root length) and Zn, Ag, Co and Fe. There were two significant negative correlations ($r = -0.89$ and -0.86. $p< 0.05$) between IT (from shoot -and total plant-weight) and the concentration of Ni in the plant tissue. These differing results suggest that a positive relationship between 'tolerance' and uptake is not obligatory, and the mechanism of tolerance can thus be different to that of uptake, accordingly to the metal and possibly the concentration employed.

The final table (Table 5.8) summarizes on a more comparative basis the IT (from root length and total plant weight) and metal content, for all populations and metals. The mean IT (from root weight) over the five populations shows that for all metals but three (Pb, Mn and Ni) the IT was about 50%. This could be taken as a reasonable justification to the concentrations employed in the experiment. The mean IT (from total plant weight) also taken over the five populations, indicates that exposure to 20 $\mu g.ml^{-1}$ Pb or 25 $\mu g.ml^{-1}$ Zn has relatively little effect on growth, whereas the chosen low concentrations of Cu, Cr and Ag strongly inhibited the growth. The metal content figures have been divided by the metal concentration used in the treatment solutions, in an attempt to standardize the data and facilitate comparisons between metals. The results summarized in this

TABLE 5.8 METAL UPTAKE AND ACCUMULATION BY SEEDLING OF VARIOUS POPULATIONS OF *T. ALPESTRE* FOR 12 HEAVY METALS IN RELATION TO I.T'S. (EXPT. XI)

Metal	Con. $\mu g.ml^{-1}$ in solution	Root IT(%)	Total weight IT (%)	Metal Content			Metal content μg^{-1} per 1 $\mu g.ml^{-1}$			
				Shoot	Root	Total	S/R	Shoot	Root	Total
Pb	20	85 + 5	98 ± 9	28	874	902	0.032	1.4	43.7	45.1
Zn	25	54 + 5	98 ± 7	2018	437	2455	4.6	80.7	17.5	98.2
Cd	5	43 ± 3	39 ± 3	217.5	66.5	284	3.3	43.5	13.3	56.8
Al	20	51 ± 4	37 ± 4	11	227	238	0.048	0.55	11.35	11.9
Ag	0.5	45 ± 2	22 ± 2	2.16	1.96	4.1	1.10	4.3	3.9	8.2
Co	5	61 ± 5	31 ± 2	207.5	11.5	219	18.0	41.5	2.3	43.8
Cr	1	60 ± 10	23 ± 1	2.4	10.4	12.8	0.23	2.4	10.4	12.8
Cu	1	45 ± 2	17 ± 0.5	5.5	26.0	31.5	0.21	5.5	26.0	31.5
Fe	6	45 ± 3	35 ± 3	20	377	397	0.053	3.3	62.8	66.1
Mn	10	88 ± 4	57 ± 2	496	48	544	10.3	49.6	4.8	54.4
Mo	10	48 ± 2	57 ±4	168	91	259	1.85	16.8	9.1	25.9
Ni	2	78 ± 4	78 ± 7	138	8.9	147	15.6	69.0	4.5	73.5
		*	*	**	**	**	**	**	**	**
a	b	c	d	e	f	g	h	i	j	k

* - Data from the mean over 5 *T. alpestre* populations.

** - Data from Whitesike *T. alpestre* populations.

a - Metals employed in the experiment.

b - Metal concentrations ($\mu g.ml^{-1}$) added to the solution culture.

c - Mean index of tolerance based on root elongation measurements.

d - Mean index of tolerance based on total plant dry weight yield.

e - Metal content in *T. alpestre* shoot ($\mu g.$)

f - Metal content in *T. alpestre* root ($\mu g.$)

g - Metal content of whole plants ($\mu g.plant^{-1}$) - e + f)

h - Shoot:root metal content ratios.

i - Metal content in the shoot relative to the concentrations added in the solution culture. (a measure of relative efficiency of shoot accumulation).

j - Metal content in the root relative to the concentrations added in the solution culture. (a measure of relative efficiency of root accumulation)

k - Metal content in the whole plant parts relative to the concentrations added in the solution culture. (a measure of relative efficiency of plant accumulation.

table also demonstrate the extensive uptake of all metals (rather less for Ag, Al and Cr than the others). When standardized for differing solution concentrations, uptake of these three metals are remarkably all of the same order of magnitude, in spite of the very different tolerance and the very different fates of the metals once absorbed into the roots.

Species differ considerably in their metal uptake characteristics and for any species these may also vary for different metals (Baker, 1981). The notable feature of *T. alpestre* to tolerate and accumulate high concentrations of Zn, Pb and Cd has been considered in the previous experiment, and the relationships between this plant and Zn is well reported in the literature (e.g. Baumann, 1885; Dvorakova, 1968). Ernst (1975) reported concentrations of 1800 μg.g^{-1} Pb, 10000 μg g^{-1} Zn,550 μg g^{-1} Cd and 80 μg.g^{-1} Cu in the leaf dry matter of *T. alpestre*, grown on naturally metalliferous soil. Very high concentrations of Ni in the leaf of specimens of *T. alpestre* have been reported (1800-4100 μg.g^{-1}, Reeves & Brooks, 1983). Concentrations of Ni found in the present study seem to fall within the range reported by the latter authors. Concentrations of other metals (e.g. Co, Cr, Fe, Mn & Mo) together with Pb, Zn, Cd, Ni & Cu were higher than the concentrations reported for shoot specimens of *M. verna* collected from five different sites in N. Greece by Kelepertsis, Andrulakis & Reeves (1985). The analyses indicated that even in treatments where metals were supplied at low concentrations, the species shows a remarkable ability to accumulate these metals. The general similarity between the different populations in their metal concentrations of any metal tested suggests constitutional mechanisms of tolerance within the species. This and the marked accumulation ability even of those metals which have not been investigated before in this species suggest the potential use of the species in biogeochemical prospecting for many different metals.

The significance of the ability to accumulate metals by *T. alpestre* must be interpreted with caution (Ingrouille & Smirnoff, 1986). As described above, there was no consistent relationship between tolerance and metal uptake. Both characteristics may be independently inherited (Smirnoff & Stewart, unpublished). Nevertheless the results described in the present study for the index of tolerance and the agreement between the IT of two populations and metal concentrations in their soil of origin and the concentrations in the plant tissue (Zn, Black Rocks and Fe, Bradford Dale) indicate that there are variations within the species in these physiological traits upon which selection can, and has acted. However, it seems likely that factors unrelated to heavy metal tolerance are probably also affecting the distributions of *T. alpestre* and *M. verna* but with stronger impact, (e.g. the extremely localized dispersal of the seed, low survival rate and low competitive ability). These will be considered in the following two chapters.

5.6 SUMMARY

1. Responses of *M. verna* and *T. alpestre* to metal treatments have been investigated under controlled experimental conditions. Four parameters of plant performance were used (seed germination, root elongation, biomass yield and metal uptake).

   Responses of the two species to metals were significantly different in most of these parameters, as were the different populations of each species.

2. In the first germination experiment, seed of *T. alpestre* showed higher percentage germination and rate than *M. verna*. No significant effect on the germination by Zn concentrations up to 50 $\mu g.ml^{-1}$ were shown in either species. In the second experiment reported, the germination of *M. verna* seeds from both populations studied was higher on Bradford Dale soil than on Tideslow Rake soil and JIPC, whereas germination of *T. alpestre* was superior on metalliferous soils than on JIPC. The percentage germination of this species was however lower than that reported in Expt. I.

3. Differences between species in their responses to metals were elucidated by tolerance tests using 3 metals (Pb, Zn & Cd) singly & in factorially combined treatments. Tolerance to a range of other metals was investigated. Differences in tolerance between species and populations were significant. *M. verna* was more tolerant than *T. alpestre* to Cu and Mo, but the latter species was more tolerant than the former to all other metals studied.

4. The uptake characteristics of the two species proved to be different, reflecting different tolerance mechanisms, whereby *M. verna* tends to restrict metal translocation from root to shoot, *T. alpestre* tends to accumulate more metals in the shoot.

5. Although significant differences were shown between the indices of tolerance of the different populations of T. alpestre, the metal concentrations in the plant tissues were similar. This may suggest that tolerance and uptake mechanisms are independently inherited.

6. Both species showed an ability to accumulate high metal concentrations, but T. alpestre showed a remarkable ability to accumulate higher concentrations even from low background concentrations, added to the soil or solution cultures. The present investigation of the uptake by T. alpestre to a range of heavy metals (some have not been employed before with this species) indicated the constitutional hyperaccumulation characteristic of this species.

7. For both species, some inter-population variation in physiological characteristics was suggested by the experiments reported in this chapter.

CHAPTER 6

POPULATION DYNAMICS OF MINUARTIA VERNA AND THLASPI ALPESTRE AT DOVEGANG LEAD MINE (A ONE YEAR INVESTIGATION)

6.1 INTRODUCTION

The reasons for the disjunct distribution of species like M.verna and T.alpestre may well lie in their postglacial history and the present discontinuity of their suitable habitats (Pigott & Walters, 1954). In the British Isles, although M.verna and T.alpestre are now occupying similar habitats, M.verna is distributed more widely than T.alpestre (see Chapter 2). The discontinuous distribution of the latter species on a national or international scale could be explained by historical factors stated above but on a local scale this explanation is not acceptable. In Derbyshire, T.alpestre is fundamentally under-distributed, and is restricted to a small area around, and to the west of, Matlock. Contrasted with this, is the ubiquitous distribution of M.verna on the metalliferous spoil heaps of Derbyshire and elsewhere in the Pennine orefield. Although M.verna invariably occurs wherever T.alpestre occurs, the reverse case does not hold. There were no differences detected in climate or soil heavy metal status between sites in which T.alpestre occurs and those where the species is not found, and both species showed high metal tolerance (see Chapters 2, 4 and 5 respectively), so the reason could lie in one or more of the plant characteristics. Such biological factors which may affect the distribution of T.alpestre include the breeding system, seed production the method of seed dispersal, seedling recruitment and survival, and the past history of the distribution (see Chapters 2, 3 and 8 for discussion of some of these factors).

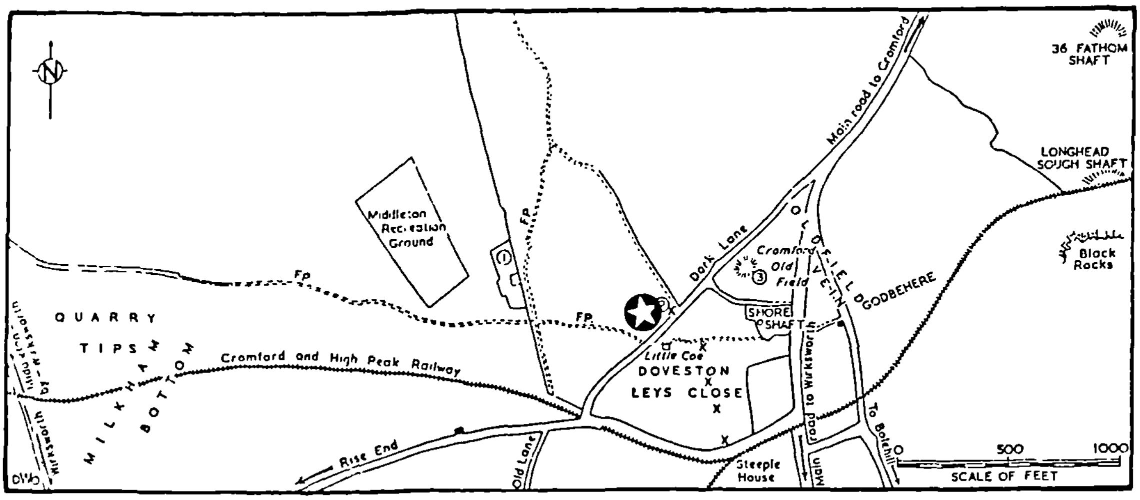
The major emphasis in the present work discussed in this chapter focussed on seedling recruitment and survival within bare and turfed areas. A site in which both species occur was chosen and a permanent quadrat was the subject of a detailed study throughout a year, in order to ascertain the number of new recruits and their survival, as well as the number of established plants, in and out of turfed areas.

The site is situated within the South Pennine orefield; within the county of Derbyshire close to the High Peak Trail at the site of Dovegang lead mine (SK 287556) (Figure 6.1).

The area was described by Kirkham (1953) as typical of deserted lead mine ground; "hummocky, uneven, with grass-lined hollows of run-in shafts, mostly rough grassland, with some stony hillocks, and light grey limestone walls". The workings on the north, dipping into Dean hollow, have scrub on them. Kirkham continued the description of the site as "uninteresting, desolate, compared with the beautiful and popular dales". It was a wide vein, and as well as lead, barytes has been mined from it (same author).

Although the site as a whole is a south-facing slope, the heaps within the site have more than one aspect. The quadrat used in this study was typical of the general slope of the site. The Dovegang area inclines onto the Carboniferous Limestone, so the spoil has high calcium carbonate content, with considerably elevated concentrations of Cd, Pb and Zn (see Chapter 4). It consists of a mixture of large stones, coarse and fine sandy particles (Plate 6.1). The substrate is

Figure 6.1 Dovegang Lead Mines, the site of study, based on Kirkham (1953).



Roads pathways, and printing in small letters. -- from OS. 25" to 1 mile map
CAPITAL LETTERS Positions known definitely but not on OS map
Sloping Letters · (see text) — ① Gang House ② Forefield Coe ③ Old Engine Pit X G.B Stones

✪ The site of study

PLATE 6.1 The permanent quadrat at the site studied.

loose and dusty, especially in bare areas, which makes it very susceptible to erosion by wind and water. Since the area is south-facing, it has very dry soil during summer.

6.2 MATERIALS AND METHODS

At Dovegang lead mine, a study plot 1 x 2 m was selected and carefully marked out in such a manner as to enable the exact position of the corners to be located again on subsequent occasions. The area chosen consisted of turf dominated by *Festuca rubra* with some *F.ovina* and herbs such as *Campanula rotundifolia*, *Cerastium fontanum*, *Galium sterneri*, *Lotus corniculatus* and *Thymus praecox* ssp.*arcticus* and areas of bare ground.

M.verna and *T.alpestre* spread onto both parts. The area chosen provided sufficient numbers of both species in turfed and bare ground. From July 1983 until July 1984 the number of established plants, seedlings from the previous year and new seedling recruits were recorded in order to define the dynamics of seedling establishment and survival for both species within the bare and turfed areas. It was hoped that this might help to understand possible reasons for the under-distribution of *T.alpestre*, by comparison with *M.verna*.

The area of the turf and bare ground in the permanent quadrat was calculated as 15.4% and 84.6% respectively. A graduated quadrat frame (1 m^2) divided with string into 400 (25 cm^2) sub-units was used to map the positions of individuals and enable a composite picture of changes with time to be built up. The actual positions of individual plants were mapped diagrammatically on graph paper and the turf/bare ground boundaries marked to facilitate easy detection of temporal changes (Figure 6.2). This technique was a modification of the horizontal distribution-mapping technique, quoted by Kershaw & Looney

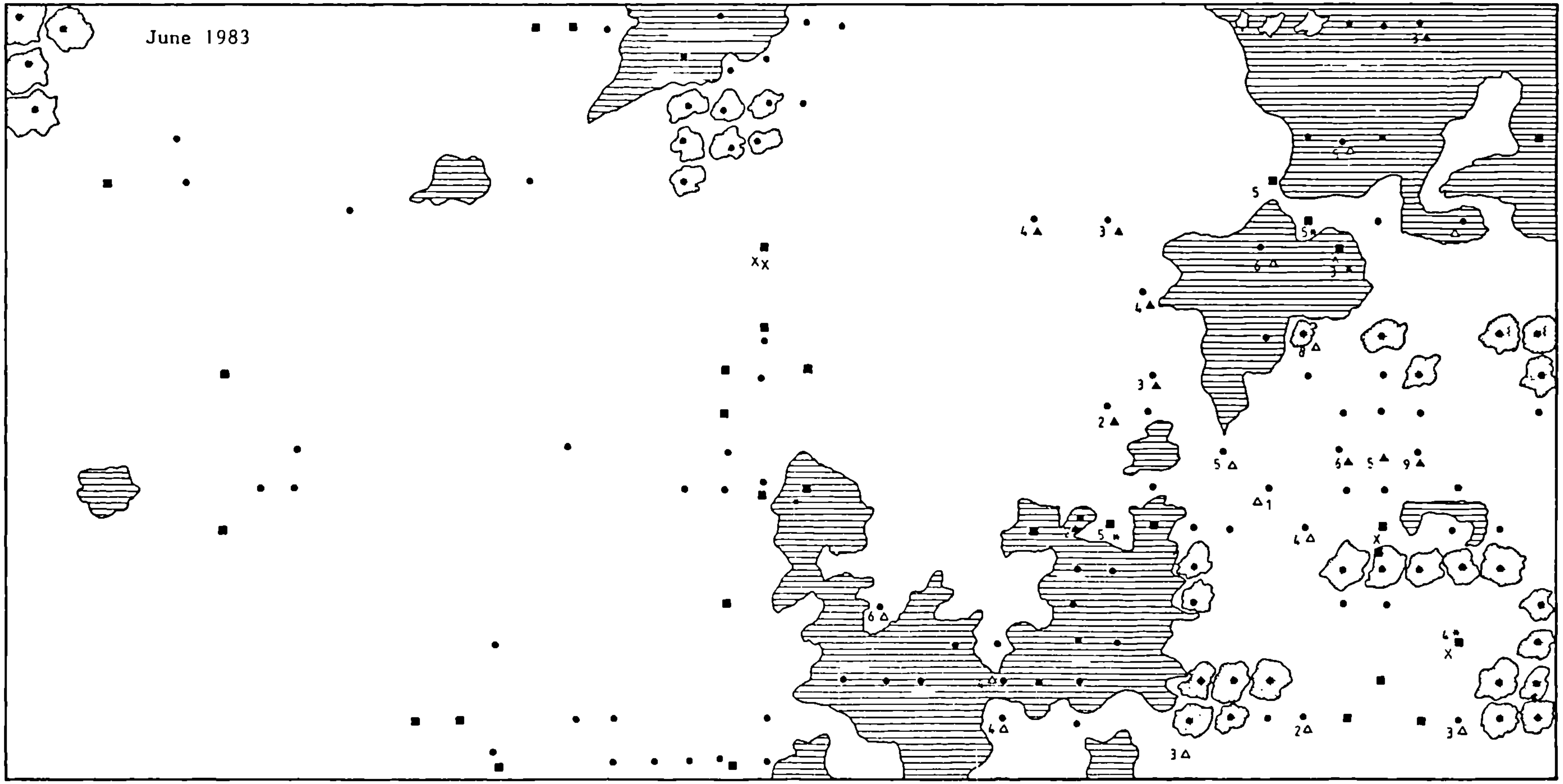
Figure 6.2

A detailed mapping record of changes in the number of established and seedling plants of <u>M.verna</u> and <u>T.alpestre</u> through one year at Dovegang Lead Mine

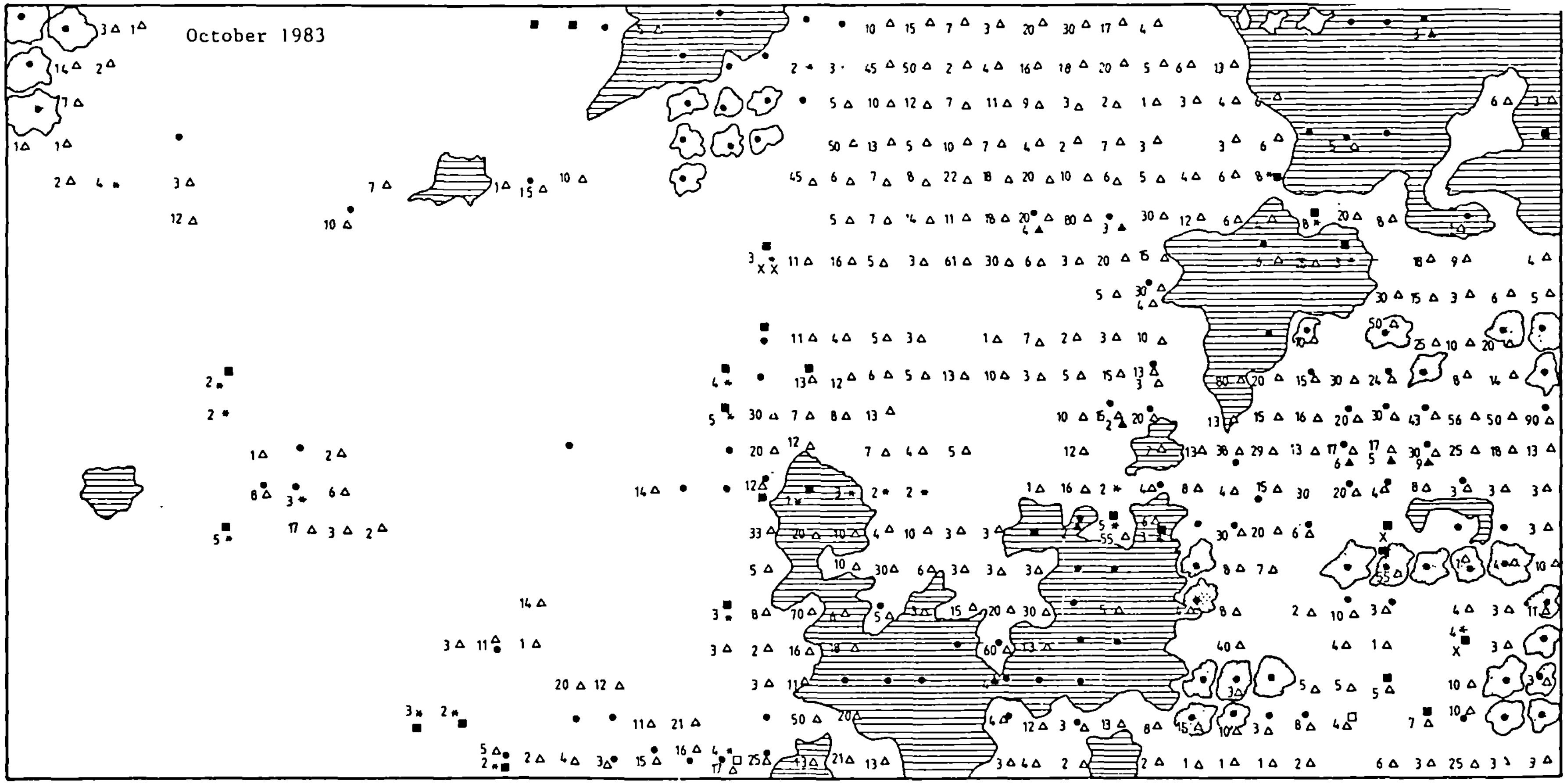
Key:

M.verna established plants	●	T.alpestre established plants	■
M.verna seedlings from 1982	▲	T.alpestre seedlings from 1982	X
M.verna seedlings from 1983	△	T.alpestre seedlings from 1983	✳
M.verna seedlings from 1984	▽	T.alpestre seedlings from 1984	✱
M.verna established plants from 1982 seedlings	○	T.alpestre established plants from 1982 seedlings	⊠

