Heavy Metal Tolerance in Plants

J. ANTONOVICS

Department of Biology, University of Stirling, Stirling, Scotland, U.K.[1]

A. D. BRADSHAW

Department of Botany, University of Liverpool, Liverpool, England, U.K.

AND

R. G. TURNER

School of Plant Biology, University College of North Wales, Bangor,[2] *Caerns., U.K.*

---

[1] Present address: Department of Botany, Duke University, North Carolina, U.S.A.

[2] Present address: Shell Research Ltd., Woodstock Laboratories, Sittingbourne, Kent, U.K.

I. INTRODUCTION

The heavy metals, defined by Passow *et al.* (1961) as those metals having a density greater than five, include about thirty-eight elements. Their common feature in relation to biological life is that in excessive quantities they are poisonous and can cause death of most living organisms. However, certain organisms are remarkable in that they possess an ability to survive under conditions of metal contamination which would prove toxic to other living things. It is the purpose of this review to bring together the literature on the plants and micro-organisms which can combat excessive quantities of heavy metal ions.

Much of the literature on the subject is scattered, but in recent years a co-ordinated picture has started to emerge. The picture is by no means complete, and this review is as much an attempt to point to directions of investigation in the future as an attempt to collate the past work on this subject.

Toxic levels of heavy metals can occur under several circumstances. Firstly, soil may itself contain large quantities of metal and it is with this type of contamination that the review will be mainly concerned.

The contamination results either from the presence of undisturbed metal ore near the soil surface causing "anomalies" as they are termed by the geochemist (e.g. Warren and Delavault, 1948; Duvigneaud and Denaeyer-de Smet, 1963; Tooms and Jay, 1964) or from the actual mining of ore bodies. Many waste products from mining activities are contaminated with metal at toxic levels and can produce large-scale pollution. Unproductive ore, tailings (see Bateman and Wells, 1917, and Griffith, 1919, for vivid descriptions) and seepage (e.g. Lackey, 1938) regularly contaminate mining areas. Smelting of ore produces contaminated slag and smoke fumes which may carry the contamination large distances (e.g. Ramaut, 1964; Hilton, 1967; Kerin, 1968). Other sources of soil contamination are less obvious and not so severe because they occur on a much smaller scale. For example, soil along main arterial roads frequently contains a high level of lead since this is an important component of "anti-knock" additive for petrol (e.g. Cannon, 1960a; Warren and Delavault, 1960, 1962; Suchodoller, 1967; Cholak *et al.*, 1968). The area below galvanized (zinc coated) fences and pylons may even have toxic zinc concentrations (Harris, 1946; Snaydon, quoted in Bradshaw *et al.*, 1965) and there may be accumulation of toxic amounts of lead on rifle ranges (McAllister, 1965). There is also evidence of increasing general environmental pollution resulting from widespread use of compounds of lead (Ruhling and Tyler, 1968, 1969) and mercury (Swedish Royal Commission, 1967).

Secondly, heavy metals are frequently used because of their toxicity as components of fungicides, pesticides or disinfectants. This source of contamination is important not only in that it may produce toxic levels of metals in the environment (Drouineau and Mazoyer, 1956; Gibson, 1958; Taschenberg *et al.*, 1961; Delas, 1963; Aomine *et al.*, 1967) and so affect the background vegetation and wild-life over which the spray is applied, but also in that the pathogen or pest over which control is being exercised may itself become resistant.

Thirdly, tolerant races can be produced in organisms growing in laboratory culture on artificial media containing heavy metals.

It is impossible to define precisely what is implied by the description "metal tolerant" because the phrase is generally used in two main senses. In one sense it refers generally to any species found occurring in an area of toxicity from which other species appear to be excluded. But it may be used more precisely to refer to specific individuals of a species which are able to withstand greater amounts of toxicity than their immediate relatives on normal soil. In the second case we are dealing with a precisely determined situation, a species normally non-tolerant but with an ability to evolve tolerant races. In the first case it will not be clear what is happening unless further investigations are made: either the species is already tolerant throughout its range (including uncontaminated sites) or it is an example of the second case and has evolved tolerant races. In those cases which have been examined it is always the latter that is found.

The theoretical problems raised by metal tolerant organisms fall into three main categories, which are separable only for the basis of argument.

1. What is the effect of heavy metal contamination on the flora (and fauna) in a given area? What organisms survive and what factors determine their distribution?
2. How has metal tolerance evolved? Is it an inherent property of some species, can it arise rapidly, and what processes are involved?
3. What is the mechanism of tolerance? How is it determined physiologically and biochemically?

The practical problems and implications of metal tolerance are several.

1. Prospecting for metal ore deposits can benefit from vegetation studies. Individual species or vegetation types may act as indicators of ore deposits. Some plants accumulate metals and deep rooted ones may bring up into their aerial parts metals which are not evident on the surface.
2. Micro-organisms play some part in the commercial extraction of metals from low-grade ores. Their role in contaminated substrates is therefore relevant.
3. Mining and smelting activities have created large areas of unsightly, and sometimes dangerous dereliction. The recolonization of these areas with vegetation is an important problem.
4. As a result of the wide use of heavy metals in sprays and other toxicants, the organisms that are being controlled may develop resistance which completely destroys the efficiency of the sprays. A knowledge of metal tolerance is necessary to minimize the chances of this happening.

The problem of heavy metal tolerance may appear to be a highly specialized topic, but it is hoped to show in this review that its study is not only of great interest and practical consequence, but also that it is a paradigm for many biological problems.

II. Ecology of Metal Tolerance

This section deals almost entirely with the plants found growing in the toxic soils that result from mining activity and natural "anomalies". The problem of races resistant to toxicants and laboratory strains will be dealt with in Section V (A2 and A3).

A. SPECIES PRESENT ON CONTAMINATED SOILS

No attempt will be made to produce comprehensive lists of species present on contaminated soils. The number of species mentioned in relation to such soils is immense and frequently there is little indication of whether they are actually growing on areas that are effectively toxic (see Section VIIA). A comprehensive list would therefore be of dubious value. This section summarizes the systems of classifying such plants, the main references to the various groups, and the most obvious features and species to emerge from the literature.

1. Vascular plants

Ferns are rarely mentioned in the literature relating to toxic areas but Vogt (1942b) mentions *Asplenium adulterium* as an indicator of nickel. Ferns are also known on serpentine soils which may be high in nickel and chromium (e.g. Kruckeberg, 1964). Wild (1968) records the ferns *Pellaea calomelanos* and *Chelianthes hirta* on copper and occasionally nickel soils in Rhodesia.

Similarly, there is no known record of Gymnosperms as consistent members of communities on contaminated soils, although they are used in biogeochemical prospecting; their leaves and twigs show enhanced metal content when growing over soil containing increased, but not toxic, levels of metal (e.g. Warren and Delavault, 1948).

Higher plants characteristic of metal-contaminated soils have been recognized for several centuries. Thalius, in 1588, noted *Minuartia verna* as a metal indicator (quoted by Ernst, 1965a) and even before 1900 there was considerable interest in these plants: for example, Williams (1830), Henwood (1857), Baumann (1885) and Jensch (1894) all quote species found consistently on metal-contaminated soils.

Plants that are largely restricted to or particularly abundant on metal-contaminated soils have been used as indicators of heavy metal ores and have therefore attracted special interest: their role in prospecting is more fully reviewed in Section VIIIA. Frequently the definition of a plant as an "indicator" is extremely vague, and often subjective. It is, generally speaking, a plant that in a given area or geographic region has been recognized as associated with a particular metal. Such plants are listed in Tables Ia and Ib.

Recently it has been realized by both the ecologists and biogeochemical prospectors that it is necessary to quantify the relative abundance of different species in relation to metal content (and other ecological factors) of the environment. The concept of indicator species is perhaps an ecologically unrealistic, although at times useful, qualitative assessment.

TABLE Ia

Plants that have been used as indicators in prospecting for heavy metals

Universal (U) or Local (L) Indicator	Species	Family	Metal	Locality	Reference
U	*Gypsophila patrini*	Caryophyllaceae	Cu	U.S.S.R.	Nestvetaylova, 1955
L	*Polycarpaea spirostylis*	Caryophyllaceae	Cu	Australia	Bailey, 1889
U	*Acrocephalus robertii*	Labiatae	Cu	Katanga	Duvigneaud, 1958
L	*Elshotzia haichowensis*	Labiatae	Cu	China	Tsung-Shan, 1957
U	*Becium* (= *Ocimum*) *homblei*	Labiatae	Cu	Rhodesia	Anon., 1959
U	*Merceya latifolia*	Moss	Cu	Sweden and Montana	Persson, 1948
L	*Eschscholtzia mexicana*	Papaveraceae	Cu	Arizona	Lovering *et al.*, 1950
L	*Armeria maritima*	Plumbaginaceae	Cu	Scotland	Henwood, 1857
U	*Tephrosia* sp. nov.	Leguminosae	Cu	Australia	Cole, 1965
L	*Polycarpaea glabra*	Caryophyllaceae	Cu	Australia	Cole, 1965
U	*Bulbostylis barbata*	Cyperaceae	Cu	Australia	Cole, 1965
U	*Fimbristylis* sp. nov.	Cyperaceae	Cu	Australia	Cole, 1965
L	*Loudetia simplex*	Gramineae	Cu	Rhodesia	Jacobsen, 1968
L	*Olax obtusifolia*	Olacaceae	Cu	Rhodesia	Jacobsen, 1968
L	*Erianthus giganteus*	Gramineae	Pb	Tennessee	Cannon, unpubl.
U	*Tephrosia* sp. nov.	Leguminosae	Pn, Zn	Australia	Cole, 1965
U	*Polycarpaea synandra* var. *gracilis*	Caryophyllaceae	Pb, Zn	Australia	Cole, 1965
L	*Tephrosia* affin. *polyzyga*	Leguminosae	Pb, Zn	Australia	Cole, 1965
L	*Gomphrena canescens*	Amaranthaceae	Pb, Zn	Australia	Cole, 1965
L	*Eriogonum ovalifolium*	Polygonaceae	Ag	Montana	Henwood, 1857
U	*Viola calaminaria*	Violaceae	Zn	Belgium and Germany	Vinogradov, 1955
L	*Philadelphus* sp.	Philadelphaceae	Zn	Washington	Yates, in Cannon, 1960a

An attempt to go beyond the simple indicator concept has been made directly in Belgium by Lambinon and Auquier (1964) who produced a semi-quantitative classification which was a considerable improvement on the crude concept of indicators. Their classification is summarized in Table II with some examples.

TABLE Ib

Plants that have been cited as indicators of metal containing soils but for which there is no clear cut evidence that they have been used for prospecting

Species	Family	Metal	Locality	Reference
Crotalaria cobalticola	Leguminosae	Co	Katanga	Duvigneaud, 1959
Silene cobalticola	Caryophyllaceae	Co	Katanga	Duvigneaud, 1959
Silene otites	Caryophyllaceae	Cu	Germany	Linstow, 1929
Viscaria alpina	Caryophyllaceae	Cu	Norway	Vogt, 1942a
Euphorbia matabelensis	Euphorbiaceae	Cu	Rhodesia	Jacobsen, 1967
Tapiphyllum velutinum	Rubiaceae	Cu	Rhodesia	Jacobsen, 1967
Combretum zeyheri	Combretaceae	Cu	Rhodesia	Jacobsen, 1967
Kyllinga alba	Cyperaceae	Cu	Rhodesia	Wild, 1968
Fimbristylis exilis	Cyperaceae	Cu	Rhodesia	Wild, 1968
Bulbostylis spp.	Cyperaceae	Cu	Rhodesia	Wild, 1968
Tephrosia longipes	Leguminosae	Cu	Rhodesia	Wild, 1968
Celosia trigyna	Amaranthaceae	Cu	Rhodesia	Wild, 1968
Vellozia equisetoides	Velloziaceae	Cu	Rhodesia	Wild, 1968
Hemizygia petrensis	Labiatae	Cu	Rhodesia	Wild, 1968
Andropogon amplectens	Gramineae	Cu	Rhodesia	Wild, 1968
Andropogon gayanus	Gramineae	Cu	Rhodesia	Wild, 1968
Astragalus declinatus	Leguminosae	Cu, Mo	Armenia, U.S.S.R.	Malyuga *et al.*, 1959
Alsine (= *Minuartia*) *verna*	Caryophyllaceae	Cu, Pb Ag, Zn	Europe	Linstow, 1929
Armeria vulgaris (cf. *halleri*, *elongata*, *maritima*, etc.).	Plumbaginaceae	Cu, Zn	Europe	Linstow, 1929
Amorpha canescens	Papilionaceae	Pb	Michigan, Wisconsin	Linstow, 1929
Rhus spp.	Anacardiaceae	Pb	Missouri	Linstow, 1929
Sassafras spp.	Lauraceae	Pb	Missouri	Linstow, 1929
Alsine setaceae	Caryophyllaceae	Hg	Spain	Linstow, 1929
Alyssum bertolonii	Cruciferae	Ni	Italy	Minguzzi *et al.*, 1948
Alyssum murale	Cruciferae	Ni	Georgia, U.S.S.R.	Doksopulo, 1961
Albizia amara	Leguminosae	Ni	Rhodesia	Wild, 1970
Dicoma macrocephala	Compositae	Ni	Rhodesia	Wild, 1970
Barleria aromatica	Acanthaceae	Ni	Rhodesia	Wild, 1970
Combretum molle	Combretaceae	Ni	Rhodesia	Wild, 1970
Dalbergia melanoxylon	Leguminosae	Ni	Rhodesia	Wild, 1970
Eminia antennulifera	Leguminosae	Ni	Rhodesia	Wild, 1970
Turraea nilotica	Meliaceae	Ni	Rhodesia	Wild, 1970
Pterocarpus rotundifolius	Leguminosae	Ni	Rhodesia	Wild, 1970
Lonicera confusa	Caprifoliaceae	Ag	Australia	Bailey, 1898
Trientalis europeae	Primulaceae	Sn	Bohemia	Linstow, 1929

TABLE Ib. contd.

Species	Family	Metal	Locality	Reference
Sempervivum soboliferum	Crassulaceae	Sn	Saxony	Linstow, 1929
Gnaphalium suaveoleus	Compositae	Sn	Brazil	Dorn, 1937
Thlaspi calaminare cf. (*alpestre*)	Cruciferae	Zn	Europe	Linstow, 1929
Silene vulgaris (= *inflata* = *cucubalus*)	Caryophyllaceae	Zn	Europe	Linstow, 1929
Arabis halleri	Cruciferae	Zn	Germany	Linstow, 1929
Thlaspi cepeaefolium	Cruciferae	Zn	Austria, Italy	Linstow, 1929
Anagallis collina	Primulaceae	Zn	Italy	Linstow, 1929
Cistus monspeliensis	Leguminosae	Zn	Sardinia	Linstow, 1929
Ruta graveoleus	Rutaceae	Zn	Brazil, U.S.A.	Dorn, 1937
Ruta latifolia	Rutaceae	Zn	Brazil	Dorn, 1937
Senecio brasiliensis	Compositae	Zn	Brazil	Dorn, 1937
Matricaria americana	Compositae	Zn	Brazil	Dorn, 1937
Populus deltoides	Salicaceae	Zn	U.S.A.	Buck, 1949
Ambrosia spp.	Compositae	Zn	U.S.A.	Buck, 1949

Plants regarded as indicators would either be metallophytes or extreme examples of elective pseudometallophytes. Malyuga (1964) has distinguished two types of indicator, namely "universal" which corresponds to the category absolute metallophyte, and "local" which corresponds to the category local metallophyte.

Recently Jacobsen (1968) from experience with a wide range of species in Rhodesia, as a result of a need to follow the extent of a copper-bearing reef, has attempted to define indicator species more precisely. He has determined the highest and lowest levels of soil copper at which individual species are found and from this calculated the specific indicator value for each species from

$$\frac{\text{highest Cu level} - \text{lowest Cu level}}{\text{average Cu level}}.$$

From this he considers good indicators to be species with an indicator value of 4 or less arising either from occurrence over a small range of copper levels or from occurrence at high average copper levels. Typical species include *Bulbostylis contexta* and *Eragrostis racemosa.* Although somewhat imprecise as used at present it is a technique that indicates very clearly how different species may be distributed in relation to different levels of metal in the soil.

TABLE II

Classification of plants on metal contaminated soils (*after Lambinon and Auquier,* 1964) *with examples from metal contaminated soils of Europe*

1. *Metallophytes*—taxa found only on metal contaminated soils.	
(a) *Absolute metallophytes*— found only on metal contaminated soil over all their distribution.	e.g. *Viola calaminaria*, *Thlaspi alpestre* ssp. *calaminare*, *Minuartia verna* ssp. *hercynica.*
(b) *Local metallophytes*— found only on metal contaminated soil within a given region but occurring also in a phyto-geographically distinct non-contaminated area.	e.g. *Armeria maritima.*
2. *Pseudometallophytes*—taxa occurring both on contaminated soils and on normal soils in the same region.	
(a) *Elective pseudometallophytes*— abundant and often more vigorous on contaminated soil.	e.g. *Agrostis tenuis*, *Campanula rotundifolia*, *Polygala vulgaris*, *Thymus pulegioides*, *Rumex acetosa.*
(b) *Indifferent pseudometallophytes*— live on contaminated soil regularly but show neither abundance nor particular vitality.	e.g. *Plantago lanceolata*, *Avena pubescens*, *Genista tinctoria*, *Linum catharticum.*
(c) *Accidental metallophytes*— usually weeds and ruderals appearing sporadically and showing reduced vigour on metal contaminated soils	

In the classification of Lambinon and Auquier (1964) the category in which any particular species is placed depends on the locality or group of localities being investigated, since the relative abundance of species on and off contaminated soils depends on other factors in addition to metal concentration (see Section IIc). This is elegantly illustrated by recent work on *Becium homblei* (Howard-Williams, 1970). This is an excellent indicator of copper in Southern Rhodesia, and has been considered an absolute metallophyte. But Howard-Williams (1970) shows it to be a local metallophyte, widely distributed on non-copper soils in Northern Rhodesia and not always restricted to copper soils in Southern Rhodesia.

The classification described by Lambinon and Auquier (1964) has been used by Auquier (1964) and by Demoulin *et al.* (1967). A related classification is used by Duvigneaud and Denaeyer-de Smet (1963), who describe in detail the vegetation on naturally occurring copper outcrops in Katanga.

Phytosociological studies in Germany (Braun-Blanquet, 1951; see also Poore, 1955a, b, c, 1956) have provided what is probably the best semi-quantitative system of classifying plant communities, and the techniques have been applied to communities on contaminated soils (see Section IIB). The method of classification is based on vegetation composition of the communities and hence provides an indirect means of classifying species characteristic of metal-contaminated soils.

It is appropriate here to give a brief outline of the phytosociological technique of classifying communities, since this has been an important technique both in providing lists of species characteristic of metal-contaminated soils and in classifying communities carried on such soils (Section IIB).

In these techniques the basic unit of classification is the "association". The associations can either be further subdivided (into e.g. sub-associations and varieties) or they can be grouped into families, orders, and classes according to their affinities. The process whereby associations are recognized initially involves describing by means of "releves" (species lists and notes on environmental factors) a series of "stands" (areas of vegetation to be classified). These "releves" are then sorted into groupings by means of the presence of sets of species which one group has in common ("differential" species or "Trennart"). If a group of releves is considered sufficiently distinct from other associations or other groups of releves, then the particular group is given association rank.

The differential species may be found in other unrelated associations, or they may be entirely restricted to that association. The latter group of species are known as "characteristic" species or "Kennart" and are used to recognize the communities: they are the indicators of particular associations.

Confounded with the concepts of differential and characteristic species are the concepts of "constancy" and "fidelity". A species has a high constancy if it occurs in most of the releves of an association (e.g. constancy is V if it occurs in 81–100% of the releves). "Constancy" therefore refers to the frequency of occurrence of species within an association. A differential species is one with a high constancy in a particular association, but low constancy in others. "Fidelity" refers on the other hand to the distribution of species among associations. There are five classes of fidelity: (V) exclusive species confined almost completely to one community; (IV) selective species found most frequently in certain communities but also rarely in others; (III) preferential species present in several communities more or less abundantly but predominantly in one and there with greater vigour; (II) indifferent species without any particular affinity for any particular community; (I) accidental species which are rare intruders or relics from another community. This latter classification is clearly related to that of Lambinon and Auquier (1964).

The Braun-Blanquet method therefore provides us with a means of indicating the relative abundance of different species in communities on metal contaminated soil (constancy and differential species) and their relative restriction or otherwise to such soils (fidelity, characteristic species and differential species).

These techniques have been used by several workers in Germany to classify the communities on contaminated soils (see Section IIB for full references). In so doing these workers have provided extensive species lists of plants on contaminated soils. It is not possible here to list all the species given by these workers, since they are too numerous and often it is not clear to what extent the soils on which some species are growing are actually toxic. However, Ernst (1965a, 1968a) has summarized the species on these soils in Europe which are characteristic of or differential to the communities (see Table III). Certain species are clearly unique components of most metal-contaminated soils, others are restricted to particular metal-contaminated communities, whereas a third group are consistently present in some communities but by no means confined to metal-contaminated soils.

All the methods of classifying species occurring on metal-contaminated soils are compared in Table IV.

2. *Mosses and Liverworts*

Several species of mosses (often belonging to the genera *Merceya* and *Mieliochhoferia*) have been given the name of "copper mosses" (Persson, 1948, 1956; Noguchi, 1956; Noguchi and Furuta, 1956): they have been used in prospecting (Persson, 1948) and it has been postulated, though with no evidence, that they are restricted to copper soils by virtue of their being able to use hydrogen from hydrogen sulphide into photosynthesis (Schatz, 1955).

Warncke (1968) observed that *Marchantia alpestris* was generally restricted to mineral-, especially copper-rich areas in Scandinavia: in Denmark it was confined to an area contaminated with copper and zinc from effluent of a sulphuric acid plant. He suggested that it too should be included under the category of "copper mosses". Further studies of mosses on mineral-rich soils have been made by Schacklette (1965), who showed that they may sometimes accumulate heavy metal ions.

Numerous species of mosses are recorded as members of communities on metal-contaminated soils (Schwickerath, 1931; Schubert, 1954a, b;

TABLE III

Differential and characteristic species of West and Central European plant communities of heavy metal soils, showing their constancy in various associations (after Ernst, 1966)

Family:	Armerion		Thlaspeion		Galio-Minuartion	
Association:	*Armerietum bottendorfensis* (Schubert, 1952)	*Armerietum halleri* (Libbert, 1930) in Ernst, 1966	*Violetum calaminariae* (Schwickerath, 1931)	*Armerietum muelleri* ass. nov.	*Violetum dubyanae* (Ernst, 1965a)	*Thlaspeetum cepeaefolii* (Ernst, 1965a)
Number of stands:	29	78	41	14	15	10
Armeria maritima ssp. *bottendorfensis*	V	...	...	...	...	...
Thymus chamaedrys	V	...	...	...	...	...
Poa badensis	IV	...	...	...	...	...
Festuca glauca	IV	...	...	...	...	...
Festuca sulcata	IV	...	...	...	...	...
Armeria maritima ssp. *halleri*	...	V	...	...	...	...
Cladonia alcicornis	V	III	...	...	...	...
Asperula cynanchica	V	III	...	...	...	...
Silene otites	IV	II	...	...	...	...
Scabiosa canescens	III	III	...	...	...	...
Potentilla heptaphylla	III	II	...	...	...	...
Viola calaminaria	...	...	V	...	...	...
Armeria maritima ssp. *calaminaria*	...	...	III	...	...	...
Armeria maritima ssp. *muelleri*	...	...	...	V	...	...
Thlaspi alpestre ssp. *calaminare*	...	...	IV	V	...	...
Festuca ovina ssp. *ovina*	I	IV	IV	V	...	...
Agrostis tenuis	III	V	III	V	...	...
Viola dubyana	...	...	...	...	V	...
Euphrasia salisburgensis	...	...	...	...	V	...
Thymus alpigenus	...	...	...	...	V	...
Thlaspi cepeaefolium	...	...	...	...	...	V
Galium anisophyllum	...	...	...	...	IV	IV
Poa alpina	...	...	...	...	IV	V
Dianthus silvester	...	...	...	...	I	III
Silene cucubalus var. *humilis*	I	V	IV	V	IV	V
Minuartia verna ssp. *hercynica*	V	IV	III	V	V	IV

Constancy classes 0–20% = I, 21–40% = II, etc., refer to number of stands showing that species in the association.

Characteristic species (underlined) = species restricted to that association.

Differential species (not underlined) = species generally common to the stands in an association.

TABLE IV

Comparison of various classifications of plants on metal-contaminated soils

Malyuga (1964)	General ecological terms	Lambinon and Auquier (1964)	Duvigneaud and Denaeyer-de Smet (1963) and Wild (1968)	Braun-Blanquet		
				Fidelity	"Type" species	Constancy
Indicator	Endemic or Bodenstet	Metallophyte	Metallophyte	Exclusive	Characteristic	Refers to the consistency with which any of the previous categories occur in a community. Characteristic and differential species have generally a high constancy.
Universal		Absolute	Eumetallophyte	Selective	Differential	
Local		Local	Local metallophyte			
	Bodenwaag	Pseudometallophyte				
		Elective	Metallophile	Preferential		
		Indifferent		Indifferent		
		Accidental		Accidental		
			Metalloresistant*			

* A separate category of ubiquitous species defined by the fact that they do not take up metal.

Lambinon and Auquier, 1964; Ernst, 1965a, 1968b), and Url (1956) studied the copper resistance of several species from a copper-contaminated region in Austria. Among the more commonly mentioned types are *Bryum caespiticum*, *Pohilia nutans* and spp., and *Weisia* spp.

Liverworts are far rarer on contaminated soils. *Cephaloziella* spp. are recorded by Koch (1932), Lambinon and Auquier (1964) and Ernst (1965a). The only other records are sporadic: there is mention of *Plectocoela crenulata* (McAllister, 1965) and *Riccia bischoffii* (Schubert, 1954b).

3. *Lichens*

Lichens are found both as colonizers of bare rock and as general components of the established vegetation on contaminated soils. Poelt (1955), Lampe and Klement (1958) and Lange and Ziegler (1963) consider the various encrusting lichens found as initial colonizers on bare rock associated with mining debris. The genera *Acarospora* and *Lecanora* are frequently mentioned; Lange and Ziegler (1963) show these and other species to contain large quantities of iron and copper mainly in the cell walls. Wild (1968) notes that a species of *Lecanora* is found on natural rocky outcrops in Rhodesia with its thallus actually growing over exposed malachite without any apparent harmful effects. This is remarkable if we consider that the algal component at least might be expected to be particularly sensitive to copper toxicity.

Other lichens are listed in work on community studies on contaminated soils. Ernst (1965a) quotes three *Cladonia* species and *Cornicularia aculeata* as characteristic species of sub-associations. *Cladonia* spp. and *Stereocaulon* spp. (Schwickerath, 1931; Lambinon, 1964; Lambinon and Auquier, 1964; McAllister, 1965; Ernst, 1965a, 1968a) appear as consistent components of mine communities. Maquinay *et al.* (1961) show that *Stereocaulon nanodes* Tück f. *tyroliense* (Nyl) M. Lamb may accumulate high levels of zinc (3300 ppm dry weight on soils with only 700 ppm).

4. *Micro-organisms, algae and fungi*

The general occurrence of micro-organisms in metal-contaminated soils is poorly documented although some of the best examples of bacteria which resist extreme conditions (for review see Brock, 1969) are those found in acid mine drainage. The most common species which are able to resist extremes of pH and copper and iron concentration are *Thiobacillus ferro-oxidans*, *Th. thio-oxidans* and *Ferrobacillus ferro-oxidans* (Bryner *et al.*, 1954). These bacteria, especially *Th. ferro-oxidans*, have been subsequently shown to be commercially important in leaching copper from low-grade sulphide ores (see Section VIIIB). The ability of these bacteria to resist extreme conditions is illustrated by the work of Booth and Mercer (1963) who showed that *Thiobacillus thio-oxidans* and *Ferrobacillus ferro-oxidans* could withstand 20 000 and 10 000 ppm of copper ions respectively. Ehrlich (1963b) reported the presence of not only bacteria of the *Thiobacillus–Ferrobacillus* group in mine water (pH 2·5, copper concentration 0·8 g/litre and iron concentration 1·06 g/litre), but also other organisms including yeasts (resembling *Rhodotorula* and *Trichosporon*) and Protozoa (an amoeba species and a flagellate resembling *Eutrepia*). It is clear that a wide variety of organisms inhabit mine water, and that a dominant role is played by chemotrophic bacteria in providing an initial carbohydrate source. As Ehrlich (1963b) states, "The microbial population in the mine water sample suggests the existence of a balanced ecological system in which carbon fixation is dependent on chemosynthetic and not photosynthetic autotrophy".

Subsequent work has shown fungi to be consistent if not abundant components of mine soils. The ability of fungi to withstand extreme conditions was illustrated by Starkey and Waksman (1943), who showed that *Acontium velatum* and another unidentified species could grow in pH 0 and 4% $CuSO_4$ solution. Several workers have isolated micro-organisms tolerant to high metal levels from contaminated soils. Seal (1970) showed that fungi (*Aspergillus* spp.) from copper-mine soil have a greater copper tolerance than related fungi from normal soil. The work reported in Hilton (1967, p. 92) demonstrated that the microbial population of contaminated slag tips was very sparse, but increased greatly with the addition of organic matter. Tonomura, Nakagami, Futai and Maeda (1968) isolated mercury-resistant bacteria from soils contaminated with mercury-containing fungicides. Williams (1970) has isolated at least six different Actinomycetes from very acid copper-mine waste, and noted the occurrence of fungi and bacteria. And Demoulin *et al.* (1967) record the occurrence of three Gasteromycetes *Scleroderma bovista* Fr., *Lycoperdon spadiceum* Pers. and *Bovista plumbea* Pers. on calamine soils often containing well over 5000 ppm zinc.

Records of algae on heavy metals are rare but Hassall (1962) notes that *Chlorella vulgaris*, a green alga, is tolerant to barium, manganese, lead and copper. Duvigneaud (1958) records an algal substratum of blue-green algae dominated by *Cirosiphon geniculatus* in cupriferous soils in Katanga (Congo). Such colonies have also been noted by Wild (1968) in Rhodesia where some acute and experienced prospectors have actually used them as copper indicators since they form characteristic black crusts at the soil surface. In this case, species of blue-greens belonging to the genera *Scytonema* and *Microcoleus* seem to be dominant but other blue-greens such as *Nostoc*, *Phormidium*, *Anabaena* and *Oscillatoria* also occur, as well as a diatom (*Hanzscha* sp.), and a green alga belonging to the genus *Bracteacoccus*. In Britain similar algal crusts are readily observable on copper and zinc mine soils, and pools heavily contaminated with metals can have vigorous growth of filamentous green algae. In marine algae *Ectocarpus* (Russell and Morris, 1970) and *Enteromorpha* (Hodgson, 1969) can be found growing on ships treated with antifouling paints (see Section VA2).