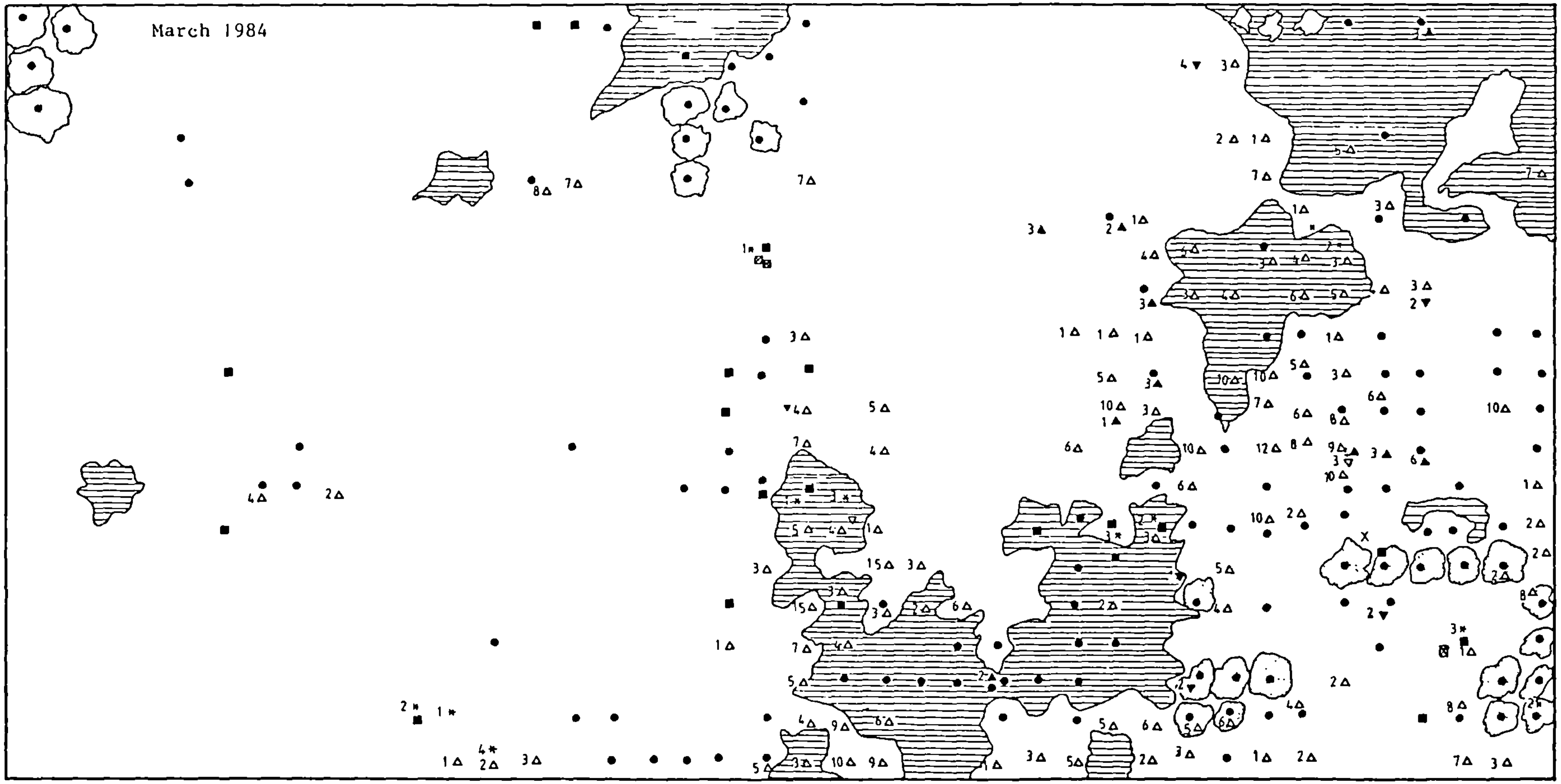
Turf	▤
Bare ground	□

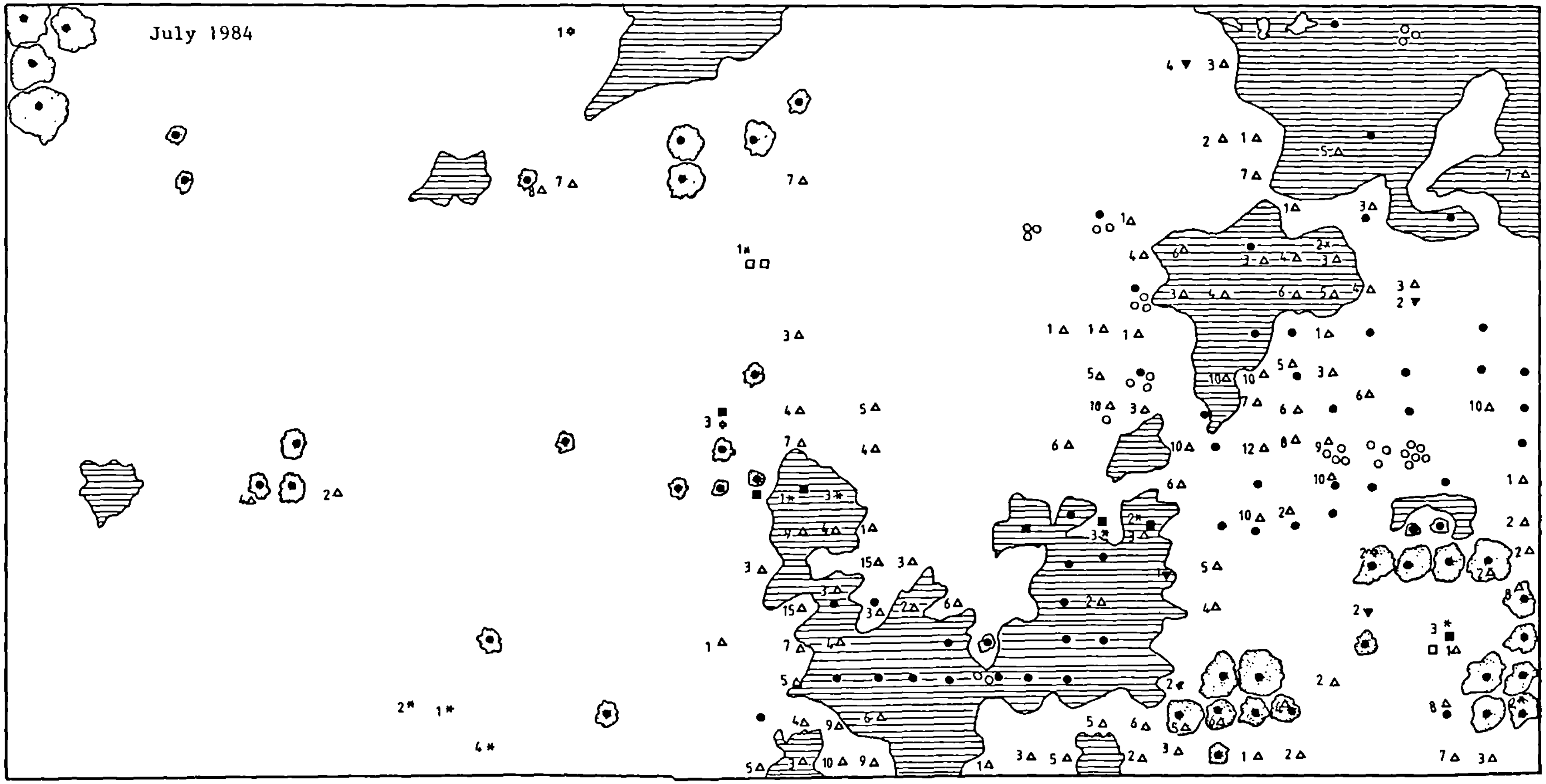


June 1983



October 1983


March 1984


July 1984

(1985) and used by Watt (1962). A density estimate was also made for both species from a 100 m^2 plot on Dovegang lead mine spoil. By using random number tables, 88 estimates were taken and the density (d) was calculated from the formula $d = 1(2 \times a)^2$ (Greig-Smith, 1964) where a is the mean distance between each random point and the nearest plant. The density was found to be 77 plant/sq. m. for *M.verna* and 4.2 plant/sq. m. for *T.alpestre*. The average number of the latter species on Derbyshire sites was estimated to be 15 plants/sq. m. by Shimwell (1968).

6.3 RESULTS

Results are summarized in Table 6.1. The abundance of *M.verna* and *T.alpestre* and seasonal variation in the number of established plants and seedlings are shown in Figures 6.2, 6.3 and 6.4 respectively. It was clear that the number of *M.verna* seedlings was larger than that of *T.alpestre* seedlings on all occasions. The scores also suggest a pronounced increase in the number of seedlings for both species in autumn, indicated by a clear peak in October and a declining trend of numbers in spring and summer. (Figure 6.4). The trend was similar for both turf and bare areas. The number of established plants was unaffected by increase in seedling numbers and remained constant during the period from July to October 1983. However, the number of these had decreased in the following spring, with considerable losses of *T.alpestre* plants; those remaining represented only 23.3% of the number observed at the start of the investigation. For *M.verna* this figure was 70%. The number of 1982 seedlings was not severely reduced suggesting that mortality can decrease as seedlings get closer to the establishing stage.

Table 6.1 Density of plants and seedlings of M.verna and T.alpestre (numbers/m^2) in turf or bare ground within the permanent quadrat at Dovegang Mine

Scoring Occasions	M. verna established plants	M. verna seedlings (1982)	M. verna seedlings (1983)	M. verna seedlings (1984)	M. verna established plants in the turf	M. verna established plants on bare ground	M. verna seedlings in the turf	M. verna seedlings on the bare gound	T. alpestre established plants	T. alpestre seedlings (1982)	T. alpestre seedlings (1983)	T. alpestre seedlings (1984)	T. alpestre established plants in the turf	T. alpestre established plants on bare ground	T. alpestre seedlings in the turf	T. alpestre seedlings on the bare ground
June 1983	65	20	25	–	94	60	68	41	15	3	17	–	13	15	16	20
Oct. 1983	65	20	1944	–	94	60	1360	2097	15	2	48	–	13	15	32	53
Mar. 1984	61	15	253	6	75	59	305	261	8	2	13	–	10	8	29	11
July 1984	46+	15	122	–	81	57	149	116	4+	2	12	3	10	2	26	10
Final No.	61		122		81	57	149	116	6		15		10	4	26	13
	M.verna established plant		M.verna seedlings						T.alpestre established plant		T.alpestre seedlings					

Figure 6.3 Seasonal variation in the number of established plants.

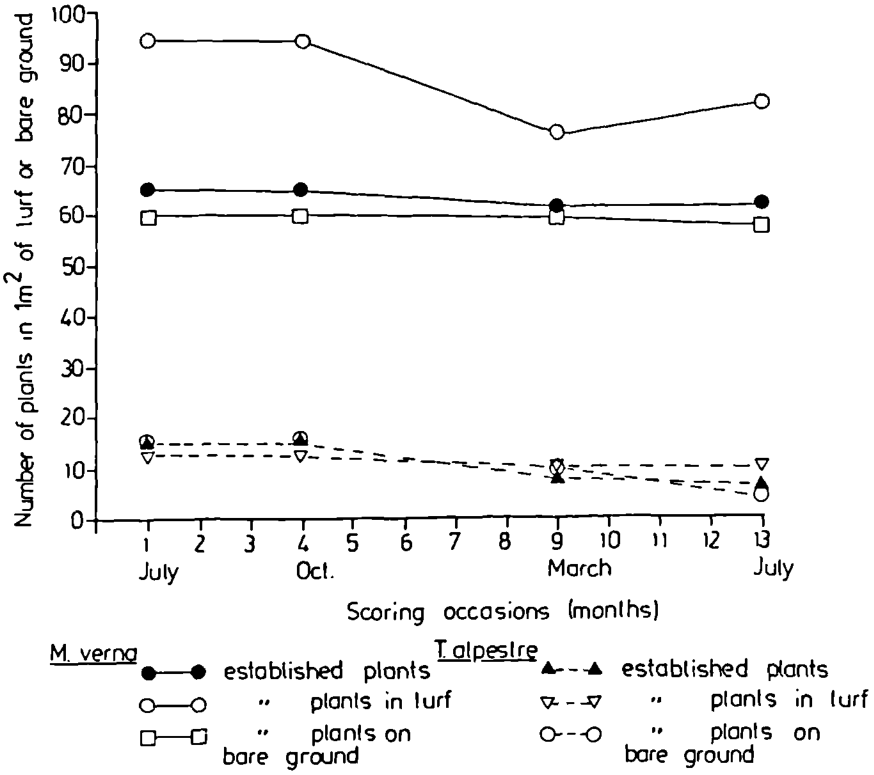
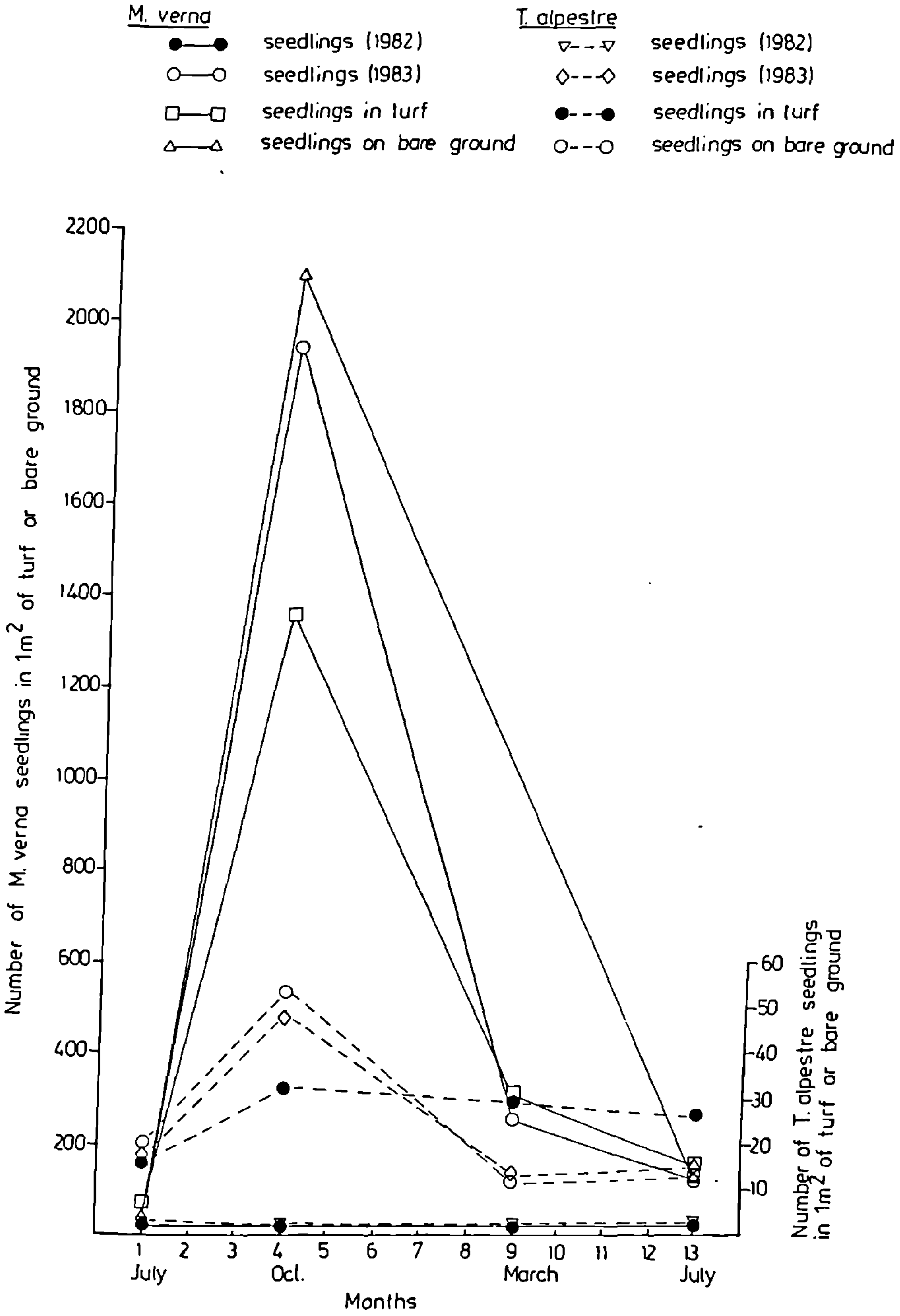


Figure 6.4 Seasonal variation in the number of seedlings.



Generally the number of the individuals was greater on bare ground but this could be the result of the bare ground contributing about 84.6% of the total area of the permanent quadrat. To compensate for this bias the number of plants/m^2 of turfed or bare area was calculated (Table 6.1) and used to present the seasonal variation data (Figures 6.3, 6.4). It was noticed that the density of established M.verna plants was higher within the turf than on the bare ground, but the density of the seedlings of this species within the turf was equal or less than that on bare ground. For T.alpestre the number was less in the turf in summer and autumn 1983 but higher in spring and summer 1984. The spatial pattern of seedlings is shown in Figure 2.6, and the configuration of this reflects a difference between M.verna and T.alpestre. The figure shows that seedlings of M.verna are spreading more away from the established plant than those of T.alpestre. Further, the number of seedlings is greater in areas close to the established vegetation (see Figure 6.2 March 1984 score).

6.4 DISCUSSION

As stated in the Introduction, there are many factors which singly or in combination, could affect the distribution of T.alpestre. Some of these factors have been studied before by other workers. Riley (1955;1956) investigated inbreeding in populations of T.alpestre and the past history of the plant distribution was considered by Pigott & Walters (1954) and Shimwell (1968) and also discussed by Antonovics et al. (1971). In the present work, the dispersal and colonization efficiency of both M.verna and T.alpestre was observed and elucidated indirectly by the distribution of new seedlings around established plants (Figure 6.2). An emphasis was placed on the plant regeneration strategies through seedling recruitment and survival in two contrasting areas within the permanent quadrat. One was highly disturbed (bare ground) and the other had a low incidence of disturbance because of the continuity of the vegetation.

Results from the present study (Chapters 2 & 4) taken together with Shimwell's (1968) observations suggest that there are no great differences in environmental factors between the sites of T.alpestre populations and similar metalliferous sites lacking the species in Derbyshire. Edaphic variations cannot therefore be implicated as causal factors in the present investigation.

In the present study although the severe drought which the site experienced for two subsequent years (1983 and 1984) possibly increased the rate of mortality among established plants and seedlings, particularly within bare areas, it was possible to detect a seasonal trend for seedling distribution, mortality, and survival within the turf and bare areas. The trend suggests that the total number of seedlings for both plants was strongly affected seasonally, with high rates of mortality during the growing season (spring and summer), which may relate to competitive exclusion by the other species within the turf, and to the effects of severe drought within the highly disturbed bare ground.

The effects of competition by other species eg. F.rubra, cannot be ignored when considering seedling survival of both M.verna and T.alpestre; this aspect is explored experimentally in Chapter 7.

These results can be interpreted in the light of what has been suggested by Grime (1974; 1977), that threats to existence fall into three categories, stress, disturbance, and competitive exclusion.

Although the total number of seedlings was affected by these factors for both species, the effect on the seedlings of T.alpestre was more severe, thus the starting number of these was always smaller for T.alpestre than for M.verna. This could be one of the most important reasons for the advantage of M.verna over T.alpestre.

The survival rates were higher for M.verna than for T.alpestre, hence the remaining number of the latter species was 33.3% for established plants and 90.9% for seedlings, while the % for the former was 93% for established plants and 486% for seedlings. Many of this large number of M.verna seedlings are liable to mortality throughout their different growing stages. However the high mortality here appears not to be important in accounting for the different distributional ranges of the two species. Also in the present context, this high number of new recruits is clearly an advantage for species growing in a hostile habitat, and is possibly a second advantage for M.verna.

These properties of M.verna, coupled with the characteristics listed below may be some of the important causes behind the differing local distribution patterns between the two species:

(i) the low horizontal growing cushion with a large number of branches contacting the soil. These branches develop a secondary root system which may provide the plant with more water, nutrients and stability on such unstable and poor substrates.

(ii) the larger area over which seeds are dispersed which may proportionately increase the chances for seedling survival and recruitment.

(iii) the lighter, smaller, more abundant and more mobile seeds.

M.verna is more likely to possess a larger seed bank as Thompson & Grime (1979) have found that in general the most abundant seeds are also the smallest, and sites with a predominance of small-seeded species tend to have larger seed banks. The spatial distribution and pattern of germination of buried seeds is of great importance in that they affect the establishment and distribution of plants of any species, but particularly so in short-lived species (Schenkeveld & Verkaar, 1984 and Thompson, 1986). Distribution patterns of seedlings shown in Figure 6.2 for both species have also indicated such importance. The lighter weight of these seeds makes them easier to carry and be disseminated by the wind. Salisbury (1942) has discussed such phenomena and stated that "the general trend for species to produce either a large number of small seeds or a small number of large seeds tends towards wider dissemination and more probable occupation of all suitable habitats". The first part of the author's statement would seem to be the case in M.verna. T.alpestre is much more restricted in its distribution over potentially suitable habitats. One of the reasons for this may be the weight of its seed. However although the seed of this species is 5 times heavier than the seed of M.verna, the author still puts them in his first category of plants of open habitats. The larger and heavier seeds of T.alpestre with very high germination rate (Riley, 1956; Ernst, 1965, Grime et.al., 1981) (see Chapter 5), can be released from their brittle siliculas by any gentle contact and germinate at the first opportunity, close to the parent plant unless otherwise moved by water or animal activity. This high rate of germination which is likely to be of survival value to a species producing less seeds and growing in a habitat where there is heavy seedling mortality, could also be a point of weakness. At any incidence of stress and/or disturbance, such as severe frost, drought, or water erosion, most seedlings will be lost. The importance of a degree of shelter for both species was observed in the present study (see Chapter 7) but such a requirement seems to be more apparent for T.alpestre, and in particular for the new recruits, especially during periods of stress.

In some cases, T.alpestre can be found in rather more closed communities than M.verna, as field observations may suggest. This could possibly be regarded as anomalous as it is generally considered to be a species of open habitats where competition from other plants is minimal. However, there is no conflict of ideas, as although the plant may require some shelter especially in the early stages, once it is established, it may need a more open habitat to grow to the subsequent established stage to continue colonization. A rather open vegetation with gaps within it could be an ideal habitat for this plant. Furthermore, T.alpestre was observed in closed vegetation on some other metalliferous wastes in Derbyshire, for example, at Clough Wood (SK 258618) on a spoil heap of the Old Mill Close Mine. Here most of the site is very open with a steep unstable south-facing slope lacking vegetation cover. The T.alpestre population here is distributed in patches mainly on the edges of the main spoil heap where the vegetation cover is more dense. The site seems to suffer from a severe drought particularly during the summer. Seedlings of this plant were observed to thrive in the sheltered areas, where the moisture regime and protection are more favourable. Conversely, the M.verna population at this site is distributed on the more open and less vegetated areas which expose the plant to more extreme conditions.

According to Shimwell (1968), T.alpestre spread from a number of relict stations in Derbyshire as a result of the development of heavy metal tolerance and was thus able to colonize nearby open sites made available by lead-mining activities. Nevertheless, this spread did not continue to all the potentially available habitats which were observed by Ray (1688), cited by Linton (1903) as the habitats for M.verna, and the reasons may be explained logically by the combination of all or some of the studied and cited factors which possibly control the distribution of the plant.

Antonovics et al. (1971) introduced the concept of 'transport endemism' for the establishment of populations on mine sites, following dispersal by human agencies associated with mining activities. This could also be involved in the explanation of the disjunct population distribution on newly-exposed spoil substrates, but cannot be reconciled with the absence of T.alpestre from suitable spoil heaps in the greater part of the mining region of Derbyshire. Hence the areas with populations of this plant represent only about one eighth of the theoretical possible distribution in Derbyshire. Finally, although the observations and results suggest that shelter can be advantageous to T.alpestre, it seems that once a dense sward has developed there is notable decrease in the gregariousness of the plant and therefore a gradual decline in population density owing to its inability to compete with grasses (Shimwell, 1968). The declining distribution in Derbyshire today can be attributed to this property coupled with human threats such as the extensive exploitation of spoil heaps for fluorspar and subsequent reclamation of these heaps and other activities which are increasingly reducing the potential habitats of this species. Such threats are urging the need for conservation work to allow this rather rare species to maintain itself in its local habitats.

6.5 SUMMARY

1. - A typical lead mine spoil heap with a population of M.verna and T.alpestre was chosen to study the population dynamics of the two species.

2. - A 2 x 1 m permanent quadrat was used to study population dynamics for one year in Festuca turf and bare areas.

3. - Four scorings were made at about four-monthly intervals from July 1983 to July 1984. The numbers of established plants and seedlings were recorded and a map of the quadrat sketched on each occasion.

4. - M.verna proved to have greater density of established plants and seedlings, and a higher rate of survival of both, within the turf and on the bare ground.

5. - The combination of the results from the scoring and field observations suggested some difference in survival strategies between the two species, which perhaps account for the greater success of M.verna.

6. - There was a seasonal effect on numbers of the two species, but the effect on T.alpestre was more dramatic, with M.verna performing better in all cases.