High concentrations of heavy metals do not, therefore, prevent the growth of micro-organisms in contaminated soils: indeed some micro-organisms appear to be able to withstand metal concentrations which are toxic to even the most tolerant higher plants. However, the general nature of the microbial communities in metal-contaminated soils is poorly understood. Such communities are of interest because they appear often to rely on chemotrophic bacteria for their energy source and because their presence appears to promote release of metal ions. This is clearly important to the ecology of organisms growing on such soils.

5. *Conclusion*

The species found on toxic soils are very varied and differ according to the local ecological conditions and geographical area. No clear-cut taxonomic pattern emerges (see Section VB).

There appears to be a close affinity between the flora of toxic soils and serpentine soils (Rune, 1953; Spence, 1970). Serpentine soils are often high in nickel and chromium, and these metals appear in part at least to determine the serpentine flora (Birrell and Wright, 1945).

However, Walker (1954) working on serpentine soils in California considered that there was insufficient evidence that nickel or chromium toxicity was of importance as compared with high magnesium/calcium levels. Soane and Saunder (1959) on the contrary produce evidence that the Great Dyke serpentine soils of Rhodesia induce intense symptoms of nickel toxicity in oats. Furthermore, the importance of the effect of nickel, in some serpentine soils, on natural vegetation, is brought out by Wild (1970). After showing that the serpentine soils of the Great Dyke produce a depauperated and stunted flora he points out that if nickel anomalies occur in serpentine mother rocks, they are characterized by an even more depauperated flora derived from the already anomalous serpentine vegetation. This makes the nickel flora under these circumstances difficult to distinguish from that of the surrounding serpentine soils without high nickel values, but this can nevertheless be done by observing rather subtle differences in the proportions and relative dominance of certain species and the presence of a few indicator species either absent or rare on normal serpentine soils. Examples are *Albizia amara*, *Barleria aromatica*, *Combretum molle*, and an undescribed species of *Dicoma* (see Section IV) which is almost exclusively endemic on Rhodesian nickel soils.

Most of the higher plants found on toxic soils are perennial herbs. The perennial habit probably makes colonization easier since it ensures persistence and a low growing habit is an adaptation to exposure.

The study of species found on contaminated soils is difficult: often it is not possible to delimit precisely the contaminated area, to determine the degree of effective toxicity, or to assess the other ecological factors involved. Studies of species on metal-contaminated soils have rarely been rigorous and are usually little more than incomplete species lists. It is often possible that the species listed are not growing on metal-contaminated soils but on distinctive, poor soils adjoining contaminated areas. From the available information it is difficult to deduce generalities about taxonomic, morphological or physiological characteristics of the species involved since they obviously occupy a wide and diverse spectrum of ecological conditions.

It is none the less clear that the plants growing on metal-contaminated soils are often characteristic of such soils. Toxic areas are usually colonized by some plants, and although the colonization of many areas is sporadic, other areas carry regular communities.

B. COMMUNITY STUDIES ON CONTAMINATED SOILS

Several workers have examined the plant communities found on metal-contaminated soil using the techniques of phytosociology pioneered by Braun-Blanquet (see Section IIA1 for full explanation). These techniques enable plant communities to be classified in a way analogous to the classification of species. The species of phytosociology is the association, and associations can be grouped into families, orders and classes.

Schwickerath (1931), Koch (1932), Schubert (1953, 1954a), Ernst (1965a, 1968a) and Baumeister (1967) have examined the communities growing on contaminated soils (zinc and copper) in Germany. This work has been extended to Holland by Heimans (1936), to France by Ernst (1966), and to Great Britain by Shimwell (1967) and Ernst (1968b, c). These workers have shown that plants on contaminated soils form definite and distinct associations, which can be easily identified and named. Keys identifying communities of a particular country or area frequently include mention of associations on metal-contaminated soils (e.g. Tuxen, 1937; Lebrun *et al.*, 1949). A full bibliography is given by Ernst (1967).

Schwickerath (1931) was the first to study in detail communities on toxic soils (lead/zinc mines near Aachen, Germany). He named these as belonging to the association *Violetum calaminariae* (order *Mesobrometum*, class *Festuco-Brometa*). He further subdivided these into vegetation types or "facies", which he regarded as representing different ecological conditions. Koch (1932) in a brief study of communities on zinc-contaminated soil near Osnabruck, Germany, considered these to belong to another association, the *Minuartia verna–Thlaspi alpestre* association. Schubert (1953, 1954a, b), studying communities on lead, zinc and copper soil, recognized further associations, *Armerietum bottendorfensis* and *Armerietum halleri*, characterized by species of the genus *Armeria*.

Heimans (1936) noted the similarity of the vegetation in Holland with the *Violetum calaminariae* of Schwickerath (1931) with the exception that *Minuartia verna* was absent, and *Agrostis vulgaris* (= *tenuis*) a dominant member.

Ernst (1965a) reconsidered the classification of mine-plant communities by previous workers. All these associations had several species in common, namely *Viola calaminaria, Thlaspi alpestre, Minuartia verna, Silene vulgaris, Armeria* spp. and *Festuca ovina*, but occasionally one or other was absent. He came to the conclusion that the similarities between these communities was so great and they were so unlike any other communities, that they should all belong to one separate class (containing only one order), the *Violetea calaminariae*. This class and order could be identified by the consistent members *Silene vulgaris* and *Minuartia verna*. Ernst (1965a), as a result of additional observations, further divided this class into three families.

1. The *Armerion halleri*, of drier habitats in central Germany.
2. The *Thlaspeion calaminariae*, of western central Europe.
3. The *Galio-Minuartion vernae*, of the alpine regions.

Ernst (1966) recognized another association in southern France, but this could be included into the *Thlaspeion calaminariae* group. The mine vegetation of Britain (Ernst, 1968b, c) could be placed within the same family, although the communities in certain areas in particular were generally species poor. A summary of the characteristic species of these associations and families is given in Table III.

The work of Ernst demonstrates clearly the power of the phytosociological methods in summarizing the variation among communities on a wide range of contaminated soil types. Certain consistent patterns emerge, related in part to habitat and geography but the reasons for the existence of such regular but different communities remain unexplained.

C. FACTORS DETERMINING PLANT DISTRIBUTION ON CONTAMINATED SOILS

1. *Metal concentration and type*

The overriding characteristic of contaminated soils is the high concentration of metals in the soil. However, although the area of contamination is frequently recognizable by a flora different from that in surrounding areas, very few workers have studied precisely to what extent metal concentration and type are important in determining the distribution of mine plants.

Part of the difficulty arises from the problem of deciding on what measure to use for the amount of metal in the soil, since the metal may be in several forms. It may be water soluble, exchangeable and bound to inorganic or organic soil components, or unavailable in stable complexes with organic substrates, or unavailable as insoluble compounds or minerals (e.g. Nicolls *et al.*, 1965).

Most workers have resorted to measuring total metal concentration since broadly speaking this has been found to reflect in a relative way on the concentration affecting the plants, and because it is easy to measure (Duvigneaud and Denaeyer-de Smet, 1963; Gregory and Bradshaw, 1965; Nicolls *et al.*, 1965).

There is no evidence that other methods (e.g. water soluble, acid soluble) reflect more accurately the amount available to plants, and at present total metal content appears the simplest and most realistic measure. The use of bioassay to solve this difficult problem has not been explored: it may be possible to use growth of genotypes of, say, *Agrostis tenuis* of differing degrees of tolerance on soils to estimate the effective concentration at particular sites in a region.

Another complicating factor is the vertical distribution of metals in the soil. This is frequently non-uniform because of leaching, accumulation of metals in humus layers, localization of ore bodies at certain depths, or spread of toxic debris on normal soils (see Malyuga, 1964, for full discussion). Clearly plants with differing root systems may encounter effectively different concentrations of toxic metals.

Soils with very high concentrations are usually barren whereas those with only slightly enhanced concentrations may not visibly affect the vegetation but may lead to increased metal content of the plants growing there (see Section VIIIA). Different plants can withstand (in the sense of tolerate or evolve tolerance of) different amounts of metal. Schwickerath (1931) recognized several vegetation types representing increasing colonization, and these could be correlated with both lead and zinc concentration of the soil. Duvigneaud and Denaeyer-de Smet (1963) and Wild (1968) recognized three types of "cuprophyte" (plants restricted to copper soils) on natural and man-made copper soils of Katanga and Rhodesia respectively. They were the "polycuprophytes" which grew in areas of 5000–10 000 ppm copper or above, the "oligocuprophytes" which were restricted to soils with 800–2000 ppm copper, and the "eurycuprophytes" which could colonize a whole range of copper concentrations. The latter were relatively rare. Ernst (1965b) recognized three initial phases in the colonization of zinc-contaminated areas: and the last of these phases which contained a considerable number of species on soils with a generally lower concentration than the first two.

The study of Nicolls *et al.* (1965) emphasizes, in a subtle way, the controlling effect of metals on vegetation. As expected, sharp changes in the vegetation coincided with changes in total contamination. However, changes in the relative amounts of lead, copper and zinc were also important. Of the various species considered, *Eriachne mucronata* seemed to tolerate high concentrations of all three metals, *Bulbostylis barbata* and *Polycarpea glabra* were found in areas of high copper, while *Tephrosia* sp. nov. was found where the copper concentration was lower. In general, changes in lead and copper were more determinant than changes in zinc.

2. *Other soil factors*

Soils contaminated with heavy metals differ from each other and from normal soils in many ways other than in metal content. The nutrient status of the soil, the organic matter content and the texture may all affect the number and types of plant growing on the soil. Their effect may be direct or they may act indirectly by influencing the toxicity of the metal in the soil.

In general, mine-tip soils are extremely low in the essential nutrients nitrogen, phosphorus and potassium. Bradshaw *et al.* (1960), and McNeilly (1965) have shown that lead- and copper-tolerant populations of *Agrostis tenuis* are also better able to grow in culture solutions containing low phosphate than are populations from pasture soils. Recent studies on reclamation of contaminated tips (Hilton, 1967; Smith and Bradshaw, 1970) (see also Section VIIIc) have shown that addition of complete fertilizer at normal agricultural rates enormously improves the growth of plants both native and foreign to mine soils: in many cases, native tolerant plants can be made to grow on previously bare mine soils only with the addition of complete fertilizer. Further indirect evidence comes from distribution studies. Horne (1967) found that greatest vegetation cover on a small area of a copper mine could best be correlated with high potassium content. Nicolls *et al.* (1965) state that phosphorus may determine the distribution of plants on a natural metal (copper and zinc) outcrop in Australia: in contrast to mine tips, the soil there had a higher phosphorus content than that of surrounding areas, and comparable nutrient content in potassium and nitrogen.

Mine-tip habitats are rich in sulphur since the metals usually occur as sulphur ores. It has been shown (Antonovics *et al.*, 1967; Turner, 1967) that *Agrostis tenuis* from a copper mine requires more sulphur for normal growth than the same species from pasture, and that sulphur oxidizing bacteria can be found in such soils (see Section IIA4).

Another element which appears to be important is calcium, either by virtue of its direct effect, or by its influence on soil pH. In Great Britain and Europe mines on acid soils differ markedly from those with a higher pH. They have generally fewer species and are usually covered with *Agrostis tenuis* and *Festuca ovina*; calcareous mines in contrast have *Agrostis stolonifera* and *Festuca rubra*. Liming of waste tips improves the performance of plants sown on mines, if fertilizer or organic matter are also added (Hilton, 1967). Schwickerath (1931) observed that his vegetation types representing different degrees of colonization, although they correlated with total lead and zinc in the soil seemed to correlate better with the calcium: zinc ratio. Since calcium may alleviate the uptake and toxic effects of zinc, this ratio is possibly a measure of zinc availability.

The importance of calcium has since been observed by other workers. Warren and Delavault (1949) found the zinc and copper content of trees growing over limestone areas was much lower than in more acid soils and concluded that "it is reasonable to assume that pH of soil is a more important factor than either the zinc or copper content of the ground water in determining the amount of zinc and copper which may be toxic". Nicolls *et al.* (1965) note that over calcareous rocks of a naturally occurring outcrop of mineralization of zinc and copper, there are different and more numerous herb species. Plants such as *Suaevola densevestita* and *Ptilotus obovatus* are listed as possible indicators of copper anomalies in calcareous areas.

The organic matter content of the soil is also of extreme importance since it forms stable complexes with metal ions, so making them unavailable (Hodgson *et al.*, 1966). Dykeman and de Sousa (1966) record concentrations of 7% copper in peat of a copper swamp yet it appears to have no toxic effects, the overlying vegetation being typical for the area. Organic matter (sewage sludge or domestic refuse) is extremely effective at alleviating the toxicity of metals in slag heaps, permitting healthy growth of various non-tolerant species for several years (Hilton, 1967).

The importance of yet other soil factors was pointed out by Ernst (1965b). He studied the ecological conditions on mines characterized by three initial stages of colonization, namely the *Silene cucubalus* (=*vulgaria*) var. *humilis* stage, the *Minuartia verna* ssp. *hercynicium* stage and the *Euphrasia* spp. stage. *Silene* grew on soils with a coarse texture, low water capacity, and low surface temperature. *Minuartia* on the other hand grew on soils with finer texture, high water capacity, but high surface temperature. The habits of these plants, namely the deep rooting *Silene* with narrower leaves than the normal forms, and the shallow-rooting but tufted habit and needle-like leaves of *Minuartia*, adapt them to their respective habitats. The *Euphrasia* phase was considered to be a succession from the above two phases, but also characteristic of soils of even higher water capacity and, as mentioned already, lower metal content. Ernst therefore provides very clear-cut evidence for the importance of factors other than metal content to plants growing on metal-contaminated soils, although the specific factor, water or nutrients, is not identifiable.

3. *Climatic factors*

Contaminated areas are frequently only sparsely colonized. The plants on such areas are therefore growing exposed in open communities. Several workers (see Section VF) have noticed morphological differences between plants growing on mine soils and those in their normal habitat. Usually these morphological differences are in the direction of plants on the mine being smaller, prostrate and apparently adapted to exposed conditions. Occasionally spreading forms are found, adapted to the sand-dune-like conditions of some tips made of tailings (Bradshaw, 1959). Bröker (1963), and Antonovics and Bradshaw (1970) have shown that these morphological differences are not pleiotropic effects of the tolerance mechanism but that they must have arisen by independent selection. This may well be selection for adaptation to climatic factors but it is possible in some instances that the small size is an adaptation not so much to exposure but to the nutrient shortage in the habitat.

As would be expected, climatic factors exert considerable control on the species to be found in different contaminated areas. Wild (1968) shows that the species found on copper soils in the neighbouring areas of Katanga and Rhodesia are different because of the greater aridity of Rhodesia: for instance, *Combretum* spp. are common in copper soils in Rhodesia and almost absent in Katanga. For this reason, Malyuga (1964) divides Russia into nineteen biogeochemical zones.

4. *Biotic factors*

Plants probably play an important part in changing the nature of the toxic habitat. Their root systems stabilize the soil and their aerial parts offer some shelter to subsequent colonizers, but above all they contribute organic matter and humus to the surface layers of the soil. The addition of humus has three results: it increases the nutrient status of the soil, it forms complexes with heavy metals and makes metal ions unavailable to plants, and it improves soil texture. The ability of organic matter to complex with heavy metals frequently results in an accumulation of heavy metals at the surface of the soil and in humus layers of the soil (Dykeman and De Sousa, 1966; Le Riche, 1968). The cyclic process of mineral absorption, death, and mineral release into upper layers of the soil has been called the Goldschmidt or Vernadskii principle (see Malyuga, 1964, for full discussion) and it is the reason why humus layers of the soil are often useful in biogeochemical prospecting (see Section VIIIA). Generally metal ions complexed to organic matter are unavailable to plants, so that the effective metal concentration of the soil is reduced.

Schwickerath (1931) produced evidence that colonization of the mine habitat by some species appeared to depend on the prior presence of others: with increasing colonization new species were distinctly associated with the *Festuca ovina* sward. Similarly Schubert (1954b) showed that colonization by *Silene vulgaris* var. *humilis* was concentrated on or near the *Minuartia verna* swards. It cannot be deduced whether such associations are due to a genuine alleviation of available metal level, or whether both species colonize patches of slightly lower toxicity or patches which are ecologically less adverse in some other way.

Schwickerath (1931), Schubert (1954b) and Ernst (1965b) recognize areas of differing colonization and assume that they represent stages of a succession. But the data can equally well be interpreted as a static situation in which a varied set of environments, and not successional processes, are the cause of the different communities. If a truly successional situation is assumed it is still not clear whether it is *autogenic*, in that the plants themselves are alleviating the toxicity, or *allogenic*, in that soil toxicity is being lowered by weathering. Schwickerath (1931) and Ernst (1965b) both show that the level of zinc is lower in the areas of increasing colonization. This suggests that either there has been allogenic succession, or more likely that the extent of colonization is related to the initial zinc content of the soil.

5. *Other factors*

Many metal-contaminated areas are created by mining activity. Since the history of mining in any given area is often well documented it might seem possible to look at the colonization of mine areas in relation to the age of the mine. The most extensive investigation has been by Schubert (1954b), who looked at copper-mine tips in Germany. He studied four main periods of mining between 1200 and the present day, but no clear-cut succession could be seen from one period to another. Differences between the periods could be explained by the various shapes of the tips determining exposure, aspect, and weathering. In the fourth period, more efficient extraction has lowered the copper content and therefore made comparison even more difficult. Antonovics (1966) briefly investigated the relationship between the age of lead mines in Wales and their colonization by *Agrostis tenuis*, but again no significant relationship was found. The classification of mine tips by age not only disregards other ecological factors, but colonization is probably quite rapid and in the order of twenty to thirty years so that historical data provide little useful evidence. The period involved is nevertheless sufficiently long to have eluded direct observation.

Ernst (1969b) has documented by pollen analysis *Armeria* and *Minuartia* on a copper-contaminated moor in Wales. By broad correlative analysis of the vegetation he considers the species to date back at least to the twelfth century AD, when both were at their peak. They were drastically reduced as a result of removing the copper turf around AD 1800. Such studies show the potential age of some populations. But some must be much older than this, perhaps dating back to the post glacial, since ore bodies could well have been exposed by glaciation and caused areas of contamination from that period onwards. In the Congo (Duvigneaud and Denaeyer-de Smet, 1963), Rhodesia (Wild, 1968, 1970) and Australia (Nicolls *et al.*, 1965) it is clear that ore bodies can have outstanding effects on vegetation without the occurrence of any mining activity (see Section VIIIA).

Another important factor in colonization is the proximity of available species. Antonovics (1966) showed that the density of populations of *Agrostis tenuis* on lead mines was greater when the populations were nearer the edge of the mine, nearer the original seed and pollen source. Although this study was inadequate in that metal concentrations in the soil were not investigated, a similar relationship was found by Schubert (1954b), who showed that *Minuartia verna* was found on tips downwind from an area where it was in great abundance. Although the evidence is again rather circumstantial, it does show the possibility that a source of suitable colonists might determine the composition of mine communities.

6. *Conclusion*

It is clear that critical evidence on the relative importance of heavy metals and other factors on the colonization of mine soils is lacking. Even though many ecologists have attempted to gauge the factors in some detail, many of their methods and assumptions will not hold up to critical examination. The situation is not peculiar to the study of metal-tolerant plants since too often factors affecting plant distribution are obtained on the basis of broad correlation principles. The main fault in the methodology used is that local distribution and habitat preference are used to define the physiological amplitude of the species involved, the successional changes that have occurred, the competitive influence of other species and so on. The arguments are then taken full circle and the factors that have been defined by distribution are then used to explain the distribution. The way out of the vicious circle is not only to define the environmental conditions more and more precisely, but to combine such studies with experimental work both in the field and in the greenhouse.

The most critical factor of all which has been rarely considered by ecologists is metal tolerance itself. Evidence will be presented in Section V that the ability to colonize mine areas requires the ability to possess or evolve tolerance: plant distribution on metal-contaminated areas has an evolutionary component. For example, the classification of plants into metallophytes, pseudometallophytes, etc. (Lambinon and Auquier, 1964) outlined earlier, may reflect not simply an ability to *tolerate* high contamination but also an ability to *evolve* such tolerance.

The crucial ecological problem is therefore whether the presence of species on metal-contaminated areas is always dependent on the evolution of tolerance or whether in some cases there are species which possess metal tolerance throughout their range; and *per contra* whether the absence of species from metal-contaminated areas is due to their inability to evolve tolerance. The evidence on this will be discussed in Section VB.

The problems of adaptation to this distinctive environment serve to highlight problems of methodology. An enlightened examination of the vegetation on metal-contaminated areas could be very instructive.

III. GEOGRAPHICAL INVESTIGATIONS

Metal-contaminated areas, either naturally occurring or man-made, are very widely distributed. They also, as has been previously shown, carry a very distinct type of vegetation which often contains species largely or wholly restricted to such areas. The geographical distribution of these species is therefore highly disjunct, and this has attracted the attention of several workers.

Schultz (1912) argued that since both *Viola lutea* and *Minuartia verna*, two plants commonly found on contaminated soil, have their main distribution in the alpine regions of Europe (and *Minuartia* also in the Arctic regions and Asiatic mountains) and show elsewhere in Europe a highly disjunct distribution (being largely limited to metal-contaminated areas), they must have been widely distributed throughout Europe at one time when conditions were favourable, in the fourth glacial period. Since then, their distribution has been limited to mine tips where they do not suffer from the effects of competition. This finds support from the evidence of post-glacial distribution for other species with disjunct distributions (Pigott and Walters, 1954; Godwin, 1956). He also considered some other heavy metal plants; he suggested *Thlaspi alpestre* and *Arabis halleri* were also of a formerly wide distribution, but there was no clear pattern for *Armeria halleri* and *Silene vulgaris*.

Similar arguments to those developed by Schultz have been used in connection with a study of *Festuca* spp. growing on contaminated areas in and around Belgium (Auquier, 1964). These fescues are considered to belong to several taxa. None of these taxa is completely restricted to contaminated soils, their relatives being often found in upland areas. However, in Belgium itself these plants are strictly confined to calamine (zinc-contaminated) soils. Auquier infers from this that these taxa represent remains of plants which once had a wide distribution during the arctic-alpine climate of the last ice age. A similar interpretation has been arrived at by Heimans (1960) studying the distribution of the zinc violet, *Viola calaminaria,* but he gives it only his tentative support.

Stebbins (1942), in discussing the interpretation of disjunct distributions, distinguished two types. The "paleo-endemic", which is a species of formerly wide distribution that is now confined to particular areas; and "neo-endemic", which is a species that has originated recently in given areas in response often to peculiar environmental conditions.

The suggestions of Schultz, Auquier, and Heimans are therefore that the species found on metal-contaminated soils are usually palaeoendemics. But the situation is difficult to interpret: a simple palaeoendemic interpretation of the geographical distribution of all the species may often be inaccurate.

Firstly, many of the so-called mine taxa are not distinct and there is no evidence that they are reproductively isolated from their relatives. These taxa could be the products of parallel evolution on different mines from relatives in the neighbouring pastures. This process, which has been shown in grasses (see Section VE), can occur rapidly; and the evolution of tolerance can occur in parallel with evolution of morphological differences. The mine taxa in this case would be neo-endemics. The interpretations of Auquier (1964) are probably erroneous since his taxa of *Festuca* are not highly distinct and could easily have arisen by recent evolution.

Secondly, contaminated soils are commonly associated with man's activities, and the widespread disjunct distribution of many mine plants may simply indicate the efficiency of dispersal by human agencies. This phenomenon could be called "transport endemism". Many of the mines are in mountainous regions and dispersal of alpine members such as *Viola lutea*, *Minuartia verna* and *Thlaspi alpestre* from upland to lowland mining areas, could give the erroneous impression that these upland plants on lowland areas are remnants from a formerly colder climate.

Thirdly, many metal mines are recent and in no way correlated with natural metal outcrops. Moreover, where outcrops occurred they have almost invariably been destroyed by mining activities. Arguments about plant distribution on contaminated areas which are the result of mining may not involve factors present more than a few hundred years ago.

All these arguments favour strongly the suggestion that most mine species are neo-endemics. Evidence that this has been the case comes from the work of Duvigneaud and Denaeyer-de Smet (1963) on the flora of mainly natural copper outcrops in Katanga. With regard to this flora they suggest that the majority of the cuprophyte species are close relatives of non-cuprophyte species which have an extended distribution and which are all found in the Zambesian region. In other respects the cuprophyte flora consists mainly of sub-species or vicarious varieties with the status of ecotypes and probably a large number of other ecotypes not morphologically differentiated. Its character is that of a young neo-endemic flora having its origin in the same area in which it is found. Some taxa have the character of relics of regional movements corresponding to the displacements which have affected the flora of the Zambesian region.

In this area, therefore, neo-endemism appears to be the rule. In those cases where a neo-endemic hypothesis cannot be applied because the possible source of the neo-endemic is far away, transport by human agency may well be an explanation.

The last and very crucial point is that even if in some instances the evidence does favour the palaeoendemic hypothesis, no plant is known that has an inherent or constitutional resistance to heavy metals throughout its distribution (see Section VB). In other words the plants on mines cannot be regarded as simple "left-overs" from a widespread distribution. The problem of the evolution of tolerance is still there.

The interpretation of the disjunct distribution of metal-tolerant plants therefore poses general questions on the nature of endemism, questions which by no means have yet been fully answered.

IV. TAXONOMIC INVESTIGATIONS

The difference in morphology of plants growing on metal-contaminated areas from plants in normal areas was noted by Baumann (1885) and Jensch (1894). Numerous workers have since then remarked on this feature of tolerant populations (see Section VF). It is therefore not surprising that taxonomists have attempted to classify these plants as separate taxa. Floras and the literature are strewn with subspecific and varietal epithets referring to mine taxa (e.g. Ernst, 1965a).

The literature on the taxonomy of plants growing on metal-contaminated soils is by far the hardest to review and it is almost impossible to come to any conclusions regarding the validity of the systems proposed for groups that have been studied. The reason for this is partly that the material concerned is in itself difficult to include in any classification system, but mostly because the taxonomy of plants is often as cursory as it is unreliable.

Firstly, there have been very few attempts to grow mine- and non-mine plants under uniform conditions to see if the differences between them (and between different tolerant populations) are inherited: conditions in metal-contaminated areas are so extreme that gross, environmentally induced effects are to be expected and examination of field material may be completely misleading taxonomically. It is interesting in this connection that Malyuga (1964) assumes changes in flower form (*Papaver* spp., *Pulsatilla patens*), flower colour (*Papaver* spp.), and changes in morphology over areas of enhanced toxicity to be the sole result of environmental effects, although there seems no good reason for this conclusion: they may well be genetic.

Secondly, there have only been a few attempts to cross mine and non-mine plants to try and assess the distinctiveness of the "taxa" (see Section VI).

Thirdly, very few taxonomists have considered the possibility that morphological differences (even if inherited) may be the result of direct adaptation to mine habitats and of quite recent origin.

The crux of the matter is that mine habitats are ecologically very distinct and often clearly demarcated from normal habitats. This makes the taxonomic recognition of forms very easy even if they are not very different from other taxa in terms of taxonomic and evolutionary distance. This is particularly so of characters such as dwarfness and hairiness. These forms are therefore given high taxonomic or even specific rank when often they are probably no more than ecotypes.

The methods of experimental taxonomy must be used if any reliable picture is to emerge. So far, not even the most elementary aspects of this approach have been attempted. A few examples will serve to illustrate the state of research in this field.

Auquier (1964) investigated the genus *Festuca*, growing on metal-contaminated soils in and around Belgium and classified the genus into two main species, one of which he divided further into subspecies, varieties and forms. This work can however be criticized from several standpoints. Firstly, material used in this study was collected directly from the field: no attempt was made to grow it under standard conditions. The taxa include the fine delimitations of variety, sub-variety and form; these could easily be environmental modifications. Secondly, even if the differences between the taxa are inherited they could easily have been achieved by parallel evolution on different mines since the characters that were measured (length of floral parts, anatomy of tillers, hairiness, vein number, leaf diameter) could easily have been affected by selection for local adaptation.

The complexities of the situation in the European genera *Silene*, *Minuartia*, *Armeria*, and *Viola* have been reviewed by Schubert (1954a) and Heimans (1960, 1966), but their views on classification of some of the genera do not escape from the controversies and problems of synonyms. Schubert quotes seven names for *Minuartia verna*; these are either synonyms or refer most probably to different ecotypes. Heimans, in his detailed review on the zinc violet (*Viola calaminaria*), quotes eight names of different ranks which are probably synonyms. With regard to the genus *Armeria*, Heimans states "Some taxonomists refer the European representatives of the genus *Armeria* to ten different species, whereas other taxonomists recognize but two". Lefebvre (1967) shows clearly some of the reasons for this confusion: populations of *Armeria* from zinc soils in Belgium are intermediate in form between *A. alpina* and *A. maritima*; the ranges of variation of the populations overlap with both these species, and populations on neighbouring contaminated sites can be very different. Nor do the populations have all the characters of another taxon with species status, *A. elongata* in which they had previously been included.

Duvigneaud (1958, 1959), Duvigneaud and Timperman (1959), and Duvigneaud and Denaeyer-de Smet (1960, 1963) recognize many new species, and new subspecies on metalliferous soils in Katanga. Wild (1968) reports several apparently distinct subspecific taxa although none at the specific level in the Rhodesia copper flora, and recently (1970, pers. comm.) has shown that one taxon previously considered to be *Dicoma macrocephala* is a new and distinct species. Again, it is difficult to assess the validity of many of the categories since the observations are made on material taken directly from the field. However, they are often well aware of the arbitrary nature of their taxonomic units.

There have been a few cytological studies of mine plants, and two examples quoted by Heimans (1960) are of interest. Firstly, his own work on the zinc violet showed that the chromosome number was $2n = 52$ (even though he and other workers had previously recorded it as

$2n = 48$) which differed from that of its supposedly nearest relative, *Viola lutea*. Heimans therefore, and on other evidence, concluded greater affinity between *Viola calaminaria* and *Viola alpestris* ($2n = 36$). He also mentions the work of Duyvendak (no reference or actual chromosome numbers given) which showed that *Festuca ovina* in the zinc area differs from all other strains found in the Netherlands, but the chromosome number agrees with those of strains found in the mountainous regions outside the Netherlands. However, Wilkins (1960) could not relate the ploidy level of *Festuca ovina* to the metal tolerance or to the relative occurrence of the two ploidy types on contaminated and uncontaminated soils. No differences in chromosome number of tolerant and non-tolerant *Agrostis tenuis* were found by Jowett (1959a).

This work on chromosome numbers is interesting in that it suggests that perhaps some of the mine races have been formed by a process similar to catastrophic selection (involving drastic chromosomal adjustments of a specially adapted type, leading to reproductive isolation) described by Lewis (1962). Further studies of chromosome numbers of tolerant and non-tolerant plants could prove rewarding from both the taxonomic and evolutionary viewpoints.