7. - The refugium theory coupled with the development of heavy metal tolerance and differing population biology of the two species may provide an explanation for the under-distribution of T.alpestre with reference to M.verna.

8. - The distribution of T.alpestre populations in Derbyshire could be in decline mostly because the human activities and competition from other species, as these progressively colonize the mine spoils in the absence of further disturbance.

CHAPTER 7

THE EFFECT OF INTERSPECIFIC COMPETITION ON THE ESTABLISHMENT AND SURVIVAL OF MINUARTIA VERNA AND THLASPI ALPESTRE

7.1 INTRODUCTION

In Chapters 2 and 6 it was shown that the distribution of T. alpestre was far more restricted than that of M. verna. The former species also shows greater tolerance of heavy metals (Chapter 5). However the results reported in Chapter 4 suggest that there are no consistent differences in metal status between sites supporting both species, and those with M. verna only.

Both M. verna and T. alpestre occur in gaps within the cover of established vegetation (Chapter 6), and the struggle for existence by these two species appears to be strongly affected by larger neighbouring perennial species.

One of the main species associated with M. verna and T. alpestre is the grass Festuca rubra, and in Derbyshire this plant has been observed to be one of the most abundant species in these metalliferous areas where it may be represented by metal-tolerant populations (see Chapter 2 & Appendix 3.2). The observations reported in Chapter 6 indicate that both M. verna and T. alpestre appear to benefit from a degree of shelter specifically at the seedling stage, but seem to be eliminated from areas with a high degree of plant cover. This phenomenon seems to be more strongly defined in T. alpestre.

In order to investigate these observations, a controlled experiment was considered necessary. The experiment was in a form which is intermediate between a field investigation and a growth room study, and was designed to investigate the interacting effects of soil toxicity, interspecific competition between seedlings of M. verna and T. alpestre and impacts of F. rubra upon establishment and survival of the two species.

Arabis hirsuta was also included in the experiment. This plant is characteristic of open turf on non-metalliferous calcareous soils, and resembled T. alpestre in morphology, and dependence upon vegetation gaps. It was therefore appropriate to include this species as a control (i.e. metal-susceptible) species in the experiment.

In both ecology and agriculture the influence of competition upon the distribution and survival of plants has been much studied. Some investigations have involved natural vegetation, (e.g. Watt, 1955; Hillier, 1984; Noble & Franco, 1986). However, the majority (e.g. DeWit, 1960 and Harper, 1961), were performed on short-lived crops, or under largely artificial conditions, and focussed upon the processes involved in short-term interactions. The experiment described here was specifically designed to simulate the conditions under which seedlings of M. verna and T. alpestre and vegetative shoots of F. rubra interact on metal contaminated sites.

7.2 MATERIALS AND METHODS

Turves of established F. rubra together with soil from the rooting zone were removed from the field and brought into the Experimental Garden at Tapton. These, together with the seeds of M. verna and T. alpestre, were collected at random from Dovegang lead mine (Chapter 6).

Non-toxic calcareous soil and the seeds of A. hirsuta were obtained from Millersdale (Nat. Grid ref.SK 155732, altitude 213 m). This site was chosen for the investigation as it is a south-facing site bearing shallow limestone soil, surrounded by large areas of exposed bedrock. The soil was freely draining and resembled the Dovegang soil in physical texture. A third soil treatment used in the experiment consisted of a 1:1 mixture of Millersdale and Dovegang soils.

The soil was prepared for use in the experiment by removing coarse rocks and roots with a 1.0 cm sieve and was then placed in a 22.5 cm diameter pots. In half of the containers of each treatment six equal sized tillers of F. rubra were planted around the perimeter of the pot. In each pot 30 newly-germinated seedlings of one species were planted, each marked by an identification ring. In the mixture pots, each of the three species was planted at the same density as in the monocultures. A description of the planting arrangement is given in the following section.

The pots were then placed inside a polythene tunnel to allow the initial phase of seedling establishment to occur under humid, sheltered conditions. The pots were then transferred to an open garden site, arranged in a randomized block design (4 blocks, Table 7.1), at the end of September, 1984.

The experiment was monitored and maintained for eighteen months. Maintenance work included the removal of alien seedlings and fallen tree leaves which covered pots in autumn.

All plants were harvested individually in April 1986. The final number, position, size and dry weight of the shoot of the surviving plants were recorded. These were then used to estimate the effect of intra - and interspecific competition on M. verna, T. alpestre and A. hirsuta and to examine the modifying influence of soil toxicity and proximity of F. rubra upon survival and performance.

7.3 PLANTING PATTERN AND EXPERIMENTAL DESIGN

The design of the experiment is shown in Table 7.1. Coloured rings (red, blue and yellow) were used as markers for the planting position of the three species (Figure 7.1a). An appropriate number of seedlings of each species (10, 8, 6, 4 or 2) was planted at random in each of five concentric zones (Figure 7.1d). In order to accurately position the rings and the seedlings, a perforated disc of fibre-glass (Figure 7.1b) was used as a template. Initially the seedlings were very small and delicate, especially those of M. verna. In order to plant the seedlings without damage, each was drawn with a small volume of water into a pipette with a modified tip (Figure 7.1c). The seedling was then released into a hole excavated inside its marker ring. This method of planting proved very successful, in that a 100% of the seedlings survived for more than two weeks, indicating that little damage resulted from this procedure.

7.4 RESULTS AND DISCUSSION

The results are presented in three main parts. First, general observations during the course of the experiment are reported. Secondly, the total number of surviving plants and their weight is considered. Thirdly, the performance of each species in each zone is examined to determine position effects on survival and dry weight.

The growth of F. rubra was similar on all three soils. Survival of the tillers of this plant was 100% on each soil, and the size of the plants was rather uniform. By the time of the harvest, the shoots of F. rubra shaded most of the area of each pot. In some pots

Table 7.1 The experimental design and layout

DESIGN								
Soils	Arabis hirsuta		Minuartia verna		T. alpestre		A.hirsuta, M. verna & T.alpestre	
	+	−	+	−	+	−	+	− *
Dovegang	✿ 1 2 3 4	5 6 7 8	9 10 11 12	13 14 15 16	17 18 19 20	21 22 23 24	25 26 27 28	29 30 31 32
Mixed	33 34 35 36	37 38 39 40	41 42 43 44	45 46 47 48	49 50 51 52	53 54 55 56	57 58 59 60	61 62 63 64
Millersdale	65 66 67 68	69 70 71 72	73 74 75 76	77 78 79 80	81 82 83 84	85 86 87 88	89 90 91 92	93 94 95 96

LAYOUT

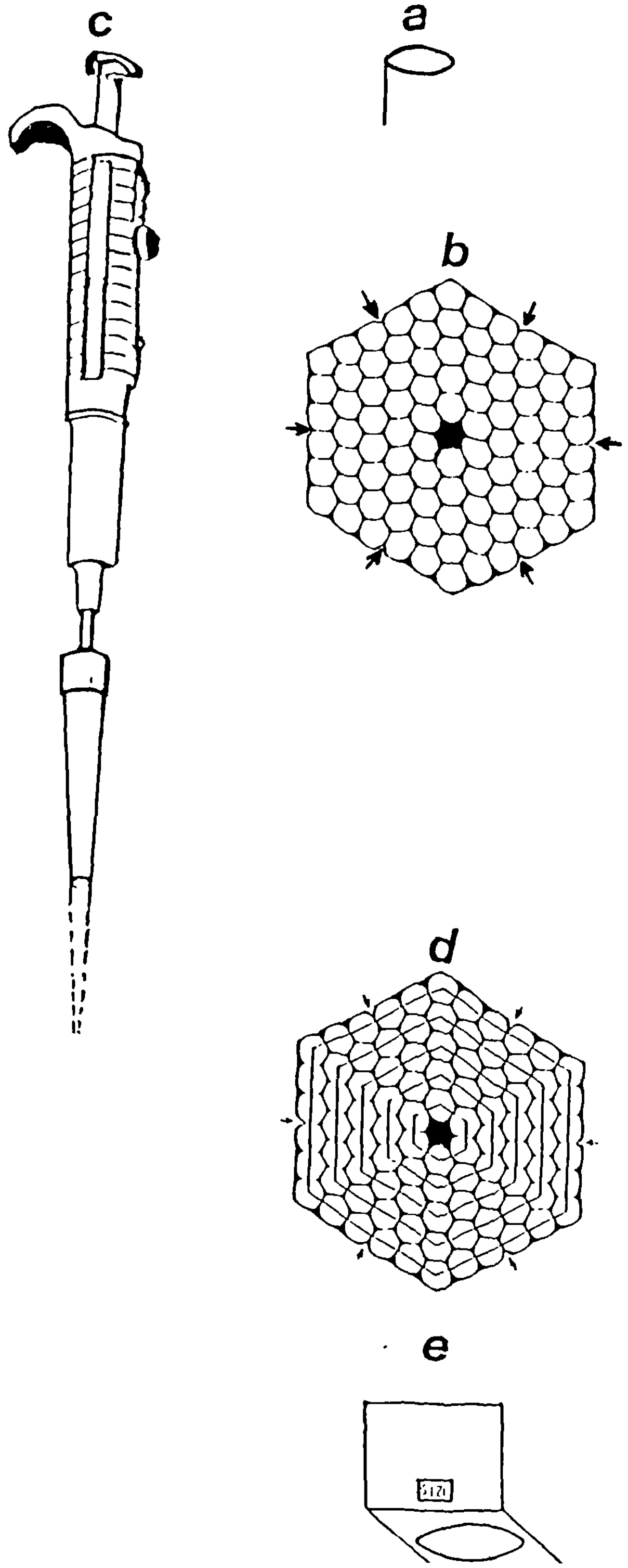
BLOCK 1	1	5	9	13	17	21	25	29	33	37	41	45	49	53	57	61	65	69	73	77	81	85	89	93
BLOCK 2	94	90	86	82	78	74	70	66	62	58	54	50	46	42	38	34	30	26	22	18	14	10	6	2
BLOCK 3	3	7	11	15	19	23	27	31	35	39	43	47	51	55	59	63	67	71	75	79	83	87	91	95
BLOCK 4	96	92	88	84	80	76	72	68	64	60	56	52	48	44	40	36	32	28	24	20	16	12	8	4

* + & − = Presence and absence of Festuca rubra

✿ = Numbers assigned to the pots.

Figure 7.1 The apparatus used in setting up and recording the competition experiment.

a – The ring used to mark the position of seedling
b – The template used to locate the positions at which seedlings were planted
c – The pipette used to plant seedlings
d – The zones used in calculation of % survival and mean yield at different distances from original planting positions of <u>F. rubra</u> (➔)
e – The design of the frame used to measure plant size, 10, 20, 30, 40, 50 & 60 mm breadth.



the central zone was not totally shaded, but an inhibition of seedling growth within this area was observed; this was possibly due to root interactions.

For each of the three remaining species, it was observed that there were very clear reductions in the number of survivors, when F. rubra was present. Moreover, in the presence of F. rubra, most of the survivors were exceedingly stunted. However some individuals had grown, and had produced flowers and fruits, but these were very small and infertile. In those treatments without F. rubra, the plants were generally larger and successfully produced flowers.

The negative effect of metal contaminated soil on the survival and growth of A. hirsuta was very clear. Thus, regardless of treatment, there was no survival of this plant on Dovegang soil and the number and size of surviving plants was markedly decreased on the mixed soil. On Millersdale soil, individuals of this species were large and vigorous.

The total number of surviving seedlings has been presented in Figure 7.2 as a percentage of the starting number. For each of the three species studied it is clear that the percentage survival was higher in those treatments lacking F. rubra. In the same figure there is also a clear negative effect of A. hirsuta on the survival of M. verna and T. alpestre. An effect of metal-contaminated soil on A. hirsuta was also apparent. The mean dry weight of survivors is presented as natural log-transformed values (Ln) in Figures 7.3a, b, c and d. The negative effect of F. rubra on the dry weight of the other three species parallels that on percentage survival. For the three species the response of dry weight yield to the treatments follows a similar pattern.

Figure 7.2 Percentage survival of plants.

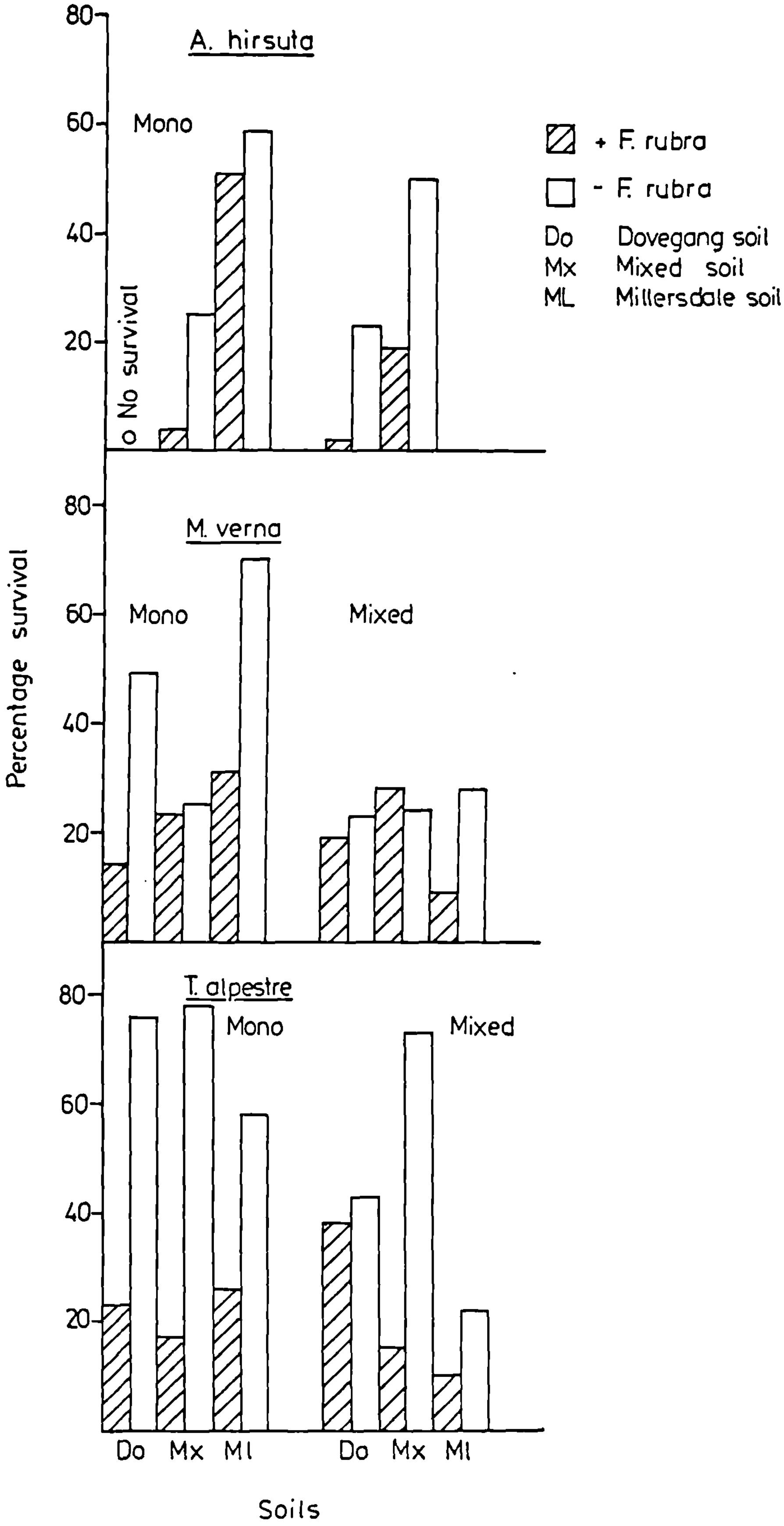
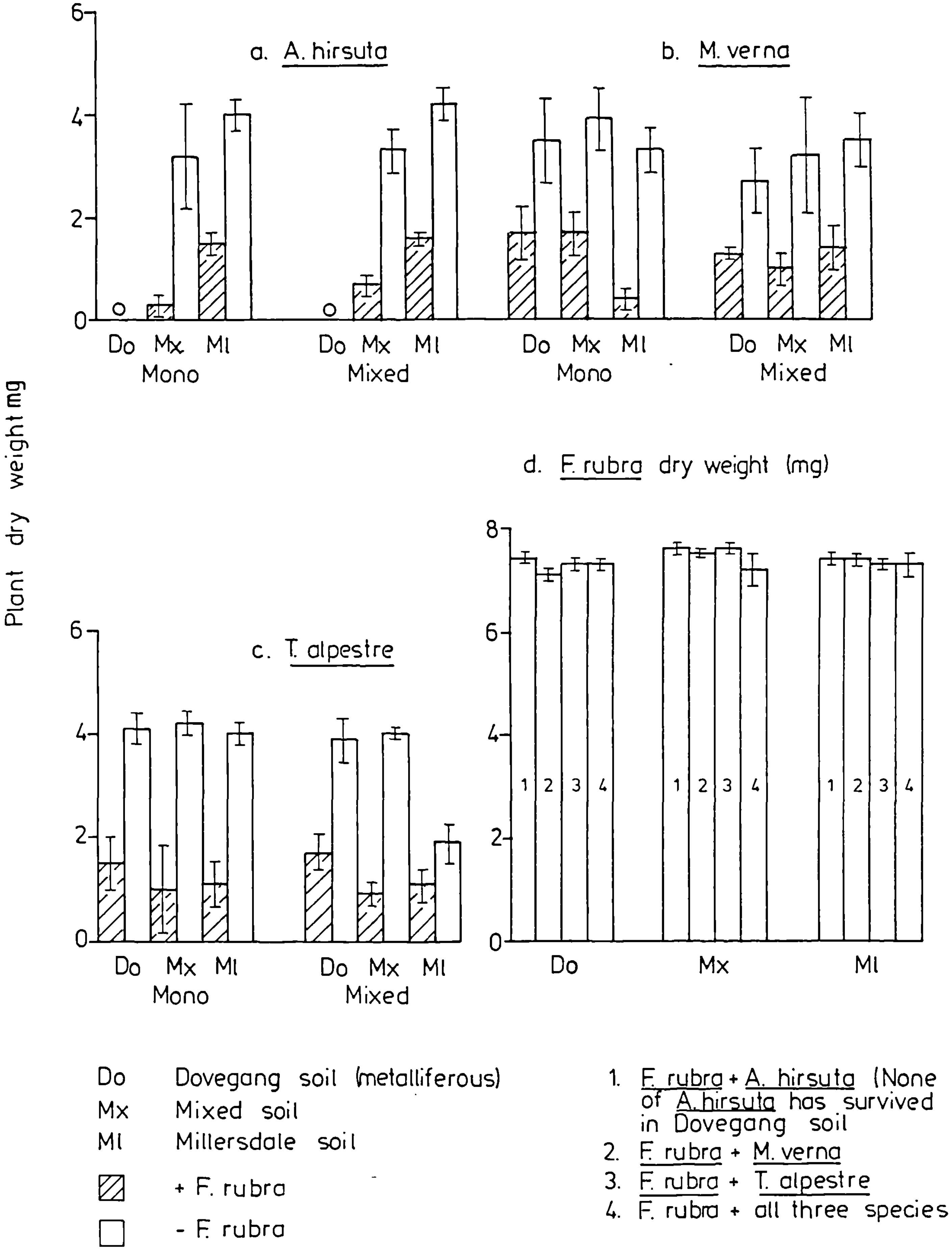


Figure 7.3 The mean dry weight of plants (natural log-transformed data).



In order to determine the effect of position on the fate of seedlings, the percentage survival and mean dry weights of the plants in each zone were calculated and presented in Figures 7.4.1, 2, 3, and 7.5.1, 2, and 3 respectively. Figures 7.4.1, 2 and 3 illustrates that the species studied showed some differences in reaction to the three soil treatments. Figure 7.4.1a provides evidence of a strong effect of metal toxicity on *A. hirsuta* and shows that this effect is exacerbated by the presence of *F. rubra*. In the absence of *F. rubra* there was no obvious effect of *M. verna* and *T. alpestre* upon *A. hirsuta*. However Figure 7.4.1b shows that *A. hirsuta* was strongly affected by the presence of these two species + *F. rubra*. In the monoculture + *F. rubra*, there is an indication of a shelter effect increasing seedling survival at 2 cm from the edge of the pot: however the plants which appeared to benefit from shelter, remained very small. As one might expect, the suppressive effect of *F. rubra* was at its greatest near the margin of the containers, where the *F. rubra* plants were originally planted. Despite the lower density of the *F. rubra* canopy at the centre of the pot, the number of survivors here was reduced. This was possibly due to competition for mineral nutrients from the roots of *F. rubra* or may have been the result of superficial desiccation of the exposed soil.

Figures 7.4.2a, b and c show the effect of position on the percentage survival of *M. verna* in metal-contaminated soil, the mixed soil and the control soil respectively. Figure 7.4.2a reveals evidence of suppression by *F. rubra* at the edges of the pot. The same figure also shows a very low percentage survival at the edges and centre of the pot in the absence of *F. rubra*; this was possibly due to inter -and intraspecific competition between seedlings. In this cushion-forming species large individuals often spread at the expense of smaller neighbours.

Figure 7.4.2b, which refers to mixed soil, again shows general suppression of this species by F. rubra with greatest severity at the edges of the pot. In the 2 cm and 4 cm zones there were clear indications of benefit from shelter, at least as far as percentage survival is concerned. Figures 7.4.2b and c also show some indications of suppression of M. verna by neighbouring individuals of T. alpestre and A. hirsuta, especially of A. hirsuta on the Millersdale soil.