The taxonomy of plants on contaminated areas needs a thorough experimental review. Speculation on the basis of present literature is probably not rewarding unless done in conjunction with experimental work. As a field of research it could again have great relevance to taxonomic methodology, especially with regard to endemic and specialized forms.

V. EVOLUTION OF METAL TOLERANCE

In the absence of any prior knowledge the existence of plants on metal-contaminated soils would immediately raise the question of whether these plants belonged to species that were for some reason inherently tolerant to metals, or whether they were races that had evolved a special tolerance not possessed by the remainder of the species.

As will be shown there is much literature demonstrating the existence of such races and this has not only exposed a new facet that must be taken into account when explaining plant distribution, but also has produced the best available example of natural selection in plants. Heavy metal tolerance in plants is probably an example of more powerful evolution in action than industrial melanism in moths.

A. EVIDENCE FOR RACES TOLERANT TO METALS

This section considers the evidence that the ability to colonize contaminated areas depends on the ability to evolve races tolerant to heavy metals.

1. Naturally occurring races on contaminated soils

The first comparative study of mine and non-mine populations is that of Prat (1934). Seed of *Melandrium silvestre* from a copper mine tip grew far better in soils with the higher concentrations of added copper carbonate than seed of plants from uncontaminated soil; at the highest concentrations the plants from uncontaminated areas died in the seedling stage. Prat considered the increased resistance of plants growing on copper mines to be the result of natural selection.

This investigation was the only one of its kind until the 1950's, when studies of mine and non-mine populations were resumed independently in Great Britain and Germany.

In Great Britain, Bradshaw (1952) reported populations of *Agrostis tenuis* tolerant to lead and zinc soils; plants from pasture soils did not survive on mine soil. Following this, Wilkins (1957, 1960) developed a rooting technique which showed *Festuca ovina* to have races tolerant to lead. The technique involved comparison of root growth in solutions with and without added metal salts, and this technique or its modifications (Gregory and Bradshaw, 1965; Holmes, 1965; Jowett, 1958; McNeilly, 1965; Gadgil, 1969) has been used to establish and quantify the tolerance of races of a wide range of species found on metal mine soils by a wide variety of authors (Table V). Work in Germany has also produced an impressive list of plants showing tolerant races. Schwanitz and Hahn (1954a, b) recording survival in water culture with different amounts of zinc showed several species to be tolerant if taken from zinc-contaminated soil, but non-tolerant if taken from normal soil. Repp (1963) and Gries (1966) measuring the cellular resistance to metal ions, by finding the concentration that produced death of epidermal cells, established tolerant races in yet other species. The results of work on races tolerant to various metals is summarized in Table V. Baumeister (1954), Baumeister and Burghardt (1956) and Wachsmann (1961) recording rate of photosynthesis and general growth, and Bröker (1963) recording dry weight in water culture, have all confirmed that *Silene inflata* forms races tolerant to zinc and to copper. Url (1956) showed that species of mosses from copper-contaminated regions had a far higher resistance to copper than species from normal soils. This work was not, however, extended to the intraspecific level.

From these results it is clear that the existence of metal-tolerant races is a common phenomenon. Many of the species can develop resistance to more than one metal, either in separate races or simultaneously in the same race if the soil is contaminated with two metals (Jowett, 1958; Gregory and Bradshaw, 1965). Generally speaking, the tolerance is metal specific: tolerance to one metal does not automatically confer

TABLE V

Species showing races tolerant to heavy metals

Species	Metal	Author
Melandrium silvestre	Cu	Prat, 1934
Taraxacum officinale	Cu	Repp, 1963
Tussilago farfara	Cu	Repp, 1963
Mimulus guttatus	Cu	Allen and Sheppard, 1971
Agrostis tenuis	Cu, Pb, Ni, Zn, Cu+Ni, Pb+Cu, Zn+Pb	Gregory and Bradshaw, 1965
	Cu	McNeilly, 1968; McNeilly and Bradshaw, 1968
	Pb	Jowett, 1958, 1964; Jain and Bradshaw, 1966
	Pb, Zn	Bradshaw, 1952
Rumex acetosa	Cu	Coackley and Dawson, 1966
	Zn	Spilling and Thomas, 1964; Schwanitz and Hahn, 1954b
Festuca ovina	Pb	Wilkins, 1957, 1960
	Zn	Gregory and Bradshaw, 1965
Agrostis stolonifera	Pb	Jowett, 1958
	Zn	Gregory and Bradshaw, 1965; Archer, 1964
Agrostis canina	Pb	Craig, 1970
	Zn	Gregory and Bradshaw, 1965
Viola lutea	Zn	Schwanitz and Hahn, 1954a
Alsine (= *Minuartia*) *verna*	Zn	Schwanitz and Hahn, 1954a; Humphreys and Farnworth, 1964
Silene vulgaris (= *inflata* = *cucubalus*)	Zn	Schwanitz and Hahn, 1954a; Baumeister, 1954; Baumeister and Burghardt, 1956; Bröker, 1963; Gries, 1966; Ernst, 1968d
	Cu, Zn	Wachsmann, 1961
Plantago lanceolata	Zn	Schwanitz and Hahn, 1954b; Williams and Morgan, 1964
Linum catharticum	Zn	Schwanitz and Hahn, 1954b
Campanula rotundifolia	Zn	Schwanitz and Hahn, 1954b
Agrostis tenuis × *stolonifera*	Zn	Gregory and Bradshaw, 1965; Archer, 1964
Festuca rubra	Zn	Gregory and Bradshaw, 1965;
Holcus lanatus	Zn	Jenkins and Winfield, 1964; Antonovics, 1966
Anthoxanthum odoratum	Zn	Gregory and Bradshaw, 1965; Putwain, 1963; Antonovics, 1966
Thlaspi alpestre	Zn	Ernst, 1968d
Armeria maritima	Zn	Lefebvre, 1968

tolerance to another (see Section VIIc). Moreover, the level of tolerance developed is related to the amount of metal in the soil. Thus Wilkins (1960) demonstrated a correlation between the degree of lead tolerance of *Festuca ovina* and amount of extractable lead in the soil. Similar relationships between degree of tolerance and amount of metal in the soils have been demonstrated in *Agrostis tenuis* on lead mines by Jowett (1964), on copper mines by Gregory and Bradshaw (1965) and McNeilly and Bradshaw (1968), and on zinc mines by Gregory and Bradshaw (1965) and Turner (1967, 1969).

2. *Metal tolerance to sprays and toxicants*

In higher plants, there is no evidence of the evolution of tolerance to fungicide and pesticide residues, almost certainly because metallic compounds have not been employed directly on plants in toxic quantities. However, in certain habitats toxic levels of metal are being approached: roadsides are frequently contaminated with lead, and the soil of fruit orchards and vineyards contain large amounts of lead, copper and mercury (see Section I for references). Metal tolerance may well be evolving in such situations.

In lower organisms there is some evidence that races tolerant to metal toxicants have evolved. Toxic paints involving compounds of copper and mercury are used as antifouling compounds on ships. In those cases where algal growth has occurred due to the presence of *Ectocarpus* spp., the populations have been shown to possess tolerance to copper many times greater than in normal populations (Russell and Morris, 1970). A similar tolerance to mercury has been demonstrated in *Enteromorpha* spp. (Hodgson, 1969).

In fungi, there is surprisingly little evidence of tolerant races. Ashida (1965), reviewing the adaptation of fungi to metal-containing toxicants, mentions only two instances, both cases of adaptation to Bordeaux mixtures. Taylor (1953) reports that spores of *Physalophora obtusa* from orchards sprayed with Bordeaux mixture are more resistant to copper than spores of the same species from unsprayed orchards. Horsfall (1956) suggests that the reduction in effectiveness of Bordeaux mixture in controlling potato blight might be due to the evolution of resistance to it by *Phytophthora infestans.*

More recently there have been several reports of resistance of *Pyrenophora avenae* to mercury-containing fungicides (Noble *et al.*, 1966; Malone, 1968; Old, 1968; Sheridan *et al.*, 1968; Bainbridge, 1969; Greenaway and Cowan, 1970). There is considerable evidence that such resistance is spreading (Malone, 1968; Old, 1968). Soil fungi of the genera *Penicillium* and *Aspergillus* may also develop resistance to mercury fungicide residues in the soil (Spanis *et al.*, 1962). Young (1961) found copper-tolerant races of several brown rot fungi regularly associated with copper fungicide failures on fence posts. This was confirmed by Da Costa and Kerruish (1964) who showed that most brown rot fungi were highly tolerant to copper sulphate and chromate. The most tolerant species, *Poria villantii*, grew on agar containing 8% copper sulphate.

There is therefore evidence that the evolution of fungi resistant to metal containing toxicants is possible, but such that examples are relatively rare. This may in part be because the resistant strains are less able to compete with virulent strains in the absence of the fungicide (e.g. poor vitality of copper-resistant *Phytophthora infestans* (D'Yakov, 1963a, b), or simply that the evolution of tolerance to metal toxicants is not a very common phenomenon. The former explanation seems more likely since there is considerable evidence for tolerance to metals in laboratory strains (see Section VA3). However, there is clearly insufficient evidence to come to any firm conclusions.

Bacteria resistant to metals have been isolated from various sources. Chatterjee *et al.* (1968) record mercury-resistant strains of *Staphylococcus pyogenes* and note that their occurrence is more common in hospitals than in the general community. Mercury resistance was common in strains responsible for hospital cross-infections and frequently also had resistance to several antibiotics.

A similar association between mercury resistance and drug resistance (to tetracyclines) was found in *Staphylococcus aureus* (Williams, 1967). Novick and Roth (1968) show that separate factors giving up to a hundredfold resistance to As, Pb, Cd, Hg, Bi, Sb and Zn are associated on a non-nuclear plasmid carrying a separate factor for *Penicillin* resistance.

Mercury-resistant strains of bacteria have been isolated from soils contaminated with mercury-containing fungicides by Tonomura, Nakagami, Futai, and Maeda (1968) (*Pseudomonas* sp.), and by Spanis, Munnecke and Solberg (1962) (*Bacillus* spp).

3. *Metal tolerance in laboratory strains*

Under laboratory conditions the evolution of metal-tolerant races appears to take place fairly frequently and apparently more readily than in nature. Much of the work on laboratory strains of filamentous fungi and yeasts has been reviewed by Ashida (1965) and this section will deal with other groups and subsequent papers on fungi. Both Ashida (1965) and Turner (1969) contain tables showing the tolerances recorded for fungi and a few other micro-organisms.

Relatively few studies have attempted to select for resistance to metal ions in bacteria, although this should be possible since such races often occur naturally (see Sections IIA4, VA2, and VIIIB).

Several recent papers (or papers not mentioned by Ashida, 1965) have been concerned with selection for metal tolerance in fungi. D'Yakov (1963a, b) showed slow adaptation to copper in *Phytophthora infestans* by successive transfer between tuber discs sprayed with copper arsenate solution. The resulting strains were (after seventeen transfers) three times more resistant than the controls, but the spores germinated poorly on transfer to normal medium and the tolerance was lost after two such transfers.

Antoine (1965) paralleled the earlier studies of Ashida and co-workers, and showed the existence of copper-tolerant races of yeast.

The work on natural selection for metal tolerance on mine tips has recently been paralleled by attempts to select artificially for tolerance to toxic soils. Seed of *Agrostis tenuis* from normal populations can be screened to produce in one generation genotypes tolerant to copper and to zinc, whereas other species of grass not normally found on contaminated soils do not produce tolerant races when screened in the same way (see Section VE for full references). However, varietal differences in the tolerance of soybeans to excess quantities of zinc (Earley, 1943) suggest that gradual selection for tolerance may be successful in species not normally colonizing mine soils.

B. THE OCCURRENCE OF TOLERANCE IN DIFFERENT FAMILIES AND SPECIES

The evolution of tolerant races appears to be such a common phenomenon that it is pertinent first of all to ask if there is any evidence that some species are inherently tolerant to metals (even when not growing on metal-contaminated soil) and are thereby able to colonize contaminated areas. The only work which suggests that inherent (or constitutional) tolerance may be important in colonizing metal mines is that of Repp (1963). She showed that the cellular resistance of *Silene vulgaris* (= *cucubalus, inflata*) from normal soil was just as high as the resistance of *S. vulgaris* from mine soils. This finding is however in contrast to the whole plant investigations of Bröker (1963) and Schwanitz and Hahn (1954a), and was quite clearly contradicted by the work of Gries (1966) where normal *Silene cucubalus* was shown to be non-resistant by a similar technique. So far, therefore, there is no evidence that a species has constitutional tolerance to heavy metals: evolution has always occurred when mine habitats are colonized.

Because of the work of particular people, it is easy to get the impression that tolerance is restricted to certain families and genera, such as the *Caryophyllaceae*, or the genera *Agrostis* and *Festuca*. But Table V shows that the more work that is done the less this seems to hold.

Good examples of tolerance newly found in separate families are *Mimulus guttatus*, *Thlaspi alpestre* and *Armeria maritima*.

If we assume, as seems reasonable, that all species occurring on high concentrations of metals have evolved tolerant populations, then there is much evidence, particularly that of Wild (1968, 1970), which shows that tolerance is very widely distributed indeed, and is to be found in families as widely separated as Cyperaceae, Compositae, Orchidaceae and Ranunculaceae. The greatest number of tolerant species is found in the Leguminosae and the Gramineae. But these of course happen also to be among the largest families in the world's angiosperm flora with correspondingly greater possibilities of evolution of tolerant species.

The special characteristics of species that are able to evolve tolerance to more than a single metal appears from the restricted evidence in Table V to be equally widely distributed, occurring in species as unrelated as *Silene vulgaris* and *Agrostis tenuis*. The evidence of species occurrence on toxic soil supports this. Many of the species in Rhodesia that occur on copper soils occur also on nickel (Wild, 1970).

However, it is interesting that while some species are able to evolve tolerance, others seem unable to do so even though they are abundant in the vicinity of metal-contaminated soils. Why this should be so is not clear. Species (not normally associated with metal-contaminated soils) have been shown to differ in their tolerance to metals (e.g. Earley, 1943; Biebl, 1947a, b, 1950) or metal uptake (e.g. Gorsline *et al.*, 1964), but no one has critically compared these aspects of plants that can and those that cannot evolve tolerance. However, Khan (1969) has preliminary evidence from a comparison of five species which are known to evolve tolerance and five species which seem not to, that the former possess genes for tolerance in normal populations while the latter do not. Another possibility is that a certain characteristic may pre-adapt certain species to colonize toxic areas: mine habitats require adaptations other than metal tolerance (see Section IIc2) and the possession of these characters (e.g. tolerance to low nutrients) would assist the development of tolerant races. Otherwise, the requirement of multiple adaptation to new features of the environment is considerably more difficult. Simultaneous selection for tolerance to two metals is considerably less successful than selection for one (Khan, 1969; see also Section VE). A paradigm for the idea that certain characters may pre-adapt populations to metals comes from the work of Horn and Wilkie (1966) who show that a respiratory mutant of yeast is advantageous to a cobalt medium.

C. THE "NEED" FOR METALS BY TOLERANT PLANTS

Several workers (e.g. Schatz, 1955) have suggested that tolerant plants may have a positive need for metals and for this reason are restricted to contaminated areas. Work on tolerant races shows that this is not the case: tolerant plants grow well in normal soil. However, there is some evidence that tolerant plants are stimulated in their growth by levels of metal considerably above the normal micro-nutrient levels, and more so than non-tolerant plants.

Baumeister (1954) and Baumeister and Burghardt (1956) grew *Silene vulgaris* at different levels of zinc in soil, sand-culture, and water-culture. At intermediate levels of zinc the tolerant type had a greater fresh weight, rate of photosynthesis, and chlorophyll content than at low levels of zinc. The effect was not so marked in the non-tolerant type.

Lefebvre (1969) showed that plants of *Armeria* from zinc-contaminated soil were much stimulated in their growth on normal soil by the addition of zinc salts.

Further evidence comes from authors who have found indices of tolerance greater than 100% (more root growth in metal than without metal). These are listed in Table VI.

TABLE VI

Species showing an index of tolerance indicative of root growth being stimulated by presence of metal ions in the testing solution

Species	Metal	Author
Agrostis tenuis	Cu	McNeilly, 1968
	Pb	Jowett, 1964
	Pb, Cu, Zn	Barker, 1967
Mimulus guttatus	Cu	Allen and Sheppard, 1971
Anthoxanthum odoratum	Zn	Putwain, 1963; Antonovics, 1966; Gadgil, 1969
Holcus lanatus	Zn	Jenkins and Winfield, 1964
Armeria maritima	Zn	Lefebvre, 1969

Although Ernst (1965b) did not use control non-tolerant populations, five out of six species from zinc soils produced a greater percentage germination and rate of germination in 50 ppm zinc than in 1 ppm. *Epilobium angustifolium*, a species from a normal area, did not show this effect.

There is therefore considerable evidence that tolerant plants are stimulated in their growth by small amounts of metal. This could be interpreted as a definite need for metal in these plants, but a fairer interpretation is probably that, because of the efficiency of the tolerance mechanism in inactivating the metals, the external trace element requirement is rather higher than the norm.

D. THE COMPETITIVE PERFORMANCE OF TOLERANT PLANTS

The apparent restriction of metal-tolerant genotypes on contaminated areas, despite their normal growth on non-contaminated soil, has led to the speculation that these plants are largely restricted to mine soils because they are competitively inferior to normal plants. This idea is supported by Kruckeberg (1954), who showed that serpentine species did not survive when grown in competition with their non-serpentine counterparts.

Putwain (1963) and Antonovics (1966) have shown that zinc-tolerant *Anthoxanthum odoratum* is competitively inferior to non-tolerant when grown on non-contaminated soil. Putwain (see Jain and Bradshaw, 1966) estimates that the selection pressure against the tolerant type on normal soils is 0·3. Antonovics (1966) showed that yield of tolerant type was progressively reduced in competition, whereas that of non-tolerant increased: after nine months the selection pressure was at 0·35 against the tolerant type.

The situation in *Agrostis tenuis* is rather different. McNeilly (1965) not only failed to find any differences in fitness between copper-tolerant and ordinary when grown as spaced plants, but also found only just significant differences when these were grown in competition. From the latter experiment the selection pressure against the tolerant type is only 0·05. Earlier work of Bradshaw (see Jain and Bradshaw, 1966) suggested that selection against lead tolerance could be as high as 0·4, but it is possible that other characters besides metal tolerance were involved.

Further competition experiments between plants in sand culture (McNeilly, 1970) have shown that the outcome of competition between tolerant and non-tolerant plants depends on phosphate level: at high phosphate levels the non-tolerant plants are competitively superior, whereas the converse is true at low phosphate levels.

Lefebvre (1970a) studying the competition of tolerant and non-tolerant ecotypes of *Agrostis tenuis*, *Anthoxanthum odoratum* and *Plantago lanceolata*, each grown with a normal herbage grass, *Lolium perenne*, showed that the tolerant ecotypes were competitively inferior to the non-tolerant.

E. THE PROCESS OF EVOLUTION

The first discovery by Prat (1934) of plant populations on metal mines tolerant to heavy metals in the soil led to his postulating that metal tolerance had arisen by natural selection.

The existence of a character that was clearly adaptive to the environment in which it was found, and the discovery by Wilkins (1957) of a rooting technique of measuring metal tolerance, has been exploited by various workers to provide one of the best documented cases of evolution in action. The account which follows is a brief summary of this work mainly on *Agrostis tenuis*.

As has already been mentioned, waste-heaps from mining activities carry tolerant populations of *Agrostis tenuis*, with specific tolerances corresponding to the type of metal in the soil (Gregory and Bradshaw, 1965). When these populations are sampled as seed it is found that they normally have a lower mean tolerance and greater variance than populations sampled as adults (Antonovics, 1966; McNeilly and Bradshaw, 1968; McNeilly, 1968). This is the most direct evidence that in natural conditions there is selection acting to preserve the high tolerance of the adult populations. Selection pressures acting to maintain the populations have been calculated (Antonovics, 1966) and are in the order of 0·3–0·7. Experimental studies have shown that most of the selection occurs at the seedling stage: if non-tolerant seed is sown on contaminated soil, most of the seed germinates (there is a slight inhibition) but the seedlings fail to root, and die before they can produce many leaves (McNeilly, 1968): this suggests that selection pressures can be up to 1·0.

Tolerant populations are normally surrounded by normal non-tolerant populations on ordinary pasture. Gene flow between the two populations is possible since the areas of contamination are usually small or there is a sharp boundary between the two populations at the edge of the mine (McNeilly, 1968). Even on large diffuse mines the background rain of pollen can reduce the level of tolerance of the seed on the mine (McNeilly and Bradshaw, 1968). The existence of gene exchange has been demonstrated by a comparison of seed populations collected in the wild and seed populations produced by adults brought back to the experimental greenhouse and grown away from non-tolerant material. The seed from natural populations has normally a lower tolerance than seed grown in isolation, showing that there is dilution of tolerance by the pollen from the surrounding non-tolerant population (McNeilly, 1968).

However, investigations of tolerance across mine boundaries show that tolerance changes abruptly at the boundary over a distance of a few feet (Jain and Bradshaw, 1966; McNeilly, 1968; Antonovics and Bradshaw, 1970). It seems puzzling that firstly, population differentiation could occur over such short distances, and secondly that there are very few tolerant individuals in adjacent pastures. The former is explicable on the basis that the selection pressures involved are sufficiently strong to maintain the populations in the face of gene flow. This process was examined theoretically using a computer simulation by Jain and Bradshaw (1966), who showed such differentiation to be not only possible but very probable. The absence of tolerant individuals in pasture populations could be explained on the basis of their poorer competitive ability (see Section VD). But as has been shown, the fitness of mine plants on ordinary soil, while less than that of pasture plants, is still relatively high; in which case the selection pressures (even if they were cumulative in perennial grasses) against tolerance in pasture would be much smaller than selection for tolerance on the mine. From this it had to be argued that to fit the findings of the computer model, gene flow on the transects that had been examined must be quite low, or selection high.

The situation was illuminated considerably by the discovery of a copper mine in a U-shaped valley with the prevailing winds in one direction, where a study was made of transects across the mine boundaries in a cross-wind and down-wind direction (McNeilly, 1968). The cross-wind transect showed a sharp cline at the mine boundary whereas in the down-wind transect the tolerance decreased gradually over a long distance (100 m) into the pasture. Moreover, whereas on the mine the seed had a lower tolerance than the parents, in the pasture downwind from the mine the seed had a greater tolerance than the parents: clearly there is selection against tolerance in the pasture. This is excellent evidence for the occurrence of natural selection and shows clearly the interaction between selection and different amounts of gene flow in determining the pattern of differentiation.

Antonovics and Bradshaw (1970) studied the way in which several characters in addition to tolerance changed across an abrupt zinc/lead mine and pasture boundary. Different clinal patterns were observed for the various characters even though the characters were all measured on the same set of plants. This was interpreted to be a consequence of different selection pressures for the different characters interacting with gene flow which must have been identical for all characters.

In addition to showing differences between the populations in a wide range of characters, this work showed that even within mine and within pasture populations there were differences between the various sites. Mine and pasture habitats were themselves heterogeneous and this led to further differentiation within the populations. There was therefore evidence of disruptive selection maintaining differences within and between the mine and pasture populations.

All this work has shown that there can be differentiation in the face of gene flow, yet that gene flow is not without effect and interacts with selection to produce various clinal patterns. It is also clear that this gene flow must be, having a deleterious effect in that it is hindering adaptation to the two environments diluting tolerance on the mine and leading to the production of ill-adapted tolerant seed in the pasture. The magnitude of such a gene-flow load was examined in a computer model by Antonovics (1968b), who showed that it was greater if the selection pressure and the gene flow were greater. In addition, the gene flow load was greater the lower the frequency of the favoured gene; it also depended on the degree of dominance of the favoured gene, being greater if the favoured gene was recessive.

It might be expected that mine and pasture populations had developed mechanisms to reduce the harmful effects of gene flow and a study was therefore made of possible breeding barriers (Antonovics, 1968a; McNeilly and Antonovics, 1968). There was no clear-cut incompatibility between tolerant and non-tolerant populations: crosses between populations were just as successful as crosses within populations. Differences in flowering time were observed between tolerant and non-tolerant populations in the direction of earlier flowering in mine populations (giving approximately 25% isolation) and evidence was presented that sometimes this is an adaptation to gene flow. The self-fertility of mine populations was shown to be considerably greater than the self-fertility of pasture populations. This change in the breeding system could not be clearly attributed to any one factor but a brief survey suggested that this too might be an adaptation to prevent the dilution of tolerance by flow of non-tolerant genes from neighbouring populations. The process of evolution in areas subjected to gene flow was studied by a parallel investigation using computer simulation (Antonovics, 1968b), where it was confirmed that the changes outlined above were quite possible and other changes, such as evolution of dominance in the face of gene flow, were predicted.

The evolution of metal tolerance therefore also sheds light on the mechanism of speciation. Geographical isolation is clearly not a prerequisite for divergence: but lack of isolation can encourage the production of breeding barriers (see Smith, 1966, for theoretical discussion).

Further indirect evidence for changes in characters that may effect changes in the breeding system (or isolation from non-tolerant populations) comes from the work of Malyuga (1964). The reports are that *Papaver macrostomium* has notched petals when growing over zinc-contaminated areas, that *Papaver commutatum* has black bands on the petals when they are growing over copper and molybdenum areas, and that *Pulsatilla patens* lacks petals on nickel silicates in the Kimpersaikii ore region. Malyuga (1950, 1964) suggests that these are direct environmental effects; but the effects are quite specific and unlike the generalized effects of metal toxicity. This suggests that they are, in fact, the result of evolutionary changes of some kind.

The changes in petal shape and markings may be isolating mechanisms (cf. Levin and Kerster, 1967) and the loss of petals suggests a change to inbreeding. It is interesting in this connection that Duvigneaud (1958) reports that *Protea goetzeana* has a creeping sterile form on toxic soils.

Differences between the corolla markings and colours in different *Gladiolus* species of the *Gladiolus robiliartianus* group have been recorded by Duvigneaud and Denaeyer-de Smet (1963). Each *Gladiolus* species appears on a single or group of adjacent copper hills (outcrops), and is characterized by its own corolla type. They differ in other respects such as the position of the inflorescence and leaf length. Such markings may clearly keep the populations isolated from each other and from related non-tolerant types, but their evolutionary origin is difficult to deduce.

A change towards inbreeding has been reported in calamine populations of *Armeria maritima*: they are much more self-fertile than their maritime counterparts (Lefebvre, 1970b). The tolerant *Armeria* populations are not immediately surrounded by non-tolerant forms and therefore self-fertility cannot be an adaptation to reduce the harmful effects of gene flow. It appears that the self-fertility originated and is maintained as a result of the difficulties of establishment of self-sterile *Armeria* on new mines and on the continually changing terrain of calamine areas. This is another example of the far-reaching evolutionary changes in metal-tolerant plants.

The evolution of metal tolerance is of additional interest since it appears to have been extremely rapid. Most mine tips are of relatively recent origin, between 50 and 100 years old (Jones, 1922), yet often carry well-established populations. However, it could be argued that in mining areas there exist long-established pockets of tolerance with these perhaps originally deriving from plants growing in metal outcrops: this is well illustrated by Duvigneaud and Denaeyer-de Smet (1963) and Nicolls *et al.* (1965).

However, tolerant populations exist on contaminated slag heaps which are the result of smelting ore: in the Swansea area of South Wales these are more than fifty miles from the nearest mine (Gregory and Bradshaw, 1965). More convincingly Snaydon (quoted in Bradshaw *et al.*, 1965) showed that plants of *Festuca ovina* and *Agrostis canina* growing under galvanized zinc fences several hundred miles from a mining area were tolerant even though the fences were only erected in 1936.

A new dimension was opened on this problem by the work of Abbot and Misir (quoted in Antonovics, 1966), who showed that it is possible to select for tolerance in *Agrostis tenuis* in one generation. Commercial seed was sown on copper-mine soil alleviated with a small amount of loam and this resulted in the survival of a few plants (1 in 7000).

These few plants, which grew well on the contaminated soil, were planted to normal soil and tested for tolerance. They were found to have a significantly greater tolerance than the original population (Antonovics, 1966). Moreover, the size of the plants on copper soil was significantly related to their tolerance. These studies showed that the evolution of tolerance can take place within one generation by the simple process of screening non-tolerant populations. Walley (1967) confirmed these results; using this technique to pick out tolerant individuals, he showed that seed collected from pasture populations one and a half miles down-wind from a large copper mine produced a significantly greater proportion of tolerant individuals than seed collected from pastures not in the vicinity of the mine. This work illustrated very clearly the long-distance transport of genes by pollen flow.

Khan (1969) not only confirmed these results of Walley but showed that the number of tolerant individuals found in pastures up-wind of the mine was much lower than down-wind. He also made a general study of the problem of evolution of tolerance *de novo* (Bradshaw *et al.*, 1969). He showed that tolerant genotypes occurred in normal populations with a frequency of about 2% for Zn soil and a similar frequency for Cu soil, yet that selection for simultaneous tolerance to two metals was far less successful, suggesting that the occurrence of the tolerances is independent. He was able to demonstrate that the survivors possessed the appropriate tolerances and that the most successful survivors had tolerances approaching that of established tolerant populations. When he selected for zinc tolerance in populations already tolerant to copper, and vice versa, he found the frequency of occurrence was the same as in normal populations lacking tolerance to either metal. Selection for zinc tolerance in a population already tolerant to copper gave survivors that were tolerant to both copper and zinc.

The results on artificial selection for tolerance, and the fact that the species are perennial, might suggest that the process of evolution of tolerance in grasses on metal mines is not a continuing process: tolerant individuals are screened from normal populations in one generation and being perennial persist in subsequent years. However, Antonovics (1966, and subsequent unpublished data) studied by mapping techniques the longevity of *Anthoxanthum odoratum* on a lead/zinc mine, and demonstrated a high rate of population turnover. Only a minute fraction of individuals (3·0%) survived for more than four years. Clearly the evolution of tolerance, and of the other differences between tolerant and pasture populations (see Section VF) is a continuous dynamic process, the characteristics of the mine plants being continually selected for in the face of the diluting effect of gene flow from pasture populations.

The study of the evolution of metal tolerance has added force to the idea that disruptive selection plays an important part in differentiating and possibly eventually completely isolating natural plant populations. Much of the current interest in metal tolerance stems from the light its study can throw on evolution in nature as opposed to the laboratory.

F. EVOLUTION IN ASSOCIATED CHARACTERS

Metal-tolerant plants may differ from normal plants in features other than tolerance. Schwanitz and Hahn (1954a, b) grew tolerant and non-tolerant populations of a range of species under standard conditions and showed that, in general, tolerant plants had smaller flowers, smaller leaves and thinner stems. These differences were reflected in the size of the epidermal cells of the leaves, but using other non-tolerant variants they showed that small cell size did not go hand in hand with tolerance. They concluded that there must be independent selection for morphology as well as tolerance. This conclusion was confirmed in *Silene vulgaris* by Bröker (1963): in F2 progeny of tolerant × non-tolerant crosses, tolerance was not associated with morphology. The first mine population of *Agrostis tenuis* to be examined morphologically (Bradshaw, 1959) showed startling morphological differences from the adjacent pasture population. However, subsequent investigations by Jowett (1964) showed that the mean differences of mine populations from pasture populations was not great.