Following the same order stated above, Figures 7.4.3a, b and c illustrate position effects on the percentage survival of T. alpestre. Figure 7.4.3a shows that F. rubra reduced the survival of this species at the edges of the pot. The data also provide evidence of a shelter effect in the 2 cm zone. The percentage survival decreased significantly in the centre of the pot in all treatments. This suggests that in T. alpestre seedling establishment is promoted by a moderate degree of shelter. It seems likely that in the present experiment shelter was provided not only by the leaf canopy of F. rubra but also to some extent by the rim of the pot. This interpretation accords with the field observations reported in Chapter 6. The pattern of survival of T. alpestre on the mixed soil is presented in Figure 7.4.3b. Strong suppression by F. rubra at the outer edge of the pot is evident. In addition, a shelter effect in the 2 cm zone is apparent. There is a general trend of decreasing survival towards the centre of the pot. Because of the sensitivity of A. hirsuta to metal toxicity, no effect of this species upon T. alpestre occurred on either of the heavy-metal-contaminated soils. In Figure 7.4.3c, the percentage survival of T. alpestre on the uncontaminated soil is presented. Strong

Figure 7.4.1 The effect of position on the percentage survival of *A. hirsuta*.

a – Mixed soil b – Millersdale soil

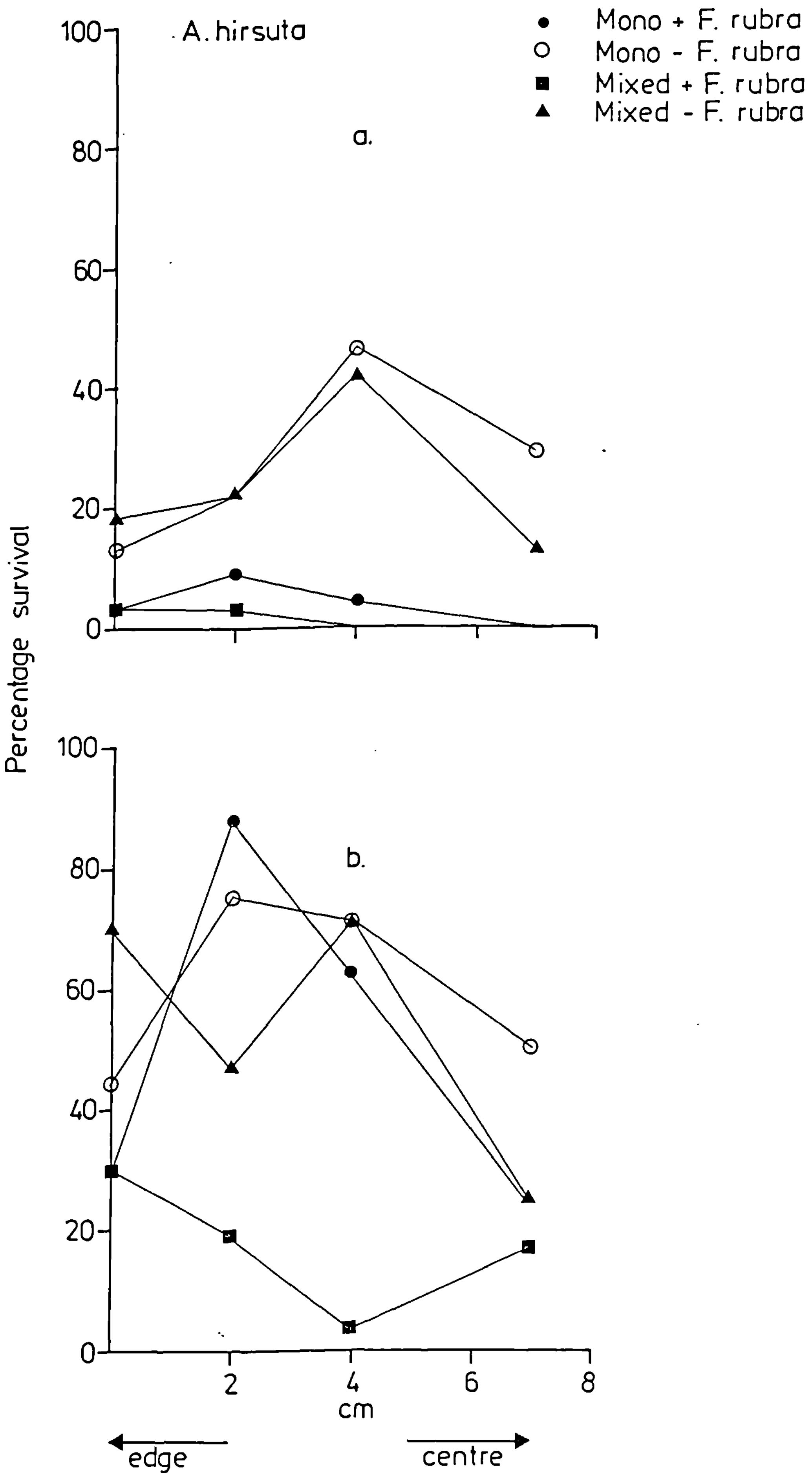


Figure 7.4.2 The effect of position on the percentage survival of <u>M. verna</u>

a – Dovegang soil

b – Mixed soil

c – Millersdale soil

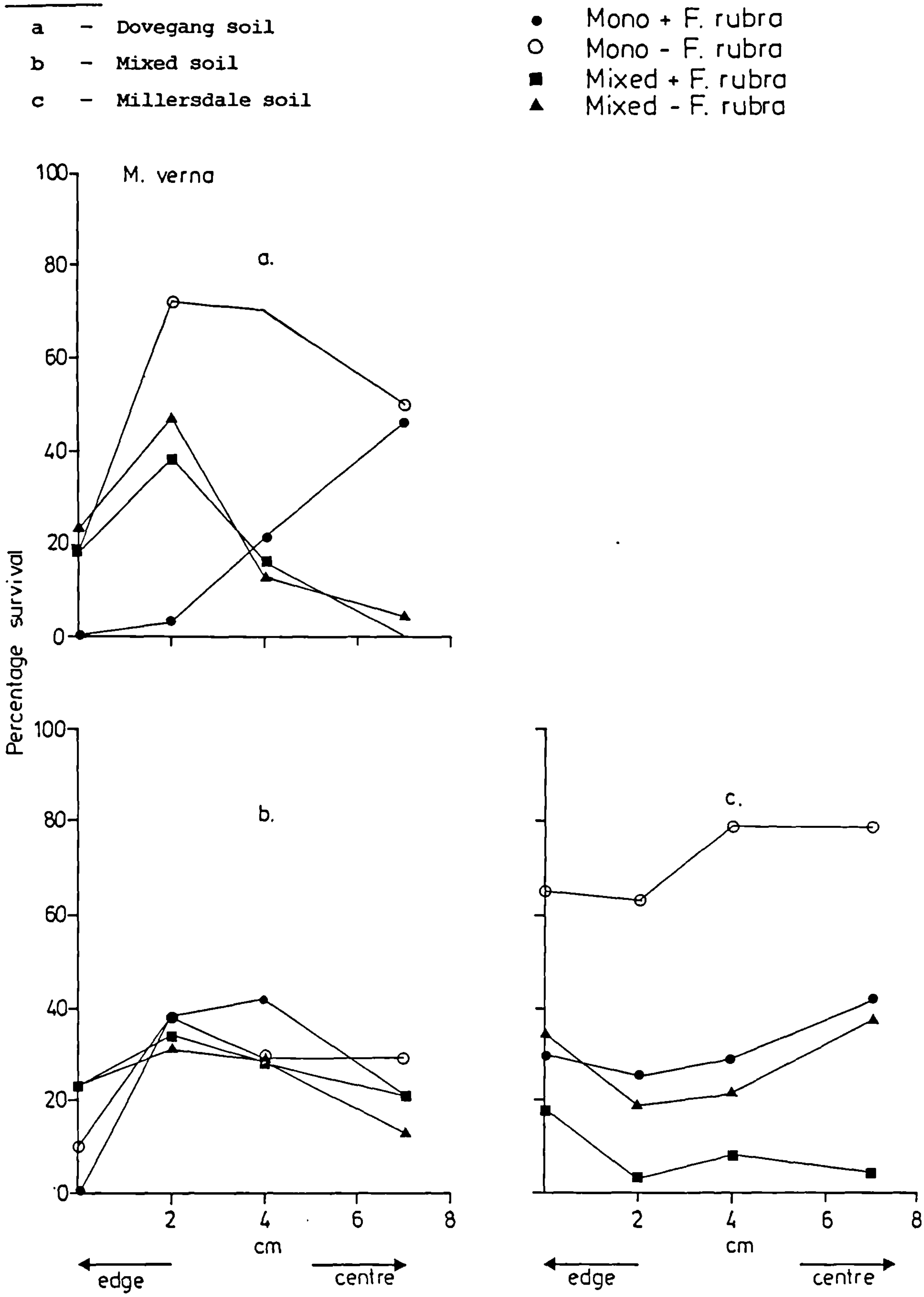
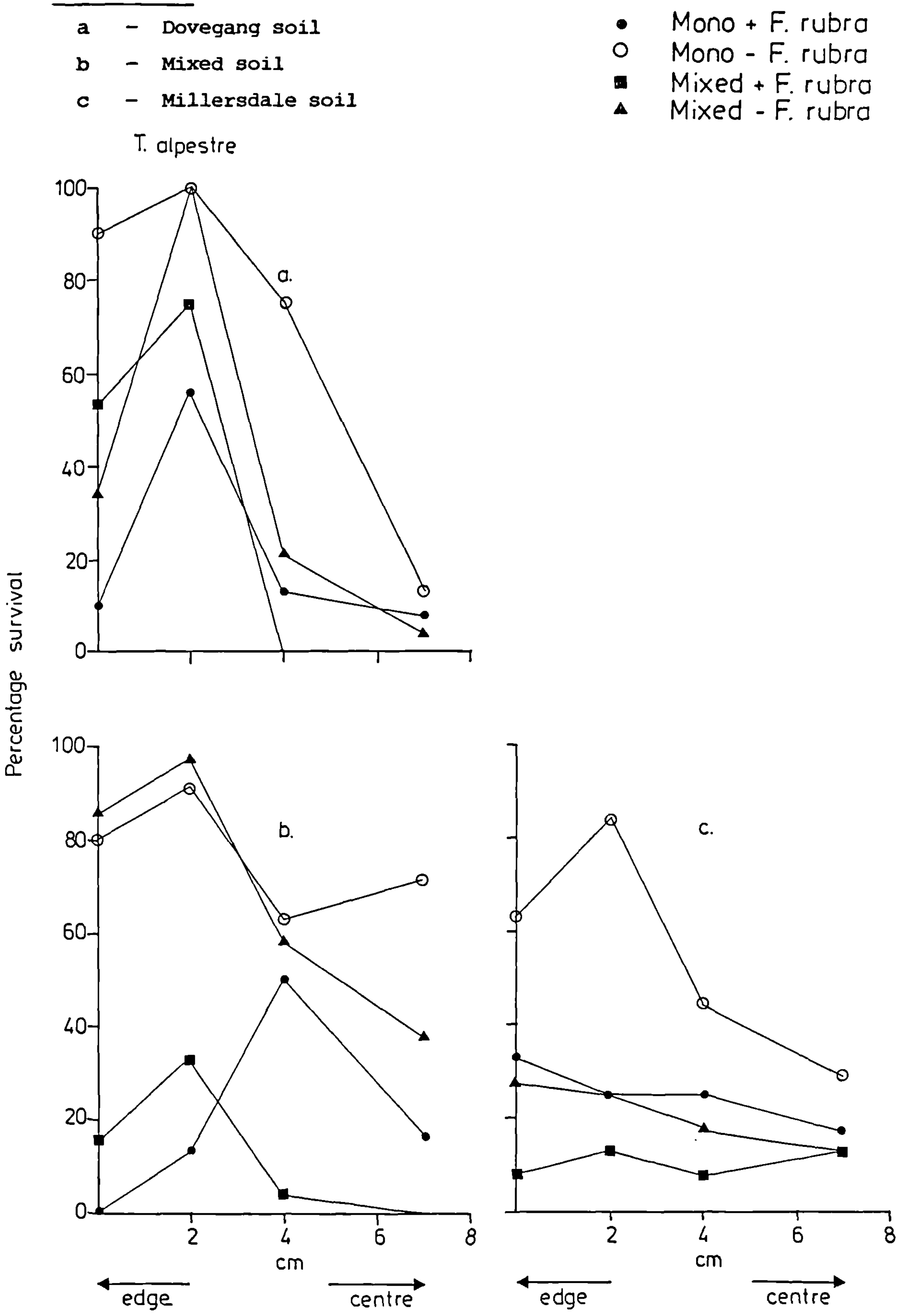


Figure 7.4.3 The effect of position on the percentage survival of T. alpestre.

a – Dovegang soil

b – Mixed soil

c – Millersdale soil



suppression by F. rubra and A. hirsuta was detected; this was probably due to the dominant impact of these two relatively robust species. As for M. verna, there was a general decrease in percentage survival of T. alpestre towards the centre of the pot.

The effect of position on the yield of A. hirsuta is examined in Figures 7.5.1a and b. Figure 7.5.1a shows clearly a strong suppression of the yield of this species by F. rubra, but there are no clear indications of position effects. Toxicity of the soil is the dominant effect on this species but Figure 7.5.1b confirms, as one might expect, that there is a strong negative effect of F. rubra on this species when the two species interact on the uncontaminated soil. There were no clear indications of position effects in all treatments, except in the mixed culture without F. rubra: here the pattern of dry weights indicated an increase towards the centre of the pot. This coincides with a decrease in percentage survival and indicates intraspecific competition. There was no clear evidence of effects of interspecific competition from M. verna or T. alpestre.

Figures 7.5.2a, b and c examine the influence of planting position on individuals of M. verna. On each soil there was a strong suppression of the yield of this species by F. rubra. In Figure 7.5.2a there is an indication of intraspecific competition. This is evident also in Figure 7.5.2b, but in Figure 7.5.2c there is no clear effect of position. In general, the effect of the mixed culture on the yield of M. verna was not as pronounced as on the percentage survival of this species.

Following the same order as above, Figures 7.5.3a, b and c show the effect of position on the yield of T. alpestre. On all soils, suppression of the dry weight by F. rubra was clear. Also, in general, there were no clear indications of position effects on the dry weight

Figure 7.5.1 The effect of position on plant dry weight

a - Mixed soil b - Millersdale soil

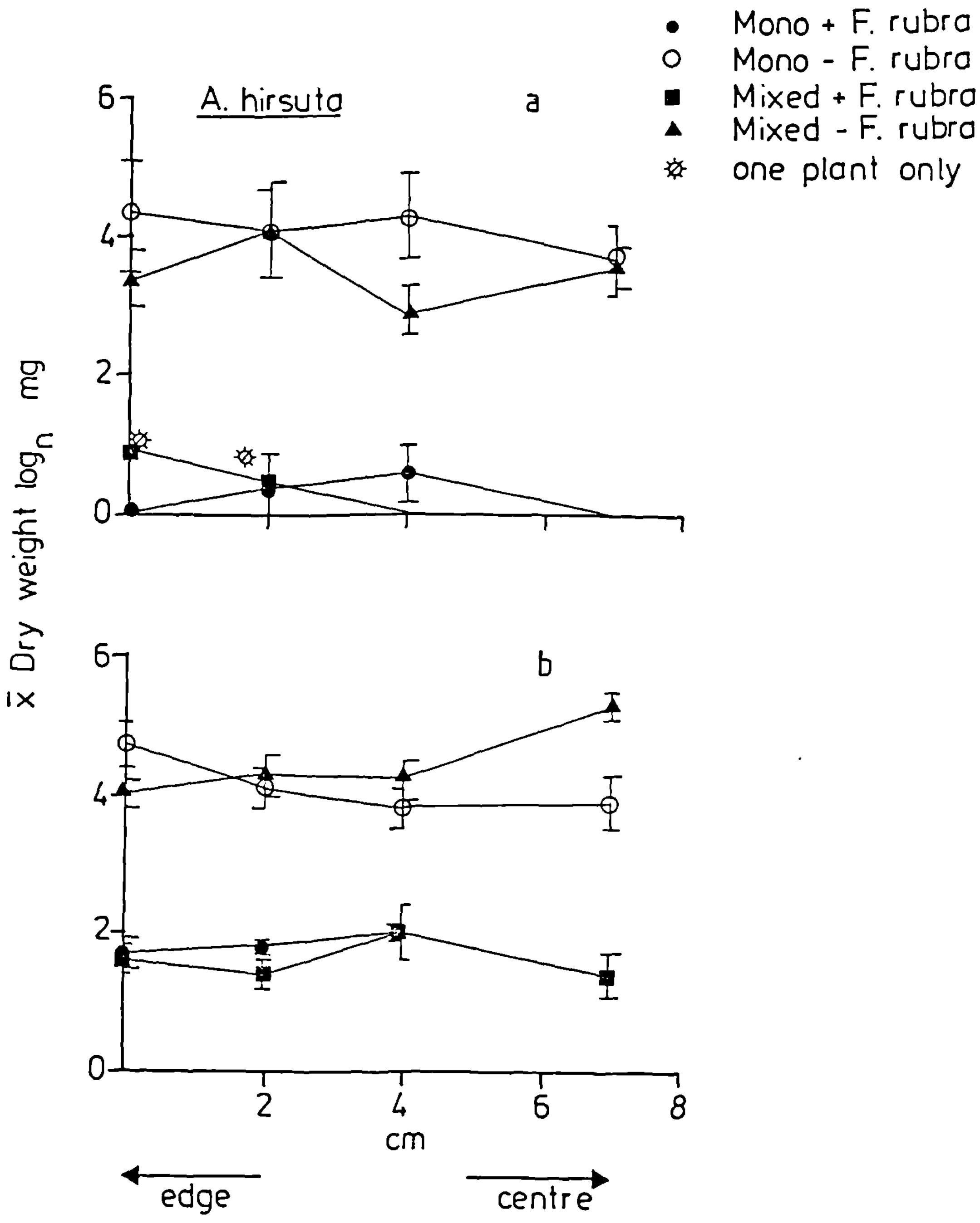


Figure 7.5.2 The effect of position on plant dry weight

a – Dovegang soil

b – Mixed soil

c – Millersdale soil

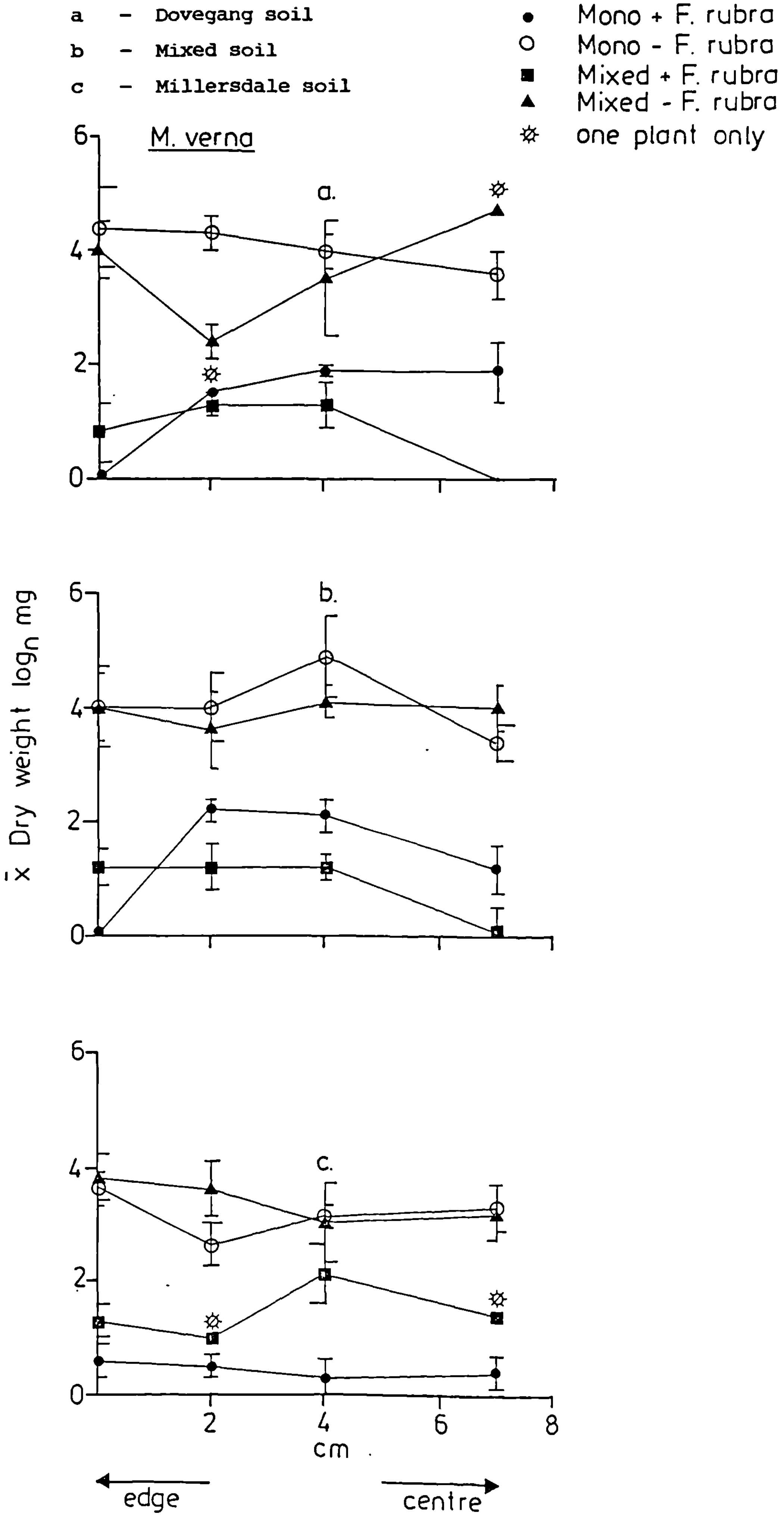
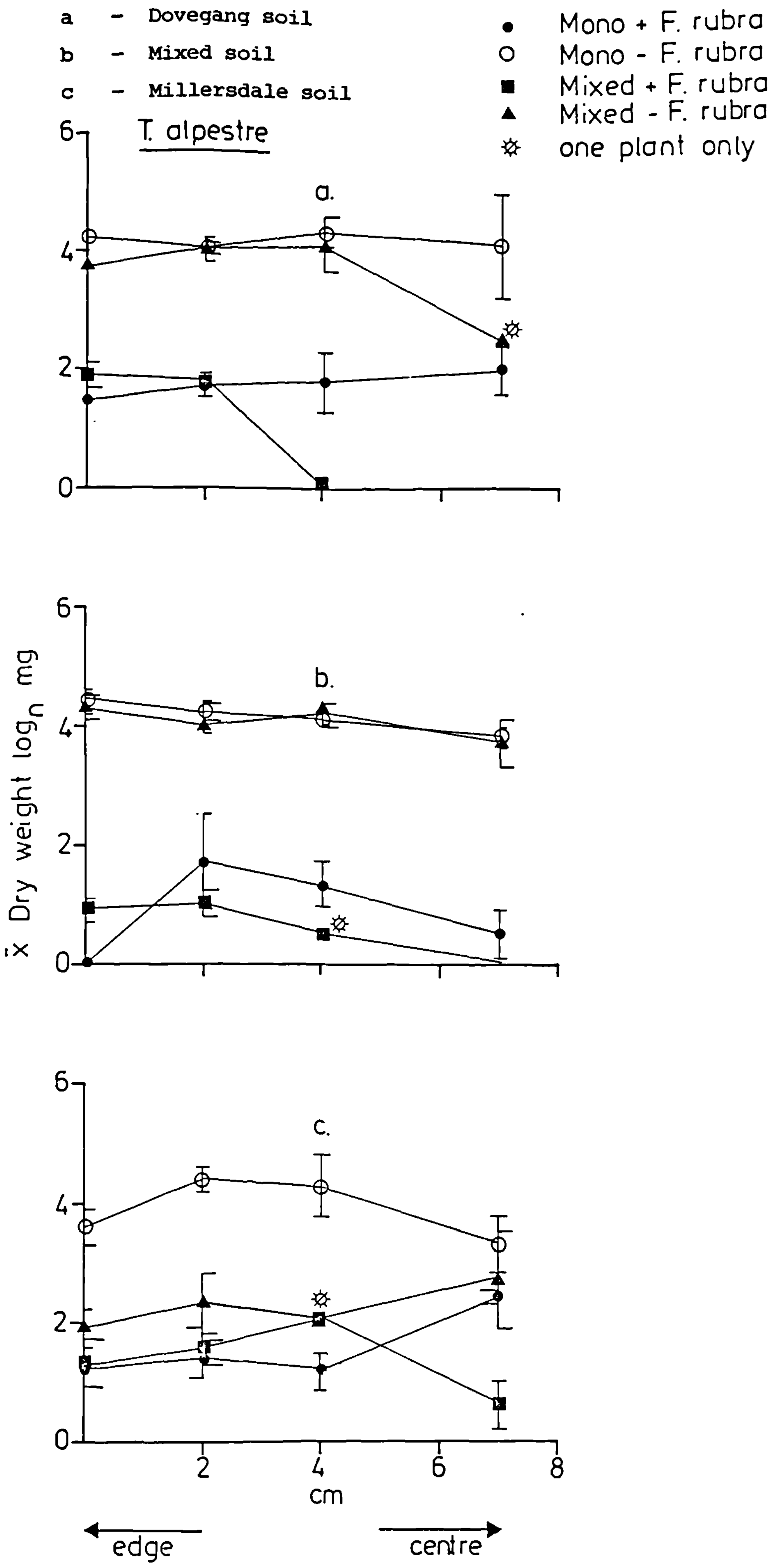


Figure 7.5.3 The effect of position on plant dry weight



of plants of this species. Effects of intraspecific competition within this species were essentially similar to those already described for A. hirsuta and M,. verna

The strong suppression of the size and dry weight of A. hirsuta, M. verna and T. alpestre by F. rubra may be illustrated photographically (Plate 7.1). In the presence of F. rubra these seedlings remained exceedingly stunted on the two metal-contaminated soils and on the non-toxic soil. However in the case of A. hirsuta, the metal-contaminated soils brought about a significant decrease in the number, size and dry weight of this species, both in the presence and in the absence of F. rubra. This suggests that A. hirsuta is very susceptible to heavy metals, and the observed occurrence of this species on some metal-mine spoils is almost certainly related to the heterogeneous nature of metal-mine substrates, where some less toxic sites may provide a suitable place for the germination and growth of this species. Support for this interpretation was obtained in the present work in that some survival of this species was recorded on the mixed, less toxic soil.