The data suggest a complex adaptation of each population to local ecological factors independent of tolerance rather than an effect of tolerance on overall size and yield. Only slight differences in morphology between copper-tolerant and non-tolerant plants were detected by McNeilly (1965).

The situation with regard to morphological characters was re-investigated using *Anthoxanthum* by Antonovics and Bradshaw (1970). It was shown that tolerant and non-tolerant individuals from adjacent populations differed in a wide range of characters. Tolerant plants were generally shorter, had a smaller number of flowering tillers, shorter flag leaves, and a lower ratio of flowering/vegetative tillers. The characters, all measured on the same set of plants, formed different clinal patterns across the mine boundary. Gene flow must have been the same for all characters and the results suggested that there are different selection pressures acting on the morphological characters. Within the mine there was no correlation between tolerance and any of these morphological characters, again confirming that selection for them is independent of selection for tolerance.

Observations on other species brought back and grown under uniform conditions (*Rumex acetosa* and *Plantago lanceolata*, unpublished data) confirm that tolerant plants are generally dwarf or prostrate compared with their pasture counterparts.

The differences between tolerant and non-tolerant plants are not confined to morphology. Physiological differences have also been found. Jowett (1959b) and Bradshaw *et al.* (1960) showed that lead-tolerant *Agrostis tenuis* was more tolerant of low calcium and low phosphate, and McNeilly (1965) confirmed the differential phosphate response in copper-tolerant and non-tolerant ecotypes. These differences could be related to the characteristics of the mine soils: they are generally low in phosphate and those studied by Jowett (1959b) were acidic mines deficient in calcium. Barker (1967) showed that *Agrostis tenuis* from a calcareous lead/zinc mine showed good calcium response. Differences between copper-tolerant and non-tolerant plants in response to varying sulphur levels have been shown by Turner (see Antonovics *et al.*, 1967). Non-tolerant plants performed relatively better at low sulphur levels than did copper-tolerant plants. This could again be related to the normally high sulphur content of the mine soil from which the plants were taken.

It is therefore seen that the evolution of tolerance to metals is but one facet to the evolution of plants on abandoned mine workings. There is also natural selection for morphological and physiological characters which adapt the plants to the harsher mine environment. The existence of such changes in associated characters is important in several ways. Firstly, it would be interesting to investigate some of the processes (e.g. gene flow, selection) found in mine populations using these characters as well as tolerance: the course of evolution should be similar. Secondly, morphological differences between tolerant and non-tolerant plants have been the basis of taxonomic distinctions between the two types (Schubert 1954a; Duvigneaud and Denaeyer-de Smet, 1963; Lambinon and Auquier, 1964). It must be noted, however, that in all these studies no attempt was made to grow the plants under standard conditions: the differences recorded could have been purely environmental. Nor was any attempt made to see if there was an absolute correlation between metal tolerance and morphology. Those studies showing differences between plants grown under standard conditions suggest that the taxa that have been recognized by the above workers may either have the status of different ecotypes or imperfectly differentiated local populations.

VI. The Genetics of Heavy Metal Tolerance

There has been very little work done on the genetics of heavy metal tolerance. Wilkins (1960) working on lead tolerance in *Festuca ovina* states that "in spite of the amount of effort devoted to refining the measurement of tolerance, the nature of the genetic mechanism controlling it has not been established with certainty". He nevertheless found that tolerance (whether high or medium range) was dominant and that a major gene with just two alleles was an inadequate model to explain the results. Whether these several alleles were at one locus or at more than one was not established.

Bröker (1963) again found dominant inheritance of zinc tolerance in *Silene vulgaris*. However, the F2 data (from selfing F1's) allowed one to conclude very little since only a few plants per family were tested: it was, therefore, again not decided whether segregation was continuous or discontinuous. These results were confirmed by Gries (1966) using cytoplasmic resistance in the same material as a measure of tolerance.

That many genes are involved in the determination of tolerance is supported by Jowett (1959a), McNeilly (1965), and Antonovics (1966), who showed tolerance to be *not* an all or nothing effect but a continuous variable in natural populations.

Walley (1967) and Khan (1969), discussed in Bradshaw *et al.* (1969), carried out experiments in which they screened normal populations for the occurrence of tolerant individuals. They both found a low frequency of survivors when seed of normal non-tolerant populations was sown on toxic soil (see Section VE). When normal populations were screened for tolerant individuals on soils in which the toxicity was ameliorated to different degrees by the addition of normal soil, the number of survivors varied markedly, increasing with decreased toxicity. At any one level of toxicity survivors and non-survivors could be readily distinguished. This strongly suggests a threshold character determined by several genes.

Antonovics (1966) obtained some evidence that the genetics of copper tolerance in *Agrostis tenuis* might differ on different mines, behaving as a dominant character in crosses from one mine, but as non-dominant in crosses from another mine. However, the number of crosses was too low to allow a firm conclusion on this.

More specific studies by Jowett (1959a) on the genetics of tolerance showed indications that the dominance of lead tolerance in *Agrostis* was variable, but that in general it was partly recessive. Jowett suggested that this could be an artefact of pre-culture conditions which were different in parents and progeny. Nevertheless, his data provide evidence for continuous variation in the character and also considerable segregation, suggesting quite marked heterozygosity of the parents.

Investigations of the genetics of copper tolerance of *Mimulus guttatus* have recently been initiated by Allen and Sheppard (1971). Copper tolerance is readily demonstrable in populations from copper-contaminated situations: populations from normal soils were non-tolerant, except in one case. There was some indication that copper-tolerant populations were also tolerant to zinc, lead and nickel, although these metals were not present in the soils. Tests on F1 progeny of crosses between tolerant and non-tolerant parents showed that there was no maternal effect and that copper tolerance was dominant at low copper concentrations, intermediate at intermediate concentrations, and recessive at high copper concentrations. This can be explained by the assumption of a threshold effect dependent on copper concentration, which would agree with the general conclusions about the expression of tolerance. There was some indication of a copper requirement by tolerant populations at low copper concentration. It was not possible to conclude whether one or several genes were involved.

Similar general evidence that the metal tolerance is inherited comes from the work of Lefebvre (1970a), whose data suggest that the character of zinc tolerance in *Armeria maritima* tends to be dominant.

McNeilly and Bradshaw (1968) compared the copper tolerance of seed and adults of different populations, and found a high correlation ($r = 0{\cdot}983$) between the two. This suggests that the character of tolerance has a high heritability. Further studies of seed obtained from a polycross of tolerant material (McNeilly and Bradshaw, 1968) gave a heritability of 0·7.

Recently Urquhart (1971) has carried out a diallel analysis of lead tolerance in *Festuca ovina*. This has shown that there is additive genetic variation and little maternal effect and that the character must be determined by several genes. Tolerance usually showed complete dominance, but this was not always the case, for in some crosses no dominance was found. This confirms the results of Jowett (1959a), who found that dominance varied between crosses but was inclined at that time to dismiss it as being due to error.

Further evidence of the genetic control of metal tolerance comes from a study of the phenomenon in yeasts. Seno (1962) found that when yeast strains were grown in high copper concentrations they produced two levels of resistance. Both levels were controlled by dominant genes which were very closely linked. Similar results have been obtained by Antoine (1965): here four alleles at one locus were considered to be responsible for copper resistance in yeast. Again the alleles for copper resistance were dominant to the non-resistance allele. It is interesting that evidence was also presented for a remarkable effectiveness of copper ions in mutating the gene for copper resistance to alleles of higher resistance: in this instance mutation appears to be directed. General mutagenic activity of copper ions has been demonstrated by several workers (e.g. Von Rosen, 1964; Bhatia and Narayanan, 1965) and may therefore be important in the evolution of metal tolerance itself. However, there is no evidence for this in higher plants.

So far most of the studies on the genetics of metal tolerance have been cursory and rather inconclusive. Antonovics (1968b) in a computer study of the evolution of metal tolerance on a small mine predicted that the plants would be highly heterozygous and that the evolution of dominance may occur readily. This would explain some of the inconsistencies in the results of Jowett (1959a), Wilkins (1960) and Antonovics (1966), as well as the consistently large amounts of segregation observed in seed from tolerant plants (McNeilly, 1968).

Clearly a full reinvestigation of the genetics of metal tolerance is needed to complement the study of the evolution of tolerance, particularly in grasses on metal mines. This reinvestigation should bear in mind that the level of gene flow into these populations will affect the level of heterozygosity of the plants: it may also promote evolution of dominance (Antonovics, 1968b). Such a study should aim to answer the following questions. What is the dominance of the character? Is it determined by one or many genes? Is it determined differently on different mines? What is the relationship between tolerances to different metals? What is the degree of heterozygosity of the genes for tolerance in the populations?

The genetics of metal tolerance would not only help to complete the evolutionary story, but it could have considerable bearing on the mechanism of tolerance, particularly since such tolerance appears to be highly specific.

VII. MECHANISMS OF METAL TOLERANCE

The mechanisms whereby the living organism can grow on apparently contaminated soil may prevent the heavy metals from reaching their sites of toxic action within the plant or they may simply be external factors that prevent the metals from entering the organism. Table VII shows more fully the possible mechanisms whereby heavy metal tolerance may be achieved. These have been adopted and augmented from the proposals of Davis (1958), Ashida (1965) and Ehrlich (1965).

A. EXTERNAL "TOLERANCE" MECHANISMS

The external mechanism of tolerance represents those circumstances which prevent entry of the metal ions. These are not "mechanisms" in the strict sense as they are not under the control of the tolerant organism, but are of considerable ecological importance (see Section IIc).

Firstly, the form of the metal may not be readily soluble in water or if dissolved is rapidly diluted by surrounding water; in both instances

TABLE VII

Possible mechanisms of metal tolerance

A. *External*

(i) Form of metal is not directly soluble in water and/or if dissolved then rapidly diluted by surrounding water.
(ii) Actual amount of freely diffusable metal ions is small compared to total amount present.
(iii) Lack of permeability to heavy metals under specific conditions.
(iv) Metal ion antagonisms.

B. *Internal*

(v) Differential uptake of ions.
(vi) Removal of metal ions from metabolism by deposition in vacuole.
(vii) Removal of metal ions from metabolism by pumping from cell.
(viii) Removal of metal ions from metabolism by rendering into an innocuous form.
(ix) Excretory mechanisms—removal of "metal storage organ".
(x) Greater requirement of enzyme systems for metal ions.
(xi) Alternative metabolic pathway by-passing inhibited site.
(xii) Increased concentration of metabolite that antagonizes inhibitor.
(xiii) Increased concentration of enzyme that is inhibited.
(xiv) Decreased requirement for products of inhibited system.
(xv) Formation of altered enzyme with decreased affinity for inhibitor or increased relative affinity for substrate compared to the competitive inhibitor.
(xvi) Decreased permeability of cell or subcellular units to metal ions.
(xvii) Alteration in protoplasm so that enzymes may function even when toxic metals replace physiological metals.

the effective concentration is low. Ehrlich (1963a), for example, demonstrated that manganese can be released from root nodules by microbial action but that the effective manganese concentration is small due to high water content. It is noticeable that vegetation growth increases considerably in wet places on toxic soils.

The majority of naturally occurring metallic ores are usually found as mixtures of the sulphides which are virtually insoluble: thus the amounts of metal available to plant life are very small.

Temple and Le Roux (1964) showed that colloidal material containing large amounts of metals could be precipitated as the sulphides by sulphate-reducing bacteria. A practical example of this mechanism being used under field conditions has been reported by Lawrence and McCarty (1965). The organisms responsible for digestion of sewage sludge are extremely sensitive to heavy metals (zinc, copper and nickel). Heavy metal toxicity (concentrations may reach 800 ppm) may be prevented by the addition of sulphate ions under anaerobic conditions so that the metals are precipitated as sulphides. Dykeman and De Sousa (1966) showed that in a peat swamp over bedrock material rich in copper, deeply circulating waters dissolve the copper and it percolates up to the surface. The peaty humus at the surface acts as an efficient natural chelating system and toxic quantities of the metal are absorbed and thus immobilized. It is interesting to speculate on the system that originally produced the organic compounds which at present act as chelators: the situation might originally need a tolerant organism to produce organic waste products, thus producing a convenient habitat for succeeding non-tolerant species. Russell (1955) showed that this type of situation can occur in micro-organisms. *Penicillium roqueforti* is apparently tolerant to the phenyl mercuric acetate fungicides used in paper production. *P. roqueforti* absorbs mercury into its mycelium, reducing the amounts of toxic mercury remaining in the medium. This allows growth of subsequent wood-rotting fungi.

A second external mechanism of heavy metal tolerance may be the lack of permeability to heavy metals under specific conditions. Alterations in the environment can produce changes in the permeability properties of the cells or in their uptake characteristics in relation to metals. Hofsten (1962) found *Escherichia coli* to be copper tolerant only under aerobic conditions and Hassall (1962) found *Chlorella vulgaris* tolerant to barium, manganese, nickel, lead and copper under anaerobic conditions. Later work (McBrian and Hassall, 1965) showed that cells under anaerobic conditions absorbed less copper than under aerobic conditions, indicating a difference in uptake potential under varying conditions of aeration. Murayama (1961) found that copper-tolerant yeasts that were respiration deficient, because they lacked part of the normal cytochrome component, were inhibited more strongly by copper under anaerobic conditions. Investigations on a wide range of wood-rotting fungi have demonstrated that copper-tolerant organisms increase their tolerance under conditions of low pH (Young, 1961). A similar situation has been recorded with *Thiobacillus thio-oxidans* and *Azotobacter indicum* (Starkey and Waksman, 1943). Starkey (1964), considering his earlier work, suggested that the ability to develop tolerance under low pH depends on the impermeability of the cells to copper. Lowering the pH to very acid levels would produce, in theory, a net positive charge at the cell surface which would reduce uptake of the metal cations.

Vernon *et al.* (1960), studying a ferrous ion oxidizing bacterium *Ferrobacillus ferro-oxidans*, showed that it had an unusual cytochrome composition that differed from the species that did not oxidize iron. Iron oxidation via the cytochrome oxidase system occurred at the cell surface and the metal entered the cell in large amounts during the microbial breakdown of iron sulphide ores. Ehrlich (1962) suggested that the ability of the same organism to oxidize copper ores was dependent on a similar system. Metal oxidation occurred at the cell surface and the oxidation products were removed by hydrolysis or precipitation. Metal sulphides could thus be regarded simply as external energy sources.

A final external mechanism may be that the effect of toxic ions is reduced by the presence of other ions. Additional ions may reduce the net concentration of toxic ions (i.e. reduce availability simply by competitive effects in solution or by complexing with the toxic ions) or they may interfere in uptake mechanisms by competing for entry sites. Gregory (1965) showed that non-tolerant plants of *Agrostis tenuis* could grow on normally toxic mine soil if a full nutrient culture was provided daily, and toxicity could be markedly reduced if only potassium nitrate was added. Sudzuki-Hills (1963) commented that copper toxicity of mine water was reduced by the presence of other ions. Calcium ameliorates lead toxicity in *Festuca ovina* (Wilkins, 1957) and *Agrostis tenuis* (Jowett, 1964) and also zinc and nickel toxicity in *Agrostis tenuis* (Barker, 1967). Chester (1965) concluded that calcium exerted a protective action against copper poisoning in yeasts. Wilkins (1957) showed the effects to be due to calcium ions independent of the anions supplied. Schmidt *et al.* (1965) found that calcium reduced zinc uptake in barley, and Somers (1963) reported that copper uptake in fungal spores was reduced by calcium. From evidence such as this and related work (e.g. Jefferies *et al.*, 1969) it may be concluded that calcium is important in cell permeability and may exert its influence on metal tolerance in this manner. Calcium may also affect the expression and the type of metal tolerance. Gregory (1965), using calcium nitrate added to the testing solution, found a zinc-tolerant clone of *Agrostis tenuis* to be co-tolerant (defined by Turner and Gregory, 1967) towards nickel and cadmium. Using a modified testing procedure but with no calcium present, Barker (1967) could find no nickel or cadmium co-tolerance in identical plant material.