A. hirsuta affected the growth of M. verna and T. alpestre when the species impinged on each other on the non-toxic soil. This could be due to the adaptation of the two latter species (mine plants) to conditions of low fertility (Antonovics et al., 1971; Baker, 1978c). A consequence of this adaptation is that such plants may be unable to respond vigorously to extra supplies of nutrients to the extent characteristic of 'normal' plants (Jowett, 1959). In fact some metal mine plants have been shown to have lower growth rates under normal conditions (Coughtrey & Martin, 1977; Cox & Hutchinson, 1981) and the non-tolerant populations have achieved higher yields than the tolerant populations on uncontaminated soil both in monoculture, and when grown

Plate 7.1 The effect of the presence of established shoots of *F. rubra* on the growth of *M. verna*, *T. alpestre* and *A. hirsuta*, on non-toxic Millersdale soil. The framed specimens were grown with *F. rubra* and the large plants were grown in the absence of *F. rubra.*

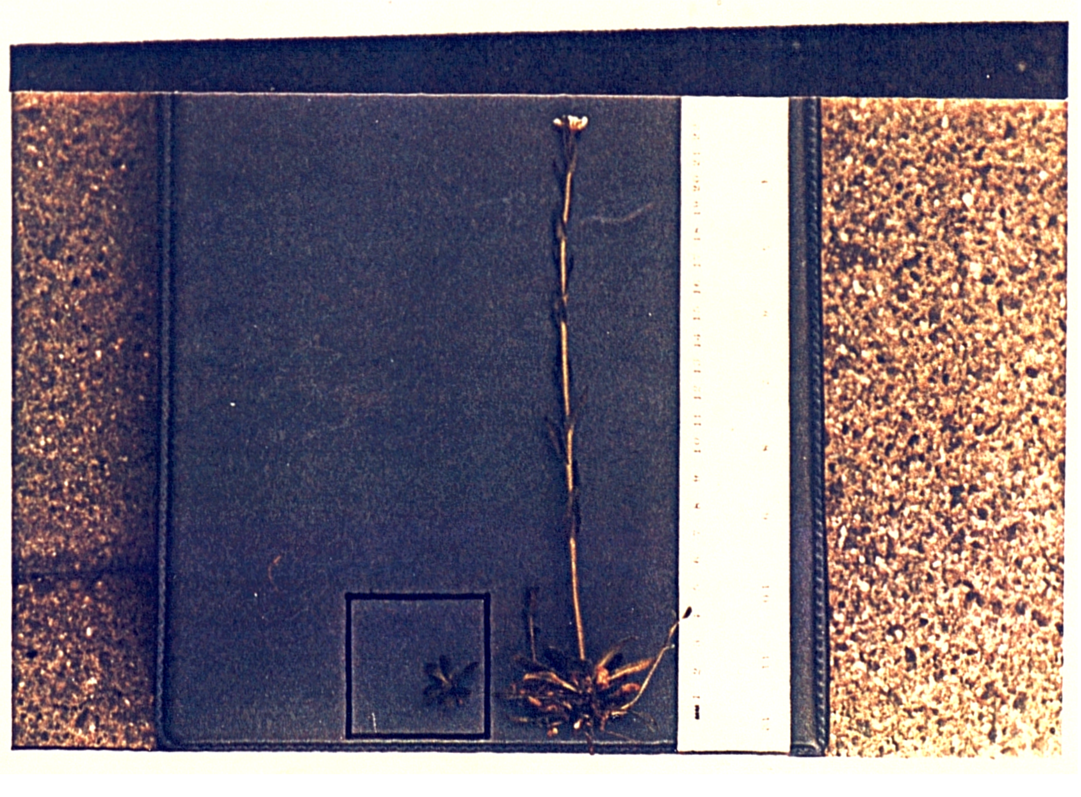
a - *M. verna*

b - *T. alpestre*

c - *A. hirsuta*

a

b



c

in competition (Cook, Lefebvre & McNeilly, 1972). These latter authors concluded that there would be selection against mine plants of (species) on normal soils by competition, if not as a result of their lower growth rates. The competitive inferiority of mine plants on normal soil has also been reported for some other species e.g. A. elatius, R. acetosa, P. lanceolata and A. odoratum (Hickey & McNeilly, 1975) and for A. odoratum (Putwain, 1963 and Antonovics, 1966, cited in Antonovics et al., 1971). The lower biomass production and loss of competitive ability under normal conditions may be due to the energy expenditure needed to maintain the mechanisms which confer metal tolerance (Ernst, 1976). A more likely explanation is that metal-tolerant genotypes from infertile habitats do not exhibit the degree of 'active foraging' (i.e. rapid re-distribution of fine roots) characteristic of strongly competitive plants of fertile soils (Grime, Crick & Rincon, 1986). It is worth noting that many of the competition experiments between metal-tolerant and 'normal' individuals cited above were carried out either in normal agricultural pasture soil or in John Innes Potting Compost, which could therefore exaggerate any differences (Shaw, 1984). Under the conditions of the present experiment (where all three soils used had low fertility) there was evidence of the competitive inferiority of M. verna and T. alpestre on both the normal and contaminated soils.

7.5 SUMMARY

1 - In order to examine the role of competition in the ecology of M. verna and T. alpestre, an experiment was designed to investigate the interacting effects of metal toxicity and competition from established perennial vegetation upon the two species.

2 - Established vegetation was represented by transplants of F. rubra, the main plant species associated with M. verna and T. alpestre in Derbyshire. The soils used in the experiment were collected from two field sites occupied by open calcareous vegetation of low productivity. One soil was contaminated by heavy metals whilst the other was non-toxic. A third soil treatment consisted of a 1:1 mixture of these two soils.

3 - The experiment was planned to measure the effect of proximity to established shoots of F. rubra on seedling establishment and yield on toxic and non-toxic soils, in both monoculture and mixed culture.

4 - A. hirsuta (a species of rather similar ecology to T. alpestre but confined to non-toxic soils) was included in the experiment as a control species.

5 - Plants were grown for 18 months and the experiment was monitored continuously and maintained.

6 - The experiment was then terminated and a final census of plant number, position, size and dry weight of the shoot of the surviving plants was recorded.

7 - A strong negative effect of F. rubra on all the other three species was detected, and a drastic effect of the toxic soil on A. hirsuta was also apparent.

8 - Effects of inter - and intraspecific competition were observed in all three species. Yield of seedlings was inhibited in close proximity to F. rubra but there was some evidence that survival of seedlings was enhanced by the shelter effects of intermediate shoot densities of F. rubra.

9 - M. verna and T. alpestre were weak competitors in comparison with F. rubra and A. hirsuta, but both species showed some capacity to persist as very small stunted plants.

CHAPTER 8

GENERAL DISCUSSION

In the introductory chapter of this thesis (Chapter 1) the ecological problem investigated in this work was stated and the various lines of observational and experimental study detailed. Each of these has been reported and discussed independently in the chapters which follow. It remains to assess the relative contribution each of these lines of study has or has not made in providing an insight into the factors which singly or together are responsible for the present-day distribution of M. verna and T. alpestre.

In many ways the problem of interpretation of the field distribution of plants is an intractable one, it is tempting to seek simple answers to what may be complex problems. Hypotheses are easily erected but frequently difficult to test experimentally. There is no doubt that a comparative autecological approach can yield useful information on the relative sensitivity of species (or populations of the same species) to single environmental factors thought to be important in controlling the field distribution of plants on the basis of field observations. Rorison (1969) has provided a protocol for the establishment of ecological inferences from laboratory experiments. Such attractive lines of study, however, can only go so far in providing solutions to complex ecological problems in that plants are considered in isolation and their performance monitored under carefully-controlled and standardized environmental conditions. In recent years, it has become increasingly apparent that demographic processes, biotic interactions and competitive effects are equally important in contributing to an understanding of plant distribution and the success of individual species in given environments. Similarly, historical factors, both ancient and recent, may be equally important in providing an understanding of the present-day distribution of plants, particularly those such as *M. verna* and *T. alpestre*, which exhibit markedly disjunct distributions. The experimental ecologist is thus faced with a situation where he can describe the field distribution of plants very precisely, but he can never provide a complete answer as to what factors have accounted for that distribution. He can, however, make reasoned judgements on the relative importance of the factors he believes to be of major importance. A further complication is the evolutionary aspect. In the field, we observe *a posteriori* the effect of recent and historical evolutionary change. The adaptability of individual species and the ease with which they evolve adapted variants at the level of individuals within populations can again be potent factors in determining the present-day success of species, particularly in changing environments. The evidence for species extinction and species expansion to new habitats on the basis of evolutionary responses (or lack of them) to environmental change is convincingly reported in an extensive literature. An insight into the nature and scale of population differentiation in both currently widespread species and those presently exhibiting more scattered distribution can emerge from comparative observations and autecological experiments at the population level. The present study has adopted all these approaches, with varying degrees of success.

Both *M. verna* and *T. alpestre* exhibit restricted and disjunct present-day distributions globally, in Europe and Scandinavia, within the British Isles, within the Pennine region and locally within Derbyshire. At every level, *T. alpestre*, a plant which according to the literature to date has similar ecological requirements to M. verna, is under-distributed or 'rarer' than the latter species. Why should this be? In order to answer this profound question, observational and experimental studies have been performed on populations of both species from the North and South Pennines where the contrasting local distribution is most noticeable. It could be argued that such an approach can never provide a complete answer as the species have not been investigated using material from the full geographical range. Conclusions derived may then only have parochial relevance. As a counter-argument however, it could be suggested that should any differences in species properties emerge from a local study then it is highly likely that these are of major relevance in understanding the wider distribution patterns. This is the premise of the present work.

Both M. verna and T. alpestre have been designated absolute metallophytes by Lambinon & Auquier (1963). The implication is that these two species currently occupy a specialized ecological niche (metalliferous soils) over the majority of their distributional range and that this has been facilitated by the evolution of heavy metal tolerance. The association of both species with metalliferous soils, particularly those newly created by metalliferous mining and smelting activities, has been recognized for many years. The two species occur in characteristic plant assemblages on mineralized ground, frequently occurring together with other associates which vary with geographical locality. Ernst (1976) gives a full phytosociological account of these communities in Europe. Field observations and survey work in Derbyshire reported in Chapter 2 confirm that both species are indeed restricted to lead mine spoil habitats in the area, the tightness of this restriction being more pronounced in T. alpestre than M. verna. Vicarious occurrences of contaminated soils are extremely uncommon and may be the result of chance dispersal to an otherwise suitable uncontaminated habitat. There the species may persist for many years but not thrive or spread further, their survival being instances of biological inertia. The British and European distributions of the two species do however show rather less convincing restriction to metalliferous soils (Chapter 2). Both species display a montane distribution in continental Europe and to a smaller extent in the British Isles. It is primarily the lowland distribution which is associated with mineralized soils. This habitat dichotomy raises intriguing questions about the post-glacial history of the species and the extent of evolutionary change.

There is still considerable debate about the origin of endemism in metallophyte floras (see e.g. Antonovics _et_ _al_., 1971). Some believe that the so-called mine taxa are 'palaeo-endemics', relict species of formerly wide distribution now confined to particular areas, while others suggest that they are 'neo-endemics', species that have originated recently in given areas in response to peculiar environmental conditions. The weight of evidence in support of either theory is not conclusive, but the palaeo-endemic theory would seem most applicable for _M. verna_ and _T. alpestre_ in view of their present distribution patterns. Such a theory would not preclude the possibility for further local evolutionary divergence between populations leading to ecotypic differentiation within the species. A major problem discussed by Antonovics _et_ _al_. (1971) is that metallophyte (mine) taxa cannot frequently be distinguished morphologically from their relatives from which they are not isolated reproductively. They suggest that such taxa could be the result of parallel evolution on different mineralized outcrops or soils from relatives in the surrounding vegetation and that evolution of both metal tolerance and morphological differences has proceeded by 'mine ecotypes'. Such could be the case for M. verna and T. alpestre in montane localities where mineralized soils are present.

The glacial history of M. verna and T. alpestre in the British Isles (Pigott & Walters, 1954; Shimwell, 1968; Pennington, 1969 and Godwin, 1975) suggests that both species were components of the Late Glacial vegetation (although no specific records of T. alpestre pollen have been identified in Quaternary deposits but brassicaceous pollen at that time was generally high), which had a high proportion of species of low competitive ability such as species of open montane habitats today. This element of the flora has survived only in those areas where refuges from the spread of woodland or peatlands existed. The pollen record thus suggests that the two species were probably more abundant in Late Glacial times than at present. Survival in situ can explain the existence of a number of montane populations of M. verna in the British Isles, as for example at Twll Du, Cwm Idwal, N. Wales, and on Widdybank Fell, Teesdale(for full list see: Halliday, 1960); and for both species in continental Europe, Shimwell, (1968) also suggested a refugium theory for the local survival of T. alpestre on remote Carboniferous limestone cliffs at Matlock Bath and elsewhere in Derbyshire. It may then be that the extended distribution of both species has resulted from the spread on to naturally metalliferous soils where an open vegetation is also maintained as a result of prevailing heavy metal toxicity. Such a habitat, particularly in upland Britain, has many similarities with the montane environment of Late Glacial times. Halliday (1960) considers that M. verna has been effectively dispersed by rivers in its montane localities, often producing transient riverine populations. Although many montane species descend in altitude with increasing latitude, M. verna occurs at sea level not only in Ireland and N. Wales but at its most southerly British station on the Lizard, while its most northerly station in Banffshire is at 530 m. Secondary dispersal of both species has been more recent as a result of the creation of mine wastes and other metal-contaminated spoils which have again provided ideal conditions for establishment. The successful spread of both species in these new habitats may well have been facilitated further by transport by man. Baker & Dalby (1980) provided a similar interpretation of the present-day inland distribution of Silene maritima in the British Isles.

The vegetation survey of M. verna and T. alpestre sites in the S. Pennines reported in Chapter 2 did not reveal any clear community preferences for the two species; both regularly occurred together but approximately half the sites recorded supported only M. verna (the same is true for the British distribution as a whole). In the DECORANA species ordination presented it was of considerable importance that the two species occupied neighbouring positions at the centre of the ordination axes. This suggests that, at least in terms of species associations, the two species are remarkably similar and that this similarity will probably extend to habitat requirements. Although no rigorous environmental records were made in conjunction with this survey, it did prove possible to review the species ordination in the light of some simple environmental indices derived from the field records. This did not reveal any major differences in habitat preference, at least in the context of the local Derbyshire survey. There was an indication that M. verna had some preference for north-facing slopes and that it occurred on soils of a slightly greater pH range than did T. alpestre. This is in general agreement with the British distribution patterns as a whole. Both species also showed a marked preference for bare ground, or at least very open vegetation which is typical of toxic mine spoil habitats. Halliday (1960) considers that the distribution of M. verna in the British Isles is intimately related to the occurrence of suitable basic or ultramafic exposures. The majority of its sites have a pH above 6.0 though the species is capable of thriving on soils with a pH as low as 5.4 provided the supply of bases is adequate. Similar comments can be applied to T. alpestre soils (Ingrouille & Smirnoff, 1986). Both species are particularly associated with the Carboniferous limestone formations of the Pennines; this rock formation is also responsible for the isolated occurrences in the Mendips and on the N. Wales coast, for both species and for M. verna in the Burren, W. Ireland; T. alpestre has also been reported from more siliceous hard rocks in North and Central Wales where it is not associated with M. verna (Smith, 1979).

Although chemical analyses were not performed on soils from all the sites in the vegetation survey, elevated heavy metal concentrations were likely as was indicated from the detailed analytical studies presented in Chapter 4. It is further true that the majority of sites in the British Isles at which M. verna and T. alpestre have been recorded are associated with elevated concentrations of soil lead, zinc, cadmium, copper or nickel and low nutrient (nitrogen and phosphorus) status. The implication is that both species have evolved heavy metal tolerance which accounts, at least in part, for their success on metal-contaminated sites. Both Halliday (1960) and Ingrouille & Smirnoff (1986) note the association of M.verna and T. alpestre respectively with metal-enriched soils; the latter authors go even further in stating that in the British Isles the distribution of T. alpestre is more strongly associated with soils contaminated with lead and zinc than with altitude. Outside the S.Pennine area, M. verna has also been recorded on copper-enriched soils (Dolfrwynog, near

Dolgellau, Wales, and Grizedale, N. Pennines (Smith, 1979)) and on nickel-rich soils (Lizard, Cornwall and various serpentine sites in Scotland (Halliday, 1960)). The soil data presented in this thesis do not suggest that, for the Derbyshire sites, there are any major differences in metal status between sites which support both M. verna and T. alpestre and those from which T. alpestre is absent. However, in a small study of soil chemistry at some N. Pennine and Irish M. verna and T. alpestre sites, Garcia-Gonzales & Clark (1985) suggested that M. verna occurs on soils having widely differing concentrations of eleven metals (Pb, Zn, Cd, Cu, Co, Ni, Mn, Fe, Ba, Ca & Mg). It appeared that T. alpestre was restricted to soils having relatively high metal status, implying that there was less variation in metal concentration between T. alpestre sites. The data presented were not convincing in view of the very limited number of sites sampled.

The biogeochemical data presented in Chapter 4 suggested that both species show a marked ability to tolerate and take up lead, zinc and cadmium from metalliferous mine spoils. There was also considerable seasonal variation in the pattern of metal uptake which could be related to changes in metal availability. The study suggested that metal tolerances in T. alpestre involved an accumulator strategy (sensu Baker, 1981) whereas, at least relative to T. alpestre, M. verna showed less accumulation and a restricted transport (exclusion strategy). This work was extended with a detailed study of inter-specific and inter-population variation in the effects of heavy metals (Pb, Zn and Cd) on seedling responses and metal uptake, which forms a substantial proportion of the experimental part of this project (Chapter 5). It established at least two important differences between the two species. Firstly, inter-population variations in metal tolerance and uptake could be detected in both specie, but these could not always be directly related to the metal status of the native soils. These variations were generally greatest in M. verna. Secondly, the unique power of metal (notably Zn) hyperaccumulation in T. alpestre emerged. Collectively the experimental results suggested that T. alpestre was substantially more metal tolerant in almost every comparison with M. verna. Furthermore, there were marked stimulatory effects of most heavy metals on the growth of T. alpestre when supplied at concentrations which are toxic to other species as metal tolerant, including M. verna. The results of these experiments would suggest that T. alpestre could grow in situations of extreme metal toxicity from which M. verna would be excluded; the field evidence did not detect such a situation. It may then be entirely fortuitous that the species differ in this way, or more likely, it is a reflection of differing internal mechanisms of metal tolerance which may be involved (see e.g. Ernst, 1976; Thurman, 1981). Mathys (1977) has linked both malate and mustard oil glucoside production with zinc tolerance in T. alpestre; similar biochemical studies have not been carried out using M. verna or for other metals. Within the Cruciferae, metal hyperaccumulating taxa are not uncommon. For example, some 50 Alyssum spp, and 20 Thlaspi spp, have been listed by Reeves & Brooks (1983) as hyperaccumulators of nickel (>1000 μg/g Ni in shoot dry matter); a number of zinc hyperaccumulating species of Thlaspi are also listed here and in Reeves (1987). Smirnoff & Stewart (cited in Ingrouille & Smirnoff, 1986) believe that from their experimental studies on T. alpestre a clear relationship between zinc tolerance and accumulation is not apparent. They suggest that the two properties may be independently inherited characters, as their 'zinc-intolerant' population (Rhum) accumulated zinc to the same degree as more tolerant mine populations.

The possibility that some plants possess a constitutional tolerance to heavy metals was originally dismissed by Antonovics *et al*., (1971). However the results presented in Chapter 5 for screening for metal tolerance to a wide range of metals in *T. alpestre* and *M. verna* could be interpreted in the light of a constitutional tolerance as, in all cases even where metal enrichment was not apparent in the parent soil, *T. alpestre* showed superior tolerance and enhanced metal uptake. There are convincing reports in the literature which suggest that species can differ widely in their thresholds of tolerance (e.g. Wu & Antonovics, 1976). Populations of the same species from diverse soil environments may show similar characteristics of metal uptake and accumulation. Thus Reeves & Baker (1984) compared the metal-uptake characteristics of a serpentine and a non-serpentine population of *Thlaspi goesingense* when grown on a range of Zn- Ni- and Co-enriched soils and found that the non-serpentine plants showed similar uptake and accumulation of these metals to the serpentine plants. This suggested the existence of a non-specific metal detoxification system in the species. More recently, Fiedler (1985) concluded from a study of heavy metal accumulation in the genus *Calochortus* (Liliaceae), that the ability to tolerate excessively high levels of Ni, Co and Cu may be a constitutional property of the whole genus. The existence of metal co-tolerances in *T. alpestre* could also provide some explanation of the broadly-based tolerance of this species. Reports of co-tolerance, whereby tolerance to one metal confers some degree of tolerance to another or others not present or enriched in the soil are not uncommon (Allen & Sheppard, 1971; Cox & Hutchinson, 1979, 1981; Symeonidis, McNeilly & Bradshaw, 1986). The work of the last authors with cultivars of the grass *Agrostis capillaris* has confirmed the existence of non-specific low-level tolerances which may be more widespread in other species than originally thought. The stimulatory growth responses of T. alpestre to metal supply perhaps suggest the operation of such mechanisms or a constitutional tolerance as indicated above, where there is an apparent requirement for an otherwise non-essential element. It would be particularly rewarding to test these theories using plants collected from truly alpine populations from elsewhere in Europe.

For both M. verna and T. alpestre it emerges that inter-population variations in metal tolerance and uptake characteristics can be detected between populations which are geographically close and growing on similar (although different in their metal concentrations) habitats. This suggests local adaption in metallophyte species is possible although the experimental data presented in Chapter 5 do not suggest direct relationships between indices of tolerance and estimates of metal activity in the native soils. Ingrouille & Smirnoff (1986) did detect a convincing relationship between indices of zinc tolerance and ammonium acetate-extractable soil zinc for four populations of T. alpestre they tested, it also emerged from their study that the one population that they included as a control from a suspected low zinc site (Rhum) also came from a soil with comparably high exchangeable soil zinc to the mine populations included. It may well be that it is difficult to detect clear trends in small-scale differences in tolerance indices between populations collected from mine sites all of which are of a high metal status. This may be particularly true of T. alpestre if a constitutional tolerance is proposed. In this case all populations may possess a certain 'blanket' tolerance which may be only slightly further enhanced in response to increases in soil metal concentrations. It is perhaps important, however, to stress that there is some variation in tolerance in both species upon which selection could act.

The study of inter-population variation in morphological characters reported in Chapter 3 revealed considerable genetically-based variation in both vegetative and reproductive characters for both species. This was in agreement with more broadly-based studies of the two species (Halliday, 1960; Riley, 1955, 1956). The two species are renowned for their variability in the field, a fact which has caused considerable taxonomic difficulties. The significance of this population variability has been discussed at some length in Chapter 3. Some of the variation can be interpreted as ecotypic in origin in the same manner as heavy metal tolerance. There was no clear evidence to suggest that morphological and physiological traits were linked in either species. What is implied however, is that both species are still in an active state of evolution as population divergence has occurred at some stage after the initial establishment of the populations from a small number of founder individuals. Local populations of T. alpestre may persist as a very small number of individuals. Subsequent evolutionary change may well be governed by the extent of geographical and reproductive isolation between populations. In the small number of populations selected for this aspect of study, it was shown that for M. verna significant morphological variation could be detected between both geographically-close (S. Pennine) and remote (N. Pennine) populations. Further, local variations in the Derbyshire populations could be partially related to both the age and size of the populations. T. alpestre populations showed rather less variability. Differences in the breeding systems of the two species and regenerative strategies are probably central to an understanding of the present-day distribution of the two species. The population biology of the predominantly inbreeding T. alpestre contrasts diametrically with the out-breeding yet self-compatible M. verna. While the self-pollination mechanism of T. alpestre can be viewed as maintaining genetic stability and high seed production (and thus be regarded as a feature of selective value in the survival of small populations), it limits the opportunities for genetic variation upon which selection may further operate. A small degree (5%) of outbreeding in T. alpestre was however detected by Riley (1956) which may be crucial in this respect. The wide-scale variation detected between populations of M. verna is to be expected from its outbreeding behaviour. The protandrous pollination mechanism in the species also favours out-crossing, or at least largely precludes selfing. The species is not self-incompatible and crossing between individual flowers on the same plant or inflorescence is possible due to variation in flower maturity. This 'insurance policy' can also be considered to be of selective importance in the survival and proliferation of populations when pollen vectors are scarce.

The distance over which pollen transfer can occur to effect cross-pollination will also be of significance in population isolation, Riley (1956) showed that when self-pollination was artificially prevented in T. alpestre, most pollen was deposited on receptive stigmas within 23 m of the male parent and that virtually none was found 90 m from this plant. Unfortunately, no estimate of the distance of M. verna pollen transport has yet been made. However, it is likely to be of greater magnitude to that of T. alpestre for at least two reasons. Firstly, the sweetly-scented and more conspicuous flowers of M. verna are likely to attract a greater range of potential pollen vectors. Halliday, (1960) noted that in the shelter of a greenhouse plants were visited by a variety of insects, particularly members of the Syrphidae, Apidae and, less frequently, the Muscidae. There are also reports of visits by members of the Empidae and the Lepidoptera. However, in spite of their attraction, Halliday noted very few insects visiting plants in the wild. Casual field observations in the present study do not agree with this observation, in that at many of the sites visited during fieldwork in June-July insect visitors were abundant. In contrast, few insects were noted on flowers or in the vicinity of plants during the flowering season of T. alpestre. Secondly, M. verna has a prolonged flowering season; in Derbyshire flowering can commence as early as May and continue until early autumn (September). Halliday, (1960) reports the longest flowering season of any M. verna population is that enjoyed by the Lizard population where flowering commences in March and may continue until October. By contrast, T. alpestre has a much more restricted flowering season (April-June in Derbyshire) at a time when insect pollen vectors are scarcer and less active. Halliday (1960) also draws attention to the high production of morphologically 'good' (viable) pollen in M. verna (generally >90%).

The role of the breeding system in determining the distributional ranges of the species is clear. However, it may well be equally important in the development and maintenance of metal tolerance in populations. In a metal-tolerant population, self-fertility will enable an establishing population of tolerant individuals to increase their number more rapidly from a few tolerant colonists, while maintaining the tolerance levels (Antonovics, 1968). This feature is probably of greatest relevance in the case of T. alpestre where population size is frequently very small, and gene flow virtually nil. It will be of less consequence in M. verna where populations are frequently large and gene flow between adjacent populations is a possibility.

The extent of gene flow may have been particularly important for M. verna during interglacial periods when previously isolated populations came into contact within newly-formed habitats with large uncolonized areas of rocky debris and detritus. In consequence, M. verna may have developed considerably greater genetical and ecological diversity even before the onset of mining operations created a new range of physically similar habitats. A mechanism for the rapid production of rather greater ecological novelty than crossing between populations, polyploid evolution, has also operated in M. verna. Polyploid evolution results from the hybridization of related taxa following the breakdown of ecological barriers due to major disturbances (Stebbins, 1971, 1980). It has presumably been responsible for the formation of M. verna ssp. collina (Neilr.) Halliday, which is tetraploid (2n = 48) and has a wide geographical distribution in S.E. Europe, where it is the commonest subspecies (Tutin et al., 1964). In contrast, polyploid evolution has not been reported in T. alpestre. This is probably because of the strong tendency of T. alpestre to be inbreeding and the fact that it is occasionally monocarpic, and hence more vulnerable to the 'bottleneck of semi-sterility' associated with the early stages of polyploid evolution (Stebbins, 1971). However while polyploid evolution may be a factor contributing to the wider geographical range of M. verna in Europe, it is not a factor of relevance to the distribution of either species in Britain, where both M. verna ssp, verna and T. alpestre are diploid (Tutin et al., 1964).

The importance of population processes and regenerative strategies in the survival and development of populations of the two species was investigated in Chapter 6. Here again, major differences in behaviour between the two species may well provide a key to their relative success in colonizing mine wastes in the S. Pennines. M.

verna can be a relatively long-lived perennial capable of regeneration from vegetative shoots, but producing large quantities of seed. T. alpestre has been referred to as a biennial, but in all the sites investigated in this study, it behaves as a short-lived perennial. It is less prolific in both flower and seed production and can regenerate effectively from small daughter rosettes which develop at the base of the parent plant and remain attached for several years. Production and dispersal of viable seed are crucial in the spread of species and the speed with which colonizing populations develop. Seed germination characteristics also determine the extent of new recruitment into populations and potential seedling survival. Although seed production and viability in T. alpestre are relatively high (Riley, 1956), the extremely localized dispersal of the heavy seeds (mean weight c, 0.4 mg) may be a major hindrance in the spread of the species within a site, and certainly to new localities. Seed of M. verna is smaller, lighter (mean weight c. 0.08 mg) and consequently more mobile. It is also produced in much larger quantities. The time at which seeds are shed and the period over which they germinate can play an important role in population dynamics (Thompson & Grime, 1979). The seeds of M. verna may germinate soon after being shed from the capsules in late summer/early autumn but some become buried during the after-ripening period and persist in the soil. This behaviour has been found in a number of small-seeded species which possess good seed banks (Grime, 1981). A persistent seed bank probably functions as a buffer against population extinction in local and precarious habitats. A more efficient dispersal is thus likely. Both field and experimental observations on T. alpestre suggested that seed release and subsequent germination are rapid and almost synchronous. Seeds are shed during late spring/early summer along with seeds of grass species such as

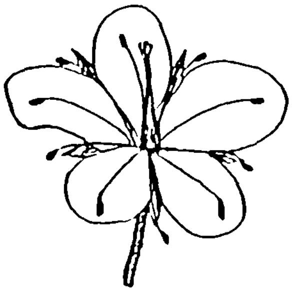
Festuca rubra, *F. ovina* and *Koeleria macrantha* (seed weight c. 0.5 mg), regarded by Thompson & Grime (1979) to be species having the ability to germinate over a wide range of temperatures in both the light and in darkness.

The result of this contrasting germination behaviour is that the majority of new recruits to a *T. alpestre* population come from successful germination of seed almost as soon as it is shed in early summer, provided conditions are conducive. In *M. verna*, some new recruits result from seed germination in the late summer/early autumn but others come from seeds germinating from the bank of viable seed maintained over the winter, in the following spring. Different cohorts of seedlings may thus be detected in the population. Colonizing ability is not restricted to species in which all or most of the seeds germinate soon after release (Grime, 1986). Some of the most successful colonists of spoil habitats, e.g. *Agrostis capillaris*, *Plantago lanceolata*, *Chenopodium rubrium*, have seeds capable of long-term survival in the soil (Grime, 1986). Distribution of buried seed also plays an important role in the distribution of mature plants in the developing population (Schenkeveld & Verkaar, 1984). Smaller seeds are also likely to be more abundant than large seeds in any seed bank (Thompson, 1986). The ecological significance of seed size and weight have long been recognized. Salisbury (1942) pointed out that the general trend for species to produce either a large number of small seeds or a small number of large seeds allow wider dissemination and more probable occupation of all suitable habitats. Thompson & Grime (1979) found that sites with an abundance of small-seeded species tended to possess large seed banks. High overall viability of seed can be a positive asset for survival or increase of population size of any species in a hostile environment, such as metalliferous mine spoil

(Riley, 1956; Hodgson, 1986c), but this may be outweighed by the detrimental effect of rapid and synchronous seed germination. In this circumstance, new recruits could be totally destroyed in the event of drought or major site disturbance, as frequently occurs in T. alpestre habitats. The germination strategy of M. verna affords some insurance against the effects of such environmental catastrophes.

In the field study reported in Chapter 6 the effects of drought (and seedling competition) were found to be more drastic on the survival of newly-recruited seedlings of T. alpestre than for those of M. verna and the numbers of the former were markedly lower than for the latter species. For both species, however, successful establishment at the seedling stage was also related to shelter effects of neighbouring plants, the effect being particularly pronounced for T.alpestre seedlings. These early beneficial effects of neighbouring plants may however give way to strong competitive interactions and exclusion at later stages of development of the communities in which M. verna and T. alpestre occur. This aspect was investigated experimentally in work reported in Chapter 7. This novel experiment was designed to assess the effects of intraspecific competition on the survival of M. verna and T. alpestre on both metalliferous and non-metalliferous soils. Competition was created with a developing 'turf' of Festuca rubra tillers, a situation not uncommon in the field. The performance of T. alpestre in this situation was also compared with that of Arabis hirsuta, a crucifer similar in morphology to T. alpestre but being restricted to open situations on non-toxic calcareous soils. This pot experiment confirmed the initial beneficial shelter effects suggested from the field study but more importantly, revealed the weak competitive ability of both M. verna and T. alpestre when growing close to perennial turf-forming species. This aspect then, has a major bearing on the success of both species, but particularly T. alpestre, in the field situation. A smaller-scale competition experiment by Rochow (1970) in the USA demonstrated a similar competitive interaction of the grass Agropyron bakeri on the performance of T. alpestre, where growth was substantially reduced as the grass developed.

The author believes that the work reported in this thesis has thrown new light on the ecology and evolutionary biology of two metallophyte species which have attracted the interests of botanists for many years. Additionally, some convincing reasons for the under-distribution of T. alpestre relative to M. verna in the S. Pennines have been suggested. It would appear that the contrasting population biology of the two species is of over-riding importance in determining the potential spread of populations on to nearby (and more distant) mine sites. The same arguments presented in this discussion chapter can be applied in the interpretation of the present-day British and European distributions of the two species. In many ways, the evolution of heavy metal tolerance in both species appears adventitious - this has enabled them to exploit a habitat to which they are in all other ways well-adapted and where they suffer minimal inter-specific competition from faster-growing species. It has allowed the two species to extend to lowland areas from their primary montane localities after the last glaciation and exploit a new range of recently disturbed habitats. M. verna emerges as a species still capable of further evolutionary adaption which is apparent from its extended distributional range within Europe; T. alpestre is restricted by its inbreeding behaviour. Indeed, this relatively rare plant on a national scale may well be in decline as a result of habitat loss from the reworking of old spoil heaps in the S. Pennines for fluorspar. As has been shown, the species is unlikely to re-establish on other suitable sites spontaneously. It appears enigmatic that two unrelated species have found a similar niche and have evolved highly efficient mechanisms of heavy metal tolerance. It remains to be determined to what extent metal tolerances are constitutional within the species throughout their present-day distributions. However, it is only possible to speculate as to whether the original montane populations also possessed this property. Further population studies on these and other metallophyte species may help to resolve this conundrum.



BIBLIOGRAPHY

Allen, S. E., Grimshaw, H. M., Parkinson, J. A. & Quarmby, C. (1974). Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford.

Allen, W. R. & Sheppard, P. M. (1971). Copper tolerance in some Californian populations of the monkey flower, Mimulus guttatus. Proceedings of the Royal Society of London, B, **177**, 177-196

Alloway, B. J. (1969). The soil and vegetation of areas affected by mining for non-ferrous metalliferous ores, with special reference to cadmium, copper, lead and zinc. Ph.D. Thesis, University of Wales.

Alloway, B. J. & Davies B. E. (1971). Heavy metal content of plants growing on soils contaminated by lead mining. Journal of Agricultural Science **76**, 321-324.

Antonovics, J. (1968). Evolution in closely adjacent plant populations. V. Evolution of self-fertility. Heredity, London **23**, 219-238.

Antonovics, J., Bradshaw, A. D. & Turner, R. G. (1971). Heavy metal tolerance in plants. Advances in Ecological Research **7**, 1-85.

Atkins, D. P., Trueman, I. C., Clarke, C. B. & Bradshaw, A. D. (1982). The evolution of lead tolerance by Festuca rubra on a motorway verge. Environmental Pollution (Series A) **27**, 233-241.

Baker, A. J. M. (1974). Heavy metal tolerance and population differentiation in Silene maritima With. Ph.D. Thesis, University of London.

Baker, A. J. M. (1970). Interclonal variation in lead tolerance in Minuartia verna. B.Sc. Dissertation, University of London.

Baker, A. J. M. (1978a). Ecophysiological aspects of zinc tolerance in Silene maritima With. New Phytologist **80**, 635-642.

Baker, A. J. M. & Dalby, D. H. (1980). Morphological variation between some isolated populations of Silene maritima With. in the British Isles with particular reference to inland populations on metalliferous soils. New Phytologist **84**, 123-138.

Baker, A. J. M. (1978b). The uptake of zinc and calcium from solution culture by zinc-tolerant and non-tolerant Silene maritima With. in relation to calcium supply. New Phytologist **81**, 321-330.

Baker, A. J. M. (1978c). Zinc-phosphorus interactions in a zinc-tolerant and a non-tolerant population of Silene maritima With. New Phytologist **81**, 331-339.

Baker, A. J. M. (1981). Accumulators and excluders - strategies in the response of plants to heavy metals. Journal of Plant Nutrition 3, 643-654.

Baker, A. J. M., Brooks, R. R., Pease, A. J. & Malaisse, F. (1983). Studies on copper and cobalt tolerance in three closely related taxa within the genus Silene L. (Caryophyllaceae) from Zaïre. Plant and Soil 73, 377-385.

Baker, A. J. M., Grant, C. J., Martin, M. H., Shaw, S. C. & Whitebrook, J. (1986). Induction and loss of cadmium tolerance in Holcus lanatus L. and other grasses. New Phytologist 102, 575-587.

Baker, H. G. (1953). Race-formation and reproductive method in flowering plants. Symp. Soc. exp. Biol. 2, 114-145.

Barry, S. A. S. & Clark, S. C. (1978). Problems of interpreting the relationship between the amounts of lead and zinc in plants and soil on metalliferous wastes. New Phytologist 81, 773-783.

Baumann, A. (1885). Das Verhalten von Zinksalzen gegen Pflanzen und im Boden. Landun. Verstnen.

Bradshaw, A. D. & McNeilly, T. S. (1981). Evolution and Pollution. Studies in Biology No. 130. Arnold, London. 76 pp.

Bradshaw, A. D., McNeilly, T. S. and Gregory, R. P. G. (1985). Industrialisation , evolution and the development of heavy metal tolerance in plants. In: Ecology and the Industrial Society (Ed. by G. T. Goodman, R. H. Edwards & J. H. Lambert), British Ecological Society Symposium No. 5, 327-343. Blackwell Scientific Publications, Oxford.

Briggs, D.. & Walters, S. M. (1984). Plant Variation and Evolution, 2nd. Edition. Cambridge University Press, Cambridge.

Brookes, A., Collins, J. C. & Thurman, D. A. (1981). The mechanism of zinc tolerance in grasses. Journal of Plant Nutrition 3, 695-705.

Brooks, R. R. (1983). Biological Methods of Prospecting for Minerals. Wiley, New York.

Brown, H. (1983). A study of cadmium tolerance in the grass Holcus Ianatus L. PhD Thesis, University of Bristol.

Carlson, R. W. & Bazzaz, F. A. (1977). Growth reductions in American sycamore (Platanus occidentalis L.) caused by Pb-Cd interaction. Environmental Pollution 12, 243-253.

Clapham, A. R., Tutin, T. G. & Warburg, E. F. (1962). Flora of the British Isles, 2nd edn. Cambridge, Cambridge University Press.

Clapham, A. R. (1964). Thlaspi L. In: Flora Europaea I. Lycopodiaceae to Platanaceae. (Ed. by Tutin, T. G., Heywood, V. H., Burgess, N. A., Valentine, D. H. Walters, S. M. & Webb, D. A.). pp. 328-322. Cambridge University Press, Cambridge.

Clapham, M. A., (1969). *Flora of Derbyshire*. Bemrose & Sons Ltd. Midland Place, Derby.

Clapham, A. R., Tutin, T. G. & Warburg, E. F. (1981). *Excursion Flora of the British Isles*, 3rd Edition. Cambridge University Press, Cambridge.

Clark, R. K. & Clarke, S. C. (1981) Floristic diversity in relation to soil characteristics in a lead mining complex in the Pennines, England. *New Phytologist* **87**, 799-815.

Cook, S. C. A., Lefebvre, C. & McNeilly, T. (1972). Competition between metal tolerant and normal plant populations on normal soil. *Evolution*, *Lancaster*, *Pa*. **26**, 366-372.

Coughtrey, P. J. & Martin, M. H. (1977). Cadmium tolerance of *Holcus lanatus* from a site contaminated by aerial fallout. *New Phytologist* **79**, 273-280.

Coughtrey, P. J. & Martin, M. H. (1978). Cadmium uptake and distribution in tolerant and non-tolerant populations of *Holcus lanatus* grown in solution culture. *Oikos* **30**, 555-560.

Coughtrey, P. J. & Martin, M. H. (1979). Cadmium, lead and zinc interactions and tolerance in two populations of *Holcus lanatus* L. grown in solution culture. *Environmental and Experimental Botany* **19**, 285-290.

Coughtrey, P. J. & Martin, M. H. (1978). Tolerance of *Holcus lanatus* to lead, zinc and cadmium in factorial combination. *New Phytologist* **81**, 147-154.

Cox, R. M. (1979). Multiple tolerance relations in native plants and their application to reclamation. *International Conference-Management and Control of Heavy Metals in the Environment*. pp. 202-205. CEP Consultants Ltd., Edinburgh.

Cox, R. M. & Hutchinson, T. C. (1979). Metal co-tolerance in the grass *Deschampsia cespitosa*. *Nature*, Lond. **179**, 231-233.

Cox, R. M. & Hutchinson, T. C. (1981). Multiple and co-tolerance to metals in the grass *Deschampsia cespitosa*: adaptation, preadaptation and 'cost'. *Journal of Plant Nutrition* **3**, 731-741.

Craig, G. C. (1977). A method of measuring heavy metal tolerance in grasses. *Transactions of the Rhodesian Scientific Association*, **58**, 9-16.

Crump, D. R. & Barlow, P. J. (1982). Factors controlling the lead content of a pasture grass. *Environmental Pollution (Series B)* **3**, 181-192.

Dale, A. (1974). *Biosystematics of selected species in the Teesdale flora*. PhD Thesis, University of Sheffield.

Dargie, T. C. D. (1984). On the integrated interpretation of indirect site ordinations: a case study using semi-arid vegetation in southeastern Spain. Vegetatio 55, 37-55.

Dargie, T. C. D. (1986). A seriation method for summarizing vegetation-environment relationships. Vegetatio 65, 91-93.

Davies, M. S. & Snaydon, R. W. (1973). Physiological differences among populations of Anthoxanthum odoratum L. collected from the Park Grass Experiment, Rothamsted. II. Response to aluminium. Journal of Applied Ecology 10, 47-55.

Denaeyer-de Smet, S. (1970). Considérations sur l'accumulation du zinc par les plantes poussant sur sols calaminaires. Bull. Inst. Roy. Sci. Nat. Belg. 46, 1-13.

Denaeyer-de Smet, S. and Duvigneaud, P. (1974). Accumulation de metaux lourds toxiques dans diverseecosystemes terrestres pollués par des rétombées d'origine industrielle. Bull. Soc. R. Bot. Belg. 107, 147-156.

DeWit, C. T. (1960). On competition. Versl. Landbouwkd. Onderz., 66, 1-82.

Dijkshoorn, W., J. Lampe, E. M. & Kowsoleea, A. R. (1975). Uptake of cadmium and zinc by ryegrass at high solution culture levels. Neth. J. Agric. Sci. 23, 285-290.

Doyle, G. J. (1982) Minuartio-Thlaspietum Alpestris (Violetea Calaminariae) in Ireland. In: Studies on Irish Vegetation. Ed. By J. White. Royal Dublin Society. pp. 143-146.

Dvorakova, M. (1968). Zur Nomenklatur einiger Taxa aux dem Formenkreis von Thlaspi alpestre (L.) L. Folia Geobot. Phytotax. (Praha), 3, 341-343.

Ernst, W.,(1964). Okologisch-soziologische Untersuchungen in den Schwermetallgesellschaften Europas unter Einschluß der Alpen. Dissertation, Munster.

Ernst, W. (1965). Uber den Einfluss des Zinks auf die Keimung von Schwermetallpflanzen und auf die Entwicklung der Schwermetallpflan zengesellschaft. Berichte der Deutschen Botanischen Gesellschaft. 78, 205-212.

Ernst, W. (1968a). Der Einfluss der Phosphatversorgung sowie die Wirkung von ionogenum und chelatisiertem Zink auf die Zink- und Phosphataufnahme einiger Schwermetallpflanzen. Physiologia Plantarum 21, 323-333.

Ernst, W. (1968b). Zur Kenntnis der Soziologie und Ökologie der Schwermetallvegetation Grossbritanniens. Berichte der Deutschen Botanischen Gesellschaft 81, 116-124.

Ernst, W. (1968c). Ökologische Untersuchungen an Pflanzengesellschaften unterschiedlich stark gestörter schmetallreicher Boden in Grossbritannien. Flora, Jena 158, 95-109.

Ernst, W. (1974). Schwermetallvegetation der Erde. Gustav Fischer--Verlag, Stuttgart.

Ernst, W. H. O. (1975). Physiology of heavy metal resistance in plants. Proc. Int. Conf. on Heavy Metals in the Environment, Vol. II, 121-136. Toronto, Canada.

Ernst, W. (1976). Physiological and biochemical aspects of metal tolerance. In: Effects of Air Polutants on Plants (Ed. by T. A. Mansfield), 115-133. S.E.B. Seminar Series Vol. 1, Cambridge University Press.

Farago, M. E. (1981). metal tolerant plants. Coordination Chemistry Reviews. **36**, 155-182.

Fiedler, P. L. (1985). Heavy metal accumulation and the nature of edaphic endemism in the genus Calochortus (Liliaceae). American Journal of Botany, **72**. 1712-1718.

Ford, T. D. & Rieuwerts, J. H. (Eds.) (1983). Lead Mining in the Peak District, 3rd Edition. Peak Park Joint Planning Board, Bakewell, Derbyshire.

Garcia-Gonzalez, A. & Clark, S. C. (1985). The soils of two metallophyte plants in the British Isles. In: Proceedings of the International Conference on Heavy Metals in the Environ ment, Athens, September 1985, Vol. 2, pp.334-336. CEP Consultants Ltd., Edinburgh.

Garland, C. J. & Wilkins, D. A. (1981). Effect of calcium on the uptake and toxicity of lead in Hordeum vulgare L. and Festuca ovina L. New Phytologist **87**, 581-593.

Gauch, H. G. Jr. (1982). Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge.

Gauch, H. G. Jr., Whittaker, R. H. & Wentworth, R. T. (1977). A comparative study of reciprocal averaging and other ordination techniques. Journal of Ecology **65**, 157-174.

Giordano, P. M. & Mortvedt, J. J. (1980). Zinc uptake and accumulation by agricultural crops. In: Zinc in the Environment (Ed. by J. O. Nriagu), pp. 401-414. John Wiley & Sons Inc., Chichester.

Godwin, H. (1975). History of the British Flora, 2nd edn., Cambridge University Press.

Grace, J. (1987). Climatic tolerance and the distribution of plants. In: Frontiers of Comparative Plant Ecology (Ed. by I. H. Rorison, J. P. Grime, G. A. F. Hendry & D. H. Lewis), New Phytologist, **106**, Special Supplement, (in press).

Gregory, R. P. G. (1965). Heavy metal tolerance in grasses. Ph.D. Thesis, University of Wales.

Gregory, R. P. G. & Bradshaw, A. D. (1965). Heavy metal tolerance in populations of Agrostis tenuis Sibth. and other grasses. New Phytologist **64**, 131-143.

Greig-Smith, P. (1964). Quantitative Plant Ecology, Second Edition, London, Butterworth's Scientific Publishers, 1964.

Grime, J. P. and Hodgson, J. G. (1969). An investigation of the ecological significance of lime-chlorosis by means of large-scale comparative experiments. In: I. H. Rorison (Ed. Ecological Aspects of the Mineral Nutrition of Plants, pp. 67-99. Oxford: Blackwell.

Grime, J. P. (1979). Competition and the struggle for existence. In: Population Dynamics (Ed. by R. M. Anderson, B. D. Turner & L. R. Taylor). Symposia of the British Ecological Society **20**, pp. 123-139.

Grime, J. P. (1986). The circumstances and characteristics of spoil colonization within a local flora. In: Proceedings of the Royal Society discussion meeting on quantitative aspects of the ecology of biological invasions, February, 1986.(Ed. by M. W. Holdgate). Phil. Trans. R. Soc. Lond. B, **314**, 637-654.

Grime, J. P. & Lloyd, P. S. (1973). An Ecological Atlas of Grassland Plants, Edward Arnold, London.

Grime, J. P. (1985). Towards a functional description of vegetation. In Population Structure of Vegetation, (Ed. by J. White & J. Beeftink). Junk, Nordrecht.

Grime, J. P. (1974). Vegetation classification by reference to strategies. Nature, Lond, **250**, 26-31.

Grime, J. P. (1977). Some evidence and theories relating to mechanisms of change in swards. British Grassland Society, Study Group III, March 1977. (cyclostyled MS).

Grime, J. P. (1981). The role of seed dormancy in vegetation dynamics. Annals of Applied Biology **98**, 555-558.

Grime, J. P., Crick, J. C. & Rincon J. E. (1986). The ecological significance of plasticity. In: Plasticity in Plants (Ed. by D. H. Jennings & A. J. Trewevas). Symposia of the Society for Experimental Biology, Number XXXX, pp. 5-29. Company of Biologists, Cambridge.

Grime, J. P., Hodgson, J. G. & Hunt, R. (1987). Comparative Plant Ecology: a Functional Approach to Common British Plants. George Allen & Unwin, London. pp. VIII + 876 (in press).

Grime, J. P., Mason, G., Curtis, A. V., Rodman, J., Band, S. R., Mowforth, M. A. G., Neal, A. M. & Shaw, S. C. (1981). A comparative study of germination characteristics in a local flora. Journal of Ecology **69**, 1017-1059.

Guha, M. M. & Mitchell, R. L. (1966). The trace and major element composition of the leaves of some deciduous trees. II. seasonal changes. Plant and Soil **24**, 90-112.

Halliday, G. (1958). Studies in the Minuartia verna complex, I. Feddes Repertorium **69**, 8-14.

Halliday, G. (1960). Taxonomic and ecological studies in the Arenaria ciliata and Minuartia verna complexes. PhD. Thesis, University of Cambridge.

Halliday, G. (1964). Minuartia L. In: Flora Europaea. Volume 1. Lyopodiaceae to Platanaceae. (Ed. by Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D. H. Walters, S. M. & Webb, D. A.), pp. 125-132. Cambridge University Press, Cambridge.

Harper, J. L. (1961). Approaches to the study of plant competition. Symp. Soc. exp. Biol., **15**, 1-39.

Harper, J. L. Lovell, P. H. & Moore, K. G. (1970). The shapes and sizes of seeds. Ann. Rev. Ecol. Systemat., **1**, 327-56.

Hassett, J. J., Miller, J. E. & Koeppe, D. E. (1976). Interaction of lead and cadmium on maize root growth and uptake of lead and cadmium by roots. Environmental Pollution **11**, 297-302.

Hegi, G. (1958). Illustrierte Flora Von Mittel-Europa: Dicotyledones, IV. Band, 1. Teil, pp. 370-373. Carl Hanser Verlag, Munchen, Germany.

Hegi, G. (1963). Illustrierte Flora Von Mittel-Europe: Dicotyledones, III. Band, (1. Teil), pp. 400-402. Carl Hanser Verlag, Munchen, Germany.

Hewitt, E. J. (1966). Sand and Water Culture Methods Used in the Study of Plant Nutrition. 2nd Edn. Technical Communication No. 22. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks.

Hickey, D. A. & McNeilly, T. (1975). Competition between metal tolerant and normal plant populations: a field experiment on normal soil. Evolution, Lancaster, Pa. **29**, 458-464.

Hill, M. O. (1973). Reciprocal averaging: an eigenvector method of ordination. Journal of Ecology **61**, 237-249.

Hill, M. O. (1979). Decorana- A Fortran program for detrended correspondence analysis and reciprocal averaging. Cornell University, Ithaca, N.Y.

Hill, M. O. & Gauch, H. G. (1980). Detrended correspondence analysis, an improved ordination technique. Vegetatio **42**, 47-58.

Hillier, S. H. (1984). A quantitative study of gap recolonization in two contrasted limestone grasslands. Ph.D. Thesis, University of Sheffield.

Hodgson, J. G. (1972). A comparative study of seedling root growth with respect to aluminium and iron supply. PhD. Thesis, University of Sheffield.

Hodgson, J. G. (1986). Commonness and rarity in plants with special reference to the Sheffield Flora. Part 1. The identity, distribution and habitat characteristics of the common and rare species. *Biological Conservation* 36, 199-252.

Hodgson, J. G. (1986). Commonness and rarity in plants with special reference to the Sheffield Flora. Part III. Taxonomic and evolutionary aspects. *Biological Conservation* 36, 275-296.

Hoiland, K. & Oftedal, P. (1980). Lead tolerance in *Deschampsia flexousa* from a naturally lead polluted area in S. Norway. *Oikos* **34**, 168-172.

Holmgren, P. K. (1971). A biosystmatic study of north American *Thlaspi montanum* and its allies. *Memoirs of the New York Botanical Garden* **21**, 1-106.

Howard-Williams, C. (1972). Factors influencing copper tolerance in *Becium homblei*. *Nature*, *Lond*. **237**, 271.

Ineson, P.R. (1981). Geological, geochemical, botanical and veterinary aspects of Pb, Zn, and Cd concentrations in part of the Pennines. In *Heavy Metals in Northern England: Environmental and Biological Aspects* (Ed. by P. J. Say & B. A. Whitton), pp. 165-180. University of Durham, Department of Botany.

Ingrouille, M. J. & Smirnoff, N. (1986). *Thlaspi caerulescens* J. & C. Presl. (*T. alpestre* L.) in Britain. *New Phytologist* **102**, 219-233.

Jalas, J. & Suominen, J. (1983). *Atlas Florae Europaeae*. Distribution of vascular plants in Europe. Caryophyllaceae and Paronychioideae). The Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo, Helsinki.

Jeffries, R. L. & Willis, A. J. (1964). Studies on the calcicole-calcifuge habit, **11**. The influence of calcium on the growth and establishment of four species in soil and sand cultures, *Journal of Ecology*, **52**, 691-707.

Johnston, W. R. & Proctor, J. (1977). A comparative study of metal levels in plants from two contrasting lead-mine sites. *Plant and Soil* **46**, 251-257.

Johnston, W. R. & Proctor, J. (1981). Growth of serpentine and non-serpentine races of *Festuca rubra* in solutions simulating the chemical condition in a toxic serpentine soil. *Journal of Ecology* **69**, 855-869.

Jones, D. A. & Wilkins, D. A. (1971). *Variation and Adaptation in Plant Species*. Heinmann Educational Books Ltd., London.

Jones, L. H. P. & Jarvis, S. C. (1981). The fate of heavy metals. In: *The Chemistry of Soil Processes* (Ed. by D. J. Greenland & M. H. B. Hayes), Ch. 11, pp. 536-620. John Wiley & Sons Ltd., Chichester.

Jones, L. H. P., Jarvis, S. C. & Cowling, D. W. (1973) Lead uptake from soils by perennial ryegrass and its relation to the supply of an essential element (sulphur). Plant and Soil **38**, 605-619.

Jordan, A. (1846). Observations sur plusiers plantes nouvelles, rare ou critique de la France. 11. Annales Societe, Linneenne de Lyon.

Jowett, D. (1958). Populations of Agrostis spp. tolerant to heavy metals. Nature, Lond, **182**, 816-817.

Jowett, D. (1959). Adaptation of a lead-tolerant population of Agrostis tenuis to low soil fertility. Nature **194**, 43.

Jowett, D. (1964). Population studies in lead tolerant Agrostis tenuis. Evolution, Lancaster, Pa. **18**, 70-80.

Karataglis, S. S. (1980). Behaviour of Agrostis tenuis populations against copper ions in combination with the absence of some macro-nutrient elements. Bericht der Deutschen botanischen Gesellschaft **93**, 417-424.

Kawano, S. (1981). Trade-off relationships between some reproductive characteristics in plants with special reference to life-history strategy. Bot. Mag. Tokyo, **94**, 285-94.

Kelepertsis, A. E. & Andrulakis, I. (1983). Geobotany-Biogeochemistry for mineral exploration of sulphide deposits in northern Greece - heavy metal accumulation by Rumex acetosella L. and Minuartia verna L. Hiern. Journal of Geochemical Exploration **18**, 267-274.

Kelepertsis, A. E., Andrulakis, I. & Reeves, R. D. (1985). Rumex acetosella L. and Minuartia verna (L.) Hiern as geobotanical and biogeochemical indicators for ore deposits in northern Greece. Journal of Geochemical Exploration **23**, 203-212.

Kendall, D. G. (1971). Seriation from abundance matrices. In: Mathematics in the Archeological and Historical Sciences (Ed. by F. R. Hodgson, D. G. Kendall, & P. Tautu), pp. 215-252.

Kendall, M. G. & Stuart, A. (1977). The Advanced Theory of Statistics. Vol. 1, pp. 231-233. Griffin, London.

Kershaw, K. A. & Looney, J. H. H. (1985). Quantitative and Dynamic Plant Ecology, Third Edition. Edward Arnold, London.

Kirkham, N. (1953). The tumultuous course of Dovegang. Bulletin of the Derbyshire Archaeological and Natural History Society. **73**, 1-35.

Lambinon, J. & Auquier, P. (1963). La flore et la vegetation des terrains calaminaires de la Wallonie septentrionale et de la Rhenanie Aixoise. Types chorologiques et groups ecologiques. Nature Mosana **16**, 113-132.

Law, R., Bradshaw, A. D. & Putwain, P. D. (1977). Life history variation in Poa annua. Evolution, **31**, 233-246.

Linstow, O. Von. (1924). Die naturliche Anreicherung der Metallsalzen und anderen anorganischen Verbindungen in den Pflanzen. Feddes Repertorium **31**, 1-151.

Linstow, O. Von. (1929). Bodenanzeigende Pflanzen (2nd ed.). Abh. preuss. geol. Landesanst., **114**.

Linton, W.R. (1903). Flora of Derbyshire. Bemrose & Son. London.

Macnair, M. R. (1981a). The uptake of copper by plants of Mimulus guttatus differing in genotype primarily at a single major copper tolerance locus. New Phytologist **88**, 723-730.

Macnair, M. R. (1981b). Tolerance of higher plants to toxic materials. In: Genetic Consequences of Made Change (Ed. by J. A. Bishop & L. M. Cooke), pp. 177-207. Academic Press, London & New York.

Martin, M. H. & Coughtrey, P. J. (1982). Biological Monitoring of Heavy Metal Pollution. Applied Science Publishers, Barking, Essex, London.

Maschmeyer, J. R. & Quinn, J. A. (1976). Copper tolerance in New Jersey populations of Agrostis stolonifera and Paronychia fastigiata Bull. Torrey Botanical Club **103**, 244-251.

Mathys, W. (1977), The role of malate, oxalate, and mustard oil glucosides in the evolution of zinc-resistance in herbage plants. Physiol. Plant 40 : 130-136. 1977.

Matthews, H. & Thornton, I. (1982). Seasonal and species variation in the content of cadmium and associated metals in pasture plants at Shipham. Plant and Soil **66**, 181-193.

Matthews, J. (1937). Geographical relationships of the British flora. Journal of Ecology **35**, 1-90.

McGrath, S. P. (1979). Growth and distribution of Holcus lanatus L. Populations with reference to nitrogen and aluminium. Ph.D. Thesis. University of Sheffield.

McGrath, S. P., Baker, A. J. M., Morgan, A. N., Salmon, W. J. & Williams, M. (1980). The effects of interactions between cadmium and aluminium on the growth of two metal-tolerant races of Holcus lanatus L. Environmental Pollution, (Series A) **23**, 267-277.

McNeilly, T. & Bradshaw, A. D. (1968). Evolutionary processes in populations of copper tolerant Agrostis tenuis Sibth. Evolution, Lancaster, Pa. **22**, 108-118.

Miller, J. E., Hassett, J. J. & Koeppe, D. E. (1977). Interactions of lead and cadmium on metal uptake and growth of corn plants. Journal of Environmental Quality **6**, 18-20.

Mitchell, R. L. & Reith, J. W. S. (1966). The lead content of pasture herbage. Journal of the Science of Food and Agriculture **17**, 437-440.

Nicholls, M. K. and McNeilly, T. (1979). Sensitivity of rooting and tolerance to copper in Agrostis tenuis Sibth. New Phytologist **83**, 653-664.

Noble, P. S. & Franco, A. C. (1986). Annual root growth and intraspecific competition for a desert bunchgrass. Journal of Ecology, **74**, 1119-1126.

Noy-Meir, I. 1974b, Multivariate analysis of the semiarid vegetation in south-eastern Australia, II. Vegetation cetenae and environmental gradients. Australian Journal of Botany **22**: 115-140.

Pennington, W. (1969). The History of British Vegetation. English University Press, London.

Perring, F. H. & Walters, S. M. (1976), Atlas of the British Flora. Second Edition, Botanical Society of the British Isles. EP Publishing Limited, Wakefield, England.

Peterson, P. J. (1971). Unusual accumulations of elements by plants and animals. Science Progress, Oxford. **59**, 505-526.

Peterson, P. J. (1975). Element accumulation by plants and their tolerance of toxic mineral soils. In: Proc. International Conference on Heavy Metals in the Environment, Ed. by T. C. Hutchinson, Vol. II, Part 1, pp.39-54. Toronto, Canada.

Pigott, C. D. & Walters, S. M. (1954). On the interpretation of the discontinuous distributions shown by certain British species of open habitats. Journal of Ecology **42**, 95-116.

Prat, S. (1934). Die Erblichklit der Resistenz gegen Kupfer. Berichte der Deutschen Botanischen Gesellschaft **52**, 65-67.

Rains, D. W. (1971). Lead accumulation by wild oats (Avena fatua) in a contaminated area. Nature, Lond. **233**, 210-211.

Rascio, N. (1977). Metal accumulation by some plants growing on zinc-mine deposits. Oikos **29**, 250-253.

Reeves, R. D. (1987), Nickel and zinc accumulation by species of Thlaspi L., Cochlearia L, and other genera of the Brassicaceae. Taxon (in press).

Reeves, R. D. & Baker, A. J. M. (1984). Studies on metal uptake by plants from serpentine and non-serpentine populations of Thlaspi goesingense Halacsy (Cruciferae). New Phytologist **98**, 191-204.

Reeves, R. D & Brooks, R. R. (1983). Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. Environmental Pollution (series A) **31**, 277-285.

Reeves, R. D. & Brooks, R. R. (1983). European species of Thlaspi L. (Cruciferae) as indicators of nickel and zinc. Journal of Geochemical Exploration **18**, 275-283.

Reilly, C. & Stone, J. (1971). Copper tolerance in Becium homblei. Nature, Lond. **230**, 403.

Riley, R. (1955). Genecological studies in T. alpestre L. Ph.D. Thesis. University of Sheffield.

Riley, R. (1956). The influence of the breeding system on the genecology of T. alpestre L. New Phytologist **55**, 319-330.

Rochow, T. F. (1970). Ecological investigations of Thlaspi alpestre L. along an elevational gradient in the central rocky mountains. Ecology 51, 649-656.

Rorison, I. H. (1964). A double shell plant growth cabinet. New Phytologist **63**, 358-362.

Rorison, I. H. (1969). Ecological inferences from laboratory experiments. In: Ecological Aspects of the Mineral Nutrition of Plants (Ed. by I. H. Rorison), BES Symp. No.99, pp.155-175 Blackwell Scientific Publications, Oxford.

Rorison, I. H. (1973). The effect of extreme soil acidity on the nutrient uptake and physiology of plants. Acid sulphate soil (Ed. by H. Dost), Proceedings International Symposium on acid sulphate soils, Wageningen 1972, pp. 223-253. International Institute of Land Reclamation and Improvement, Wageningen.

Rorison, I. H., Sutton, F. & Hunt, R. (1986). Local climate, topography and plant growth in Lathkill Dale NNR. I. A twelve-year summary of solar radiation and temperature. Plant, Cell and Environment **9**, 49-56.

Rother, J. A., Millbank, J. W. & Thonton, I. (1982). Seasonal fluctuations in nitrogen fixation (acetylene reduction) by free-living bacteria in soils contaminated with cadmium, lead and zinc. Journal of Soil Science **33**, 101-113.

Salisbury, E. J. (1942). Reproductive capacity of plants. G. Bell & Sons, Ltd., London.

Schenkeveld, A. J. & Verkaar, H. J. (1984). The ecology of short-lived forbs in chalk grasslands: distribution of germinative seeds and its significance for seedling emergence. Journal of Biogeography **11**, 251-260.

Shaw, S. C. (1984). Ecophysiological studies on heavy metal tolerance in plants colonizing Tideslow Rake, Derbyshire, Ph.D. Thesis, University of Sheffield.

Shaw, S. C., Rorison, I. H. & Baker, A. J. M. (1984). Solubility of heavy metals in lead mine spoil extracts. Environmental Pollution (Series B) **8**, 23-33.

Shimwell, D. W. (1968). Notes on the distribution of T. alpestre L. in Derbyshire. Proceedings of the Botanical Society of the British Isles 7, 373-376.

Shimwell, D. W. & Laurie, A. E. (1972). Lead and zinc contamination of vegetation in the southern Pennines. Environmental Pollution 3, 291-301.

Simon, E. & Lefebvre, C. (1977). Aspects de la tolerance aux metaux lourds chez Agrostis tenuis Sibth., Festuca ovina L. et Armeria maritima (Mill.) Willd. Oecologia. Plantarum, **12**, 95.

Smith, R. F. (1979). The occurrence and need for conservation of metallophytes on mine wastes in Europe. Minerals and the Environment **1**, 131-147.

Stebbins, G. L. (1971), Chromosome Evolution in Higher Plants, Edward Arnold, London.

Stebbins, G. L. (1980). Polyploidy in plants: unsolved problems and prospects. In: Polyploidy-Biological Relevance, (Ed. by W. H. Lewis), pp. 495-520. Plenum Press, New York and London.

Steele, B. (1955). Soil pH and base status as factors in the distribution of calcicoles. Journal of Ecology **43**, 120-132.

Symeonidis, L., McNeilly, T. & Bradshaw, A. D. (1985). Differential tolerance of three cultivars of Agrostis capillaris L. to cadmium, copper, lead, nickel and zinc. New Phytologist, **101**, 309-315.

Thompson, J. & Proctor, J. (1983). Vegetation and soil factors on a heavy metal mine spoil heap. New Phytologist **94**, 297-308.

Thompson, K. (1986). Small-scale heterogeneity in the seed bank of an acidic grassland. Journal of Ecology **74**, 733-738.

Thompson, K. & Grime, J. P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology **67**, 893-921.

Thurman, D. A. (1981), Mechanism of metal tolerance in higher plants. In: Effect of Heavy Metal Pollution on Plants (Ed. by N. W. Lepp), Vol. 2., pp. 239-249. Applied Science Publishers, Barking.

Timperley, M. H. Brooks, R. R. & Peterson, P. J. (1970). Prospecting for copper and nickel in New Zealand by statistical analysis of biogeochemical data. Economic Geology **65**, 505-510.

Tinker, P. B. (1981). Levels, distribution and chemical forms of trace elements in food plants. Philosophical Transactions of the Royal Society of London, **B 294**, 41-55.

Turner, R. G. & Marshall, C. (1972). The accumulation of zinc by subcellular fractions of roots of Agrostis tenuis Sibth. in relation to zinc tolerance. New Phytologist **71**, 671-676.

Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., & Webb, D. A. (eds) (1964-80). Flora Europaea, Cambridge University Press, Cambridge.

Wainwright, S. J. & Woolhouse, H. W. (1975), Pysiological mechanisms of heavy plant metal tolerance in plants. In: The Ecology of Resource Degradation and Renewal, British Ecological Society Symposium No. 15. (Ed. by M. J. Chadwick & G. T. Goodman), pp. 231-257. Blackwell Scientific Publications, Oxford.

Walley, K. A., Khan, M. S. I. & Bradshaw, A. D. (1974). The potential for evolution of heavy metal tolerance in plants. I. Heredity 32, 309-319.

Watt, A. S. (1962). The effect of excluding rabbits from grassland A (Xerbrometum) in Breckland 1936-1960. Journal of Ecology 50, 181-198.

Wilkins, D. A. (1957). A technique for measurement of lead tolerance in plants. Nature, Lond. 180, 37-38.

Wilkins, D. A. (1978). The measurement of tolerance to edaphic factors by means of root growth. New Phytologist 80, 623-633.

Windsor, J. (1865). On Thlaspi alpestre, The Naturalist 2, 108-110.

Wong, M. H. & Bradshaw, A. D. (1982). A comparison of the toxicity of heavy metals using root elongation of ryegrass, Lolium perenne. New Phytologist 91, 255-261.

Woolhouse, H. W. (1983). Toxicity and tolerance in the reponses of plants to metals. In: Encyclopedia of Plant Physiology, New Series, Vol. 12C. (Ed. by O. L. Lange, P. S. Nobel, C. B. Osmond & H. Ziegler), pp. 245-300.

Wu, L. & Antonovics, J. (1975). Zinc and copper uptake by Agostis stolonifera tolerant to both zinc and copper. New Phytologist 75, 231-237.

Wu, L. & Antonovics, J. (1976). Experimental ecological genetics in Plantago, II. Lead tolerance in Plantago lanceolata and Cynodon dactylon from a roadside. Ecology, 57, 205-208.

Wu, L., Huff, D. R. & Johnson, J. M. (1981). Metal tolerance of Bermudagrass cultivars. In: Proceedings of the 4th International Turfgrass Conference, pp.

Wu, L., Thurman, D. A., & Bradshaw, A. D. (1975). The uptake of copper and its effect upon respiratory processes of roots of copper-tolerant and non-tolerant clones of Agostis stolonifera. New Phytologist 75, 225-229.

APPENDIX 1

ANOVA TABLES,

CHAPTER 3, FIGURE 3.2

PLANT HEIGHT

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	406.3	101.6	121.1	***	0.78
WITHIN POPULATIONS	145	121.6	0.8367			
TOTAL	149	527.9				

PLANT BREADTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	1590	397.4	623.5	***	0.68
WITHIN POPULATIONS	145	92.43	0.6374			
TOTAL	149	1682				

LEAF LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	19.62	4.906	9.475	***	0.61
WITHIN POPULATIONS	145	75.07	0.5178			
TOTAL	149	94.7				

* - P < 0.001 = ***
P < 0.01 = **
P < 0.05 = *

FLOWER BREADTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	198	49.51	109.8	***	0.57
WITHIN POPULATIONS	145	65.4	0.451			
TOTAL	149	263.4				

PETAL LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	22.98	5.746	114.8	***	0.19
WITHIN POPULATIONS	145	7.255	5.003E-2			
TOTAL	149	30.24				

SEPAL LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	19.46	4.865	140.1	***	0.16
WITHIN POPULATIONS	145	5.034	3.472E-2			
TOTAL	149	24.49				

CAPSULE LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	38.2	9.549	163.2	***	0.21
WITHIN POPULATIONS	245	8.485	5.852E-2			
TOTAL	149	46.68				

SEED WEIGHT

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	6.256E-2	1.564E-2	234.1	***	0.12
WITHIN POPULATIONS	25	1.67E-3	6.679E-5			
TOTAL	29	6.423E-2				

APPENDIX 1 (CONTINUED)

ANOVA TABLES

CHAPTER 3, FIGURE 3.3

PLANT HEIGHT

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	2.251E5	5.628E4	58.63	***	15.7
WITHIN POPULATIONS	145	1.392E5	959.9			
TOTAL	149	3.643E5				

RACEME LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	6.504E4	1.626E4	36.96	***	17.82
WITHIN POPULATIONS	145	6.379E4	439.9			
TOTAL	149	1.288E5				

STEM LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	8.329E4	2.082E4	47.79	***	17.74
WITHIN POPULATIONS	145	6.318E4	435.7			
TOTAL	149	1.465E5				

ROSETTE BREADTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	5154	1289	4.959	***	13.70
WITHIN POPULATION	145	3.768E4	259.8			
TOTAL	149	4.283E4				

LEAF LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	1365	341.2	4.887	***	7.10
WITHIN POPULATIONS	145	1.012E4	69.81			
TOTAL	149	1.149E4				

LEAF BREADTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	97.33	24.33	8.817	***	1.41
WITHIN POPULATIONS	145	400.2	2.76			
TOTAL	149	497.5				

APPENDIX 1 (CONTINUED)

ANOVA TABLES

CHAPTER 3, FIGURE 3.4

CAPSULE LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	9.274	2.318	7.226	***	0.48
WITHIN POPULATIONS	145	46.52	0.3208			
TOTAL	149	55.79				

CAPSULE BREADTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	8.688	2.172	10.69	***	0.38
WITHIN POPULATIONS	145	29.47	0.2032			
TOTAL	149	38.16				

BREADTH INDEX

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	5.579	1.395	7.468	***	0.64
WITHIN POPULATIONS	145	27.08	0.1868			
TOTAL	149	32.66				

NOTCH BREADTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	1.115	0.2787	9.507	***	0.15
WITHIN POPULATIONS	145	4.251	2.932E-2			
TOTAL	149	5.366				

NOTCH DEPTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	1.011	0.2528	35.98	***	0.05
WITHIN POPULATIONS	145	1.019	7.027E-3			
TOTAL	149	2.03				

STYLE LENGTH

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	0.2676	6.691E-2	3.775	***	0.08
WITHIN POPULATION	145	2.57	1.772E-2			
TOTAL	149	2.837	0.01772			

FRUIT NUMBER

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	5923	1481	23.65	***	4.8
WITHIN POPULATIONS	145	9079	62.61			
TOTAL	149	1.5E4				

NUMBER OF SEEDS IN EACH CAPSULE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	138.4	34.61	25.14	***	1.02
WITHIN POPULATIONS	145	199.6	1.377			
TOTAL	149	338.1				

SEED WEIGHT

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	6.008E-3	1.502E-3	74.31	***	0.0067
WITHIN POPULATIONS	25	5.053E-4	2.021E-5			
TOTAL	29	6.513E-3				

APPENDIX 1 (CONTINUED)

ANOVA TABLES

CHAPTER 3, FIGURE 3.5

RACEME LENGTH: STEM LENGTH RATIOS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	17.77	4.442	22.03	***	0.38
WITHIN POPULATIONS	145	29.23	0.2016			
TOTAL	149	47				

NUMBER OF CAPSULES: RACEME LENGTH RATIOS (RACEME DENSITY)

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	2.525	0.6311	18.84	***	0.16
WITHIN POPULATIONS	148	4.958	3.35E-2			
TOTAL	152	7.483				

CAP L: CAP Br RATIOS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	0.3115	7.788E-2	2.951	*	0.44
WITHIN POPULATIONS	145	3.827	2.639E-2			
TOTAL	149	4.138				

Bd.I./CAP. L. RATIOS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	3.278E-2	8.194E-3	1.636	NS	0.07
WITHIN POPULATIONS	145	0.7261	5.008E-3			
TOTAL	149	0.7589				

BD.I. CAP. Br. RATIOS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	4.712E-2	1.178E-2	1.769	NS	0.069
WITHIN POPULATION	145	0.9656	6.659E-3			
TOTAL	149	1.013				

CAP. L.: NOT.D. RATIOS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	6724	1681	57.43	***	4.60
WITHIN POPULATIONS	145	4244	29.27			
TOTAL	149	1.097E4				

NOT.D/NOT Br. RATIOS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	0.691	0.1727	34.64	***	0.06
WITHIN POPULATIONS	145	0.7231	4.987E-3			
TOTAL	149	1.414				

ST.L.: NOT.D. RATIOS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	144.5	36.13	42.8	***	0.78
WITHIN POPULATIONS	145	122.4	0.844			
TOTAL	149	266.9				

CAP. Br: NOT. Br. RATIOS

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F. RATIO	PROBABILITY	LSD
BETWEEN POPULATIONS	4	20.16	5.04	14.41	***	0.50
WITHIN POPULATIONS	145	50.71	0.3497			
TOTAL	149	70.87				

Appendix 2

Percentage occurrence of M. verna, T. alpestre and associate species, recorded from five different sites in South Pennines

	S	I	T	E	S
Species	1	2	3	4	5*
Agrostis tenuis	25				
Arabis hirsuta					5
Campanula rotundifolia	5		5	35	5
Centaurea nigra			5		
Cerastium fontanum	5	5	5	15	5
Euphrasia officinalis	5	15	45	75	5
Festuca ovina	35		5		45
Festuca rubra	75	100	75	100	
Galium sterneri				5	
Hieracium pilosella			5		
Koeleria cristata	15				5
Leontondon autumnalis			5		
Linum catharticum				5	
Lotus corniculatus			5		
Minuartia verna	35	100	45	100	35
Plantago lanceolata			5		5
Ranunculus acris				5	
Rumex acetosa	35	75	45	100	5
Seneccio jacobaea			5		
Thlaspi alpestre	15	15	15		
Thymus drucei		5			15

*1 - Bradford Dale
2 - Black Rocks
3 - Clough Wood
4 - Grattondale
5 - Tideslow rake

APPENDIX 3

COMPOSITION OF NUTRIENT SOLUTIONS

The basal culture solution used was Rorison nutrient solution (Hewitt 1966) made up at one-tenth strength as follows using 'Analar' grade reagents and deionised water:

Element	Concentration mg dm^{-3}	Source	Concentration mg dm^{-3}
Ca	8.0	$Ca(NO_3)_2.4H_2O$	47.24
N	5.6		
Mg	2.4	$MgSO_4.7H_2O$	24.65
S	3.2		
K	7.8	K_2HPO_4	17.40
P	3.1		
Fe	0.3	Fe-citrate.$5H_2O$	1.80
Mn	0.05	$MnSO_4.4H_2O$	0.203
B	0.05	H_3BO_3	0.286
Mo	0.01	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.018
Zn	0.01	$ZnSO_4.7H_2O$	0.044
Cu	0.01	$CuSO_4.5H_2O$	0.039

pH was adjusted to 6.5 using H_2SO_4 or NaOH.

APPENDIX 4

MEANS AND STANDARD ERRORS OF ROOT ELONGATION (MM) OF FIVE T. ALPESTRE POPULATIONS, GROWN IN SOLUTION CULTURE

(EXPT. VII, (CHAPTER 5)

Populations	control	Pb	Zn	Cd	Al	Ag	Co	Cr	Cu	Fe	Mu	Mo	Ni
Black Rocks	85 ± 5	84 ± 7	57 ± 3	43 ± 5	44 ± 2	42 ± 3	59 ± 2	49 ± 1	46 ± 2	40 ± 3	84 ± 8	41 ± 1	77 ± 3
Bradford Dale	126 ± 6	93 ± 2	59 ± 2	58 ± 3	60 ± 2	63 ± 2	69 ± 2	51 ± 4	55 ± 2	59 ± 3	95 ± 3	58 ± 2	96 ± 2
Clough Wood	116 ± 4	90 ± 5	52 ± 8	40 ± 1	51 ± 3	47 ± 2	57 ± 2	49 ± 2	48 ± 2	44 ± 2	97 ± 4	48 ± 3	80 ± 3
Whitesike	104 ± 5	98 ± 2	66 ± 2	53 ± 3	70 ± 4	44 ± 6	62 ± 2	66 ± 6	41 ± 4	55 ± 3	98 ± 3	58 ± 5	83 ± 7
Eller Beck	99 ± 2	79 ± 3	47 ± 1	43 ± 3	40 ± 2	40 ± 2	51 ± 4	95 ± 3	45 ± 2	39 ± 1	87 ± 3	51 ± 3	72 ± 3

APPENDIX 4.1 ANOVA TABLE FOR THE GERMINATION RESPONSE (EXPT. I, CHAPTER 5)

Source of Variance	DF	Sum of Squares	Mean Square	Variance Ratio	P	Species
Zn conc.	4	0.969	0.242	20.2	***	M. verna from Bradford Dale
Scoring dates	7	0.178	0.255	212.4	***	
Interaction	28	0.247	0.881	0.7	NS	
Residual	160	0.192	0.120			
Total	199	0.210				
Zn conc.	4	0.250	0.626	6.3	***	M. verna from Tideslow Rake
Scoring dates	7	0.143	0.205	206.4	***	
Interaction	28	0.427	0.153	1.5	NS	
Residual	160	0.159	0.991			
Total	199	0.166				
Zn conc.	4	0.420	0.105	13.2	***	T.alpestre from Bradford Dale
Scoring dates	7	0.963	0.138	173.1	***	
Interaction	28	0.737	0.263	3.3	***	
Residual	160	0.127	0.795			
Total	199	0.121				

ANOVA TABLE FOR THE GERMINATION RESPONSE (EXPT. II, CHAPTER 5)

Source of Variance	DF	Sum of Squares	Mean Square	Variance Ratio	P*	Species
Soils	2	0.512	0.256	67.3	***	M. verna from Bradford Dale
Scoring dates	7	0.374	0.534	14.1	***	
Interaction	14	0.124	0.882	2.3	**	
Residual	96	0.365	0.380			
Total	119	0.137				
Soils	2	0.449	0.224	40.6	***	M. verna from
Scoring dates	7	0.425	0.608	11.0	***	
Interaction	14	0.152	0.109	1.97	*	
Residual	96	0.530	0.553			
Total	119	0.156				
Soils	2	0.379	0.189	27.1	***	T. alpestre from Bradford Dale
Scoring dates	7	0.214	0.306	4.4	***	
Interaction	14	0.243	0.174	0.3	NS	
Residual	96	0.670	0.698			
Total	119	0.129				

* – P < 0.001 = ***
P < 0.01 = **
P < 0.05 = *

APPENDIX 4.1 (CONTINUED)

ANOVA TABLE FOR ROOT LENGTH
EXPT.III, CHAPTER 5)

Source of Variance	DF	SS	SS%	MS	VR	p
POPULATION	4	32209.43	4.91	8052.36	112.311	***
SPECIES	1	136385.13	20.79	136385.13	1902.247	***
Pb	4	146922.81	22.40	36730.70	512.306	***
POPUL.SPECIES	4	19632.53	2.99	4908.13	68.457	***
POPUL. Pb	16	27191.14	4.15	1699.45	23.703	***
SPECIES Pb	4	9374.91	1.43	2343.73	32.689	***
POPUL. SPEC.Pb	16	8201.86	1.25	512.62	7.150	***
Residual	3850	276032.94	42.08	71.70		
Total	3899	655950.75	100.00	168.24		
Grand Total	3899	655950.75	100.00			
Population	4	32209.43	4.91	8052.36	91.532	***
Species	1	136385.13	20.79	136385.13	1550.301	***
Zn	4	101258.05	15.44	25314.51	187.752	***
POPUL.SPECIES	4	19632.53	2.99	4908.13	55.791	***
POPUL. Zn	16	16073.64	2.45	1004.60	11.419	***
Species Zn	4	5086.92	0.78	1271.73	14.456	***
POPUL.SPEC.Zn	16	6607.71	1.01	412.98	4.694	***
Residual	3850	338697.31	51.63	87.97		
Population	4	32209.43	4.91	8052.36	80.774	***
Species	1	136385.13	20.79	136385.13	1368.090	***
Cd	4	52297.43	7.97	13074.36	131.150	***
POPUL.Species	4	19632.53	2.99	4908.13	49.234	***
POPUL. Cd	16	13025.38	1.99	814.09	8.166	***
Species Cd	4	4246.42	0.65	1061.61	10.649	***
POPUL. SPEC.Cd	16	14347.13	2.19	896.70	8.995	***
Residual	3850	383807.25	58.51	99.69		

ANOVA TABLE FOR ROOT LENGTH
(EXPT. IV, CHAPTER 5)

Source of Variation	DF	SS	SS%	MS	VR	p
SPECIES	1	527506.00	81.14	527506.00	5604.668	***
Pb	1	16.26	0.00	16.26	0.173	NS
Zn	1	19140.63	2.94	19140.63	19140.63	***
Cd	1	13894.26	2.14	13894.26	147.624	***
Species Pb	1	136.90	0.02	136.90	1.455	NS
Species Zn	1	2830.81	0.44	2830.81	30.077	***
Species Cd	1	3071.26	0.47	3071.26	32.632	***
Pb. Zn	1	319.23	0.05	319.23	3.392	NS
Pb. Cd	1	360.00	0.06	360.00	3.825	NS
Zn. Cd	1	154.06	0.02	154.06	1.637	NS
Species Pb.Zn	1	4080.40	0.63	4080.40	43.354	***
Species Pb.Cd	1	7195.81	1.11	7195.81	76.454	***

APPENDIX 4.1 (CONTINUED)

Species Zn.Cd	1	4040.10	0.62	4040.10	42.925	***
Pb.Zn.Cd	1	235.23	0.04	235.23	2.499	NS
Species Pb.Zn.Cd.	1	8395.51	1.29	8395.51	89.201	***
Residual	624	58730.29	9.03	94.12		
Total	639	650106.50	100.00	1017.38		
Grand Total	639		100.00			

ANOVA TABLE FOR ROOT LENGTH
(EXPT. V, CHAPTER 5)

SOURCES OF VARIATION	DF	SS	SS%	MS	VR	p
Species	1	24042.60	31.88	24042.60	421.083	***
Species Pb	6	9583.95	12.71	1597.32	27.976	***
Residual	732	41795.05	55.42	57.10		
Total	739	75421.59	100.00	102.06		
Species					350.611	***
Species Zn		1183.22	1.57	197.20	2.876	*
Residual		50195.78	66.55	68.57		
Species					351.219	***
Species Cd		1270.17	1.68	211.69	3.092	**
Residual		50108.83	66.44	68.45		
Species					350.214	***
Species Al		1126.28	1.49	187.71	2.734	*
Residual		50252.72	66.63	68.65		
Species					369.936	***
Species Ag		3805.40	5.05	634.23	9.759	***
Residual		47573.59	63.08	64.99		
Species					351.665	***
Species Co		1333.65	1.77	222.28	3.251	**
Residual		50045.35	66.35	68.37		
Species					349.899	***
Species Cr		1081.12	1.43	180.19	2.622	*
Residual		50297.88	66.69	68.71		
Species					388.903	***
Species Cu		6125.61	8.12	1020.93	16.514	***
Residual		45253.39	60.00	61.82		
Species					349.820	***
Species Fe		1069.77	1.42	178.29	2.594	*
Residual		50309.23	66.70	68.73		
Species					473.051	***
Species Mn		14175.40	18.79	2362.57	46.485	***
Residual		37203.60	49.33	50.82		
Species					354.893	**
Species Mo		1788.82	2.37	298.14	4.401	***
Residual		49590.19	65.75	67.75		
Species					351.207	***
Species Ni		1268.36	1.68	211.39	3.088	**
Residual		50110.64	66.44	68.46		

APPENDIX 4.1 (CONTINUED)

ANOVA TABLE FOR ROOT LENGTH
(EXPT. VI, CHAPTER 5)

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	p
Species	1	2134.1539	1.869	2134.1539	75.6295	***
Chme	12	93636.7503	82.004	7803.0625	276.526	***
Interaction	12	10344.4295	9.059	862.0358	30.5486	***
Residual	298	18414.9293	16.127	61.7951		
Total	311	114185.833	100.00			

ANALYSIS OF VARIANCE
(EXPT. VII, CHAPTER 5)

VARIATE: ROOTL

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
POPUL	4	28461.7	7.16	7115.4	16.676***
POPUL. Pb	5	40771.3	10.25	8154.3	19.111***
RESIDUAL	770	328549.3	82.60	426.7	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.059***
POPUL.Zn	5	5502.7	1.38	1100.5	2.329*
RESIDUAL	770	363817.9	91.46	472.5	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.607***
POPUL. Cd	5	18275.4	4.59	3655.1	8.017***
RESIDUAL	770	351045.2	88.25	455.9	
TOTAL	779	397782.3	100.00	510.6	
GRANT TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.180***
POPUL. Al	5	8393.3	2.11	1678.7	3.581**
RESIDUAL	770	360927.3	90.73	468.7	
TOTAL	779	397782.3	100.00	510.6	
GRANT TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.684***
POPUL Ag	5	19991.5	5.03	3998.3	8.813***
RESIDUAL	770	349329.1	87.82	453.7	

APPENDIX 4.1 (CONTINUED)

TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	14.911***
POPUL. Co	5	1875.3	0.47	375.1	0.786*NS
RESIDUAL	770	367445.3	92.37	477.2	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.260***
POPUL. Cr	5	10277.3	2.58	2055.5	4.408**
RESIDUAL	770	359043.3	90.26	466.3	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.717***
POPUL. Cu	5	20732.0	5.21	4146.4	9.159***
RESIDUAL	770	348588.6	87.63	452.7	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.598***
POPUL. Fe	5	18061.9	4.54	3612.4	7.919***
RESIDUAL	770	351258.8	88.30	456.2	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	17.285***
POPUL. Mn	5	52351.9	13.16	10470.4	25.435***
residual	770	316968.7	79.68	411.6	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.317***
POPUL. Mo	5	11631.9	2.92	2326.4	5.008**
RESIDUAL	770	357688.7	89.92	464.5	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		
GRAND MEAN	63.93				
TOTAL NUMBER OF OBSERVATIONS	780				
POPUL	4	28461.7	7.16	7115.4	15.721***
POPUL. Ni	5	20811.6	5.23	4162.3	9.196***
RESIDUAL	770	348509.1	87.61	452.6	
TOTAL	779	397782.3	100.00	510.6	
GRAND TOTAL	779	397782.3	100.00		

APPENDIX 4.1 (CONTINUED)

GRAND MEAN	63.93
TOTAL NUMBER OF OBSERVATIONS	780

ANOVA TABLES FOR PLANT MATERIAL DRY WEIGHT
(EXPT.VIII, CHAPTER 5)

SOURCE OF VARIATION	DF	SS	MS	F RATIO	%	VARIATE	METAL	PLANT
AMONG GROUPS	5	2.723	0.5447	1.8	NS	ROOT W	Pb	M. Verna
RESIDUAL	10	3.027	0.3027					
TOTAL	15	5.75						
AMONG GROUPS	5	88.73	17.75	2.517	*	SHOOT W		
RESIDUAL	10	70.051	7.051					
TOTAL	15	159.2						
AMONG GROUPS	5	108.8	21.76	2.259	*	TOTAL w		
residual	10	96.35	9.635					
TOTAL	15	205.2						
AMONG GROUPS	5	277.6	55.52	1.633	NS	ROOT W	Pb	T.alpestre
RESIDUAL	12	408.0	34					
TOTAL	17	685.6						
AMONG GROUPS	5	3276.0	655.2	2905	2	SHOOT W		
RESIDUAL	12	2707.0	225.6					
TOTAL	17	5982.0						
AMONG GROUPS	5	5365.0	1073	2.534	*	TOTAL W		
RESIDUAL	12	5081.0	423.4					
TOTAL	17	1.045E4						
AMONG GROUPS	5	13.57	2.713	2.254	*	ROOT W	Zn	M. verna
RESIDUAL	9	10.83	1.204					
TOTAL	14	24.4						
AMONG GROUPS	5	232.2	46.43	0.9722	NS	SHOOT W		
RESIDUAL	9	429.8	47.76					
TOTAL	14	662.0						
AMONG GROUPS	5	334.6	66.91	1.054	NS	TOTAL W		
RESIDUAL	9	571.2	63.46					
TOTAL	14	905.7						
AMONG GROUPS	5	929.7	185.9	0.7168	NS	ROOT W	Zn	T.alpestre
RESIDUAL	10	2594.0	259.4					
TOTAL	15	3524.0						
AMONG GROUPS	5	2568.0	513.6	1.436	NS	SHOOT W		
RESIDUAL	10	3576.0	357.6					
TOTAL	15	6144.0						
AMONG GROUPS	5	5422.0	1084.0	0.9452	NS	TOTAL W		
RESIDUAL	10	1.147E4	1147.0					
TOTAL	15	1.689E4						
AMONG GROUPS	5	5.767	1.153	2.708	*	ROOT W	Cd	M. verna
RESIDUAL	9	3.833	0.4259					
TOTAL	14	9.60						
AMONG GROUPS	5	205.90	41.19	2.592	*	SHOOT W		
RESIDUAL	9	143.0	15.89					
TOTAL	14	348.9						

APPENDIX 4.1 (CONTINUED)

AMONG GROUPS	5	278.9	55.78	2.716	*	TOTAL W		
RESIDUAL	9	184.8	20.54					
TOTAL	14	463.7						
AMONG GROUPS	5	418.4	83.69	6.46	**	ROOT W	Cd	T.alpestre
RESIDUAL	11	142.5	12.95					
TOTAL	16	560.9						
AMONG GROUPS	5	942.9	188.6	0.8875	NS	SHOOT W		
RESIDUAL	11	2337.0	212.5					
TOTAL	16	3280.0						
AMONG GROUPS	5	2259.0	451.8	1.281	NS	TOTAL W		
RESIDUAL	11	3880.0	352.7					
TOTAL	16	6139.0						

ANOVA FOR METAL CONCENTRATIONS IN PLANT TISSUE GROWN AT DIFFERENT METAL CONCENTRATIONS IN SOLUTION CULTURE

(EXPT. VIII, CHAPTER 5)

SOURCE OF VARIATION	DF	SS	MS	F. RATIO	p	VARIAT	METAL	PLANT
AMONG GROUPS	5	1.368E5	2.736E4	22.36	***	ROOT	Pb	M.verna
RESIDUAL	10	1.223E4	1223					
TOTAL	15	1.49E5						
AMONG GROUPS	5	1.576E4	3153	47.02	***	SHOOT		
RESIDUAL	10	670.5	67.05					
TOTAL	15	1.643E4						
AMONG GROUPS	5	1.953E7	3.906E6	14.1	***	ROOT	Pb	T.alpestre
RESIDUAL	12	3.324E6	2.77E5					
TOTAL	17	2.285E7						
AMONG GROUPS	5	3.554E5	7.108E4	56.91	***	SHOOT		
RESIDUAL	10	1.499E4	1249					
TOTAL	15	3.704E5						
AMONG GROUPS	5	2.494E4	4987	2.948	*	ROOT	Zn	M. verna
RESIDUAL	9	1.523E4	1692					
TOTAL	14	4.016E4						
AMONG GROUPS	5	9.306E6	1.861E6	65.67	***	SHOOT		
RESIDUAL	9	2.551E5	2.834E4					
TOTAL	14	9.561E6						
AMONG GROUPS	5	5.519E7	1.104E7	25.01	***	ROOT	Zn	T.alpestre
RESIDUAL	10	4.413E6	4.413E5					
TOTAL	15	5.961E7						
AMONG GROUPS	5	1.708E8	3.416E7	8.239	***	SHOOT		
RESIDUAL	10	4.146E7	4.146E6					
TOTAL	15	2.122E8						
AMONG GROUPS	5	1.994E5	3.987E4	2.817	*	ROOT	Cd	M.verna
RESIDUAL	9	1.274E5	1.416E4					
TOTAL	14	3.268E5						
AMONG GROUPS	5	5.238E5	1.048E5	106.9	***	SHOOT		
RESIDUAL	9	8821	980.1					
TOTAL	14	5.326E5						

APPENDIX 4.1 (CONTINUED)

AMONG GROUPS	5	1.16E7	2.32E6	14.97	**	ROOT	Cd T.alpestre
RESIDUAL	11	1.705E6	1.55E5				
TOTAL	16	1.331E7					
AMONG GROUPS	5	1.539E8	3.079E7	9.802	***	SHOOT	
RESIDUAL	11	3.455E7	3.141E6				
TOTAL	16	1.88E8					

TWO-WAY ANOVA TABLES FOR METAL CONCENTRATIONS
(EXPT. VIII, CHAPTER 5)

SOURCE OF VARIATION	DF	SS	MS	F RATIO	p	VARIAT
SPECIES	1	1.166E7	1.166E7	11.63	***	ROOT UPTAKE
METAL	2	1.641E6	8.204E5	0.8183	NS	
INTERACTION	2	1.3.008E7	6.087E5	0.6072	NS	
RESIDUAL	30	3.008E7	1.003E6			
TOTAL	35	4.459E7				

SPECIES	1	1.044E8	1.044E8	25.13	***	SHOOT UPTAKE
METAL	2	6.961E7	3.481E7	8.379	***	
INTERACTION	2	5.219E7	2.61E7	6.283	**	
RESIDUAL	30	1.246E8	4.154E6			
TOTAL	35	3.508E8				