This action in reducing metal cation uptake is not restricted to calcium, and interference from other metals may occur. For example, copper interferes with zinc uptake in barley (Schmidt *et al.*, 1965). Hoekstra (1964) proposed that certain physical chemical properties that are similar between ions will explain these antagonisms. Vose and Randall (1962), showed that manganese and aluminium-resistant varieties of ryegrass (*Lolium perenne*) are characterized by having a low root cation exchange capacity when compared to susceptible varieties. The authors suggested that a low cation exchange capacity will permit entry of monovalent cations whilst rejecting divalent metal cations; tolerance to heavy metals would therefore be achieved.

B. UPTAKE STUDIES

Physiological investigations on plants growing on metal-contaminated soils have in the past been largely limited to measuring the levels of the various metals in the plants, and in the soil on which they grow. These studies throw some light on the tolerance mechanism since they indicate whether plants are able to tolerate metals by some exclusion mechanism, or whether the mechanism is internal. Almost invariably these studies deal with uptake into the shoot. Uptake by roots has rarely been studied in nature since it is difficult to remove contaminated soil particles from the root system.

1. Zinc

The occurrence of zinc in plants on contaminated soils has been established since the last century (Baumann, 1885; Jensch, 1894). In all these studies, the exact quantities of zinc found are probably not very reliable because of the dangers of contamination from chemicals, glassware and other apparatus. However, all these workers found extremely high values of zinc in plants on zinc soil (and low values on plants on normal soil). Since then the high level of zinc in plants on zinc soils has been consistently, if often briefly, confirmed (e.g. Linstow, 1929; Bertrand and Andreitcheva, 1933; and Dorn, 1937).

There have, however, been more extensive studies which have thrown considerable light on the characteristics of zinc uptake on contaminated soils. The following characteristics have been observed by Robinson *et al.* (1947); Maquinay and Ramaut (1960); Maquinay *et al.* (1961); Lambinon (1964); Lambinon *et al.* (1964); Ernst (1965a); Nicolls *et al.* (1965), and Baumeister (1967).

1. Different species, even though they come from the same contaminated area, differ in the degree to which they take up zinc.

Average values for the more common members of calamine soils are as follows (Ernst, 1965b):

	Zinc content in ppm dry wt	No. of sites investigated
Thlaspi alpestre ssp. *calaminaria*	7757	7
Armeria maritima ssp. *halleri*	3328	5
Minuartia verna ssp. *hercynica*	3007	17
Silene cucubalus (= *vulgaris*) var. *humilis*	1719	27
Armeria maritima ssp. *calaminaria*	1895	2
Viola calaminaria	686	4

2. Different plant organs accumulate different quantities of zinc. Generally, roots and leaves take up most zinc, and stems and inflorescences least, but in some species the flower heads seem to accumulate more. The pattern of distribution depends both on the species and on the metal concentration (Ernst, 1965b; Nicolls *et al.*, 1965; Cole *et al.*, 1968; Howard-Williams, 1969).

3. The quantity of zinc in plants changes with the growing season and often shows an increase throughout the season (Ernst, 1965b; Howard-Williams, 1969).

4. The quantity of zinc in plants is related to the amount of zinc in the soil often in a clearly linear pattern (Nicolls *et al.*, 1965). Occasionally the plants seem to take up more zinc than is present either in "available" form (e.g. *Thlaspi alpestre*, Ernst, 1965a) or in total amount (e.g. various lichens, Maquinay *et al.*, 1961; Lambinon *et al.*, 1964).

5. The cellular resistance of plants to solutions of different zinc concentrations is related to the amount of zinc they absorb (Baumeister, 1967).

These findings are confirmed by studies of zinc uptake by tolerant plants in water culture. Baumeister (1967) found a fairly linear relation between the amount of zinc in *Thlaspi alpestre* and *Armeria maritima* and the amount in the culture solutions even though the absolute amounts in the two species differed enormously. Turner and Gregory (1967) found increasing uptake with increasing concentration of zinc by *Agrostis tenuis* (tolerant and non-tolerant) in water culture. The concentrations (ppm dry weight) in the plants were in excess of the concentration (ppm in solution) in the solutions after only seven days at all concentrations. The roots showed far greater accumulation than the shoots, particularly at higher concentrations. This suggests that the values found by workers for shoots of plants growing in the field may underestimate the overall uptake of zinc by plants. Ernst (1968c) looked briefly at the zinc content of roots as well as shoots of plants in the field. *Thlaspi alpestre* showed greater accumulation in shoots than roots, whereas *Minuartia verna* showed similar root and shoot contents. There therefore appears to be variation between species. However he also showed (Ernst, 1968d) that under experimental conditions uptake was affected by phosphate: at high phosphate concentrations zinc was much higher in roots than in shoots. Zinc uptake clearly requires further study.

Zinc therefore is readily taken in by plants growing on zinc-contaminated soil, and nowhere in the literature is there any evidence of these plants having an exclusion mechanism to enable them to survive on contaminated soils. The tolerance mechanism of zinc must be internal.

2. *Copper*

Studies on copper uptake have been fewer and less extensive than those on zinc uptake.

The earliest reference is probably that of Bateman and Wells (1917), who found appreciable quantities of copper in plants (both living and dead) on copper-contaminated soils. Figures of 2000–6000 ppm were obtained for *Plantago, Agropyron* and *Dasiophora,* while lower values were found in *Medicago, Equisetum* and *Trifolium.* Dead vegetation on the whole contained more copper than living plants. Prat and Komarek (1934) found that plants of *Agrostis alba* (*stolonifera*) and *Melandrium sylvestre* growing on soils rich in copper (1–39% Cu) contained 0·2–3·25% copper in the ash. Persson (1956) again found that "copper mosses" do in fact take up this element in appreciable quantities.

The only detailed investigation has been on plants on metal outcrops in Australia (Nicolls *et al.*, 1965) and it has revealed a pattern common to several species. Copper uptake in the above ground parts stayed low and constant at low levels of soil copper, but at certain higher soil copper levels this "resistance" to uptake seemed to break down. Above this level the quantity in the plant tops increased abruptly and at only slightly higher levels in the soil no plants were found. Species differed both in the overall copper content and in the level at which the sudden increase in copper uptake was seen. These differing reactions to copper in the soil suggest that the copper did not just become available to the plant at a given total soil level, but that a genuine exclusion mechanism was in action at low copper levels. This is supported by the finding that values of copper even a little above the level at which there is greatly increasing uptake are lethal. Moreover, the species found in the most toxic areas, *Polycarpaea glabra,* takes up very little copper (a maximum of 20 ppm in the leaves) on soils containing 10 000 ppm. If the results quoted by Ernst (1965a) are re-examined in the light of this work of Nicolls *et al.* (1965), a remarkably similar pattern emerges, with both *Silene inflata* and *Minuartia verna* showing constant low uptake at low levels but a sudden increase to high uptake at high level. Although the results are based on far fewer samples than those of Nicolls *et al.*, the similarity is startling.

There is therefore evidence that the mechanism of copper uptake is different from the uptake of zinc. These results are supported by other less extensive studies. Vogt *et al.* (1943) and Vogt and Bugge (1943) analysed plants growing over an exposure of copper and showed that they did not differ in copper content from those growing off the ore. However, there were marked differences in zinc content.

Duvigneaud and Denaeyer-de Smet (1963), who fully review the literature on copper content of plants on normal soils, studied a wide range of species on different soils. They found that most species growing on soils containing often 1000 ppm copper, only showed slightly enhanced copper contents (two to three times that of plants on normal soil in the same area). Only three out of twenty-four species accumulated more than 1000 ppm dry weight. Ernst (1968b) found appreciable quantities of copper in *Minuartia verna*, but low values in four other species. Roots consistently contained greater amounts of copper than shoots: shoot values may therefore underestimate the copper content of plants. As with zinc, the relative distribution of copper between root and shoot would repay further study.

Lange and Ziegler (1963) again found variations between species of lichens in copper content, those on copper soils generally containing slightly more copper.

These results are further supported by investigations on copper and zinc in plants growing on normal soil. McHargue and Roy (1932) found that there was little variation in copper content of tree leaves over the growing season, but that zinc showed considerable variations. Holmes (1964) noted that whereas copper in plants rarely varies more than 5–15 ppm the zinc content can vary from 20 ppm to 10 200 ppm.

Despite the general consistency of patterns of uptake of copper, the recurring evidence that species may differ cannot be dismissed. Jacobsen (1967) provides perhaps the best evidence for the plants occurring on a single natural contaminated area in Rhodesia. Following the method of Thyssen (1942), he determines an "enrichment factor" which is effectively the copper in twigs as a percentage of that in the soil. This gives values of up to 5% for some species such as *Combretum zeyheri* and only 0·5% for other species such as *Brachystegia spiciformis*. The species of the first group tend to be capable of growing on high copper soils. In all species, however, the amount of metal in the plant again seems to remain remarkably constant over a wide range of soil copper values.

There has only been one study of copper uptake from culture solution, namely that of Bradshaw *et al.* (1965). Copper-tolerant and non-tolerant *Agrostis tenuis* showed a slight marginally significant increase in copper content of the shoots (leaves) with increasing copper concentration in solution. However, the roots showed a large highly significant increase.

The roots absorbed far more copper (maximum 3253 ppm dry weight) than the shoots (maximum 41 ppm dry weight). The level of copper concentrations used was low (0–1·5 ppm), showing a remarkable accumulation of copper by *A. tenuis*. These results emphasize the importance of studying both root and shoot in uptake studies.

In the absence of extensive and detailed studies of uptake from solutions, it is difficult to interpret studies relating soil copper content to plant copper content. The situation is further complicated since high copper contents may go hand in hand with low copper availability (see Sections IIc4 and VIIA). Nevertheless, some tentative conclusions can be drawn. Firstly, there are large species differences in pattern of copper uptake. Secondly, copper uptake occurs and tolerance in some species must be due to an internal mechanism. Thirdly, in other species an exclusion mechanism is probably also involved, but its precise nature needs re-examination. The mechanism may well not be a true exclusion mechanism, but a complexing mechanism in the plant roots which prevents copper from reaching the upper parts of the plant until the root system is swamped. Fourthly, the pattern of copper uptake seems very different from that of zinc uptake.

3. *Lead*

Studies on the uptake of lead have been very few. Jensch (1894) showed that whereas *Tussilago farfara* and *Polygonum aviculare* on contaminated soils contained zinc, no lead was detectable, even though the soil contained 0·72–1·06% of this element. These results are similar to those found by Nobbe, Bressler and Will (quoted in Schwickerath, 1931, but no reference given): if equal quantities of lead and zinc are given to a plant, then a smaller quantity of lead than zinc is taken up.

Nicolls *et al.* (1965) also dealt with lead uptake. The pattern here resembles that of copper rather than zinc, in that the uptake is constant with increasing levels of soil lead, till a certain point is reached when uptake becomes unrestricted, and rises abruptly. The species also are rarely present when the soil lead value is above the level at which there is a sudden increase in lead uptake.

The pattern of lead uptake by plants has, therefore, been rarely studied, and little can be concluded about the mechanism involved, except that it seems to be similar to copper.

4. *Nickel*

There has been little systematic work on nickel uptake. However, preliminary work by Wild (1970) shows that for many species the pattern of uptake appears to resemble that of copper: levels in the aerial parts of the plant remain low (around 100 ppm) irrespective of external concentration. However, this was not true for two species, *Vellozia equisetoides* and *Dicoma macrocephala*, where the uptake reached 1000 ppm. Such findings raise very interesting problems and suggest that mechanisms of tolerance to nickel may differ in different species.

C. INTERNAL MECHANISMS

From the previous sections it is clear that higher plants on contaminated soils frequently take up toxic levels of metal ions and that the tolerance mechanism must at least in part be internal. This is almost certainly true for other organisms in toxic areas or laboratory races selected for tolerance. As might be expected, the mechanisms whereby such tolerance is achieved are diverse. But only two groups of organisms, the yeasts (especially *Saccharomyces cerevisiae*) and higher plants (especially *Agrostis tenuis*), have been studied in any detail in this respect and they will be considered first.

1. Yeasts

The mechanisms whereby laboratory-selected strains of yeast achieve tolerance to a wide variety of metals has been reviewed by Ashida (1965) and more briefly by Turner (1969). The following section will therefore summarize the work rather than attempt an exhaustive review. Copper-tolerant strains of yeast *Saccharomyces cerevisiae* appear to differ from non-tolerant strains most obviously in their sulphur metabolism. Tolerant strains appear brown in colour when cultured with copper as a result of the production of a large amount of copper sulphide. Hydrogen sulphide appears to be released as a by-product of an overactive cysteine pathway; it combines with copper and the sulphide is deposited in and around the cell wall.

This however does not appear to be the only mechanism of copper resistance in these strains: strains resistant to very high copper concentrations produce less hydrogen sulphide, respiration-deficient strains produce no hydrogen sulphide and yet are still copper resistant, and genetic recombinants can be obtained which have high copper resistance but low hydrogen sulphide production. Other mechanisms may include the presence in the cell of copper-binding substances such as cysteine or thiol-based compounds. There is also evidence of a cuproporphyrin complex in tolerant strains. Metabolic differences include a greater dependence on aerobic respiration in the resistant strain (since the fermentation pathway is more sensitive to metal ions) and a greater dependence on NADP as opposed to NAD-linked reactions in the tricarboxylic acid cycle. There was evidence of changes in the RNA of copper-resistant strains of a related species, *S. ellipsoideus*.

Tolerance to other metals (Cd, Co, Ni, Ag) do not appear to depend on changes in sulphur metabolism. They do not show clear-cut cross-tolerances with each other or with copper, suggesting that different mechanisms are almost certainly involved in the different strains.

2. *Higher plants*

The mechanism of metal tolerance in higher plants from mine soils has been reviewed and studied with particular reference to zinc tolerance in *Agrostis tenuis* by Turner and Gregory (1967), Turner (1969, 1970) and Peterson (1969). Turner (1967) showed that sulphur deficiency appeared not to affect the tolerance of zinc- and copper-resistant strains differentially from non-tolerant strains, suggesting that changes in sulphur metabolism are not important in the tolerance mechanism in higher plants. Subsequent studies involving cell fractionation and differential centrifugation (Turner and Gregory, 1967; Turner, 1969, 1970; Turner and Marshall, 1971) showed that in tolerant strains zinc and copper are concentrated in the cell walls, and thereby prevented from entering more sensitive sites in cell metabolism. The level of zinc absorbed by the cell wall was correlated with zinc tolerance of different plants. Further attempts at localization showed that zinc was not released by proteases, suggesting localization in a non-protein, possibly carbohydrate, fraction although Woolhouse (1970) disagrees with this.

Alteration in the external level of one heavy metal did not influence the distribution of the other metal, suggesting the mechanisms are specific for each metal (cf. Gregory and Bradshaw (1965), who failed to find cross-tolerance for copper and zinc). These results were confirmed by Peterson (1969) using chemical fractionation of the cells of zinc-tolerant *Agrostis tenuis* and *A. stolonifera.* He found that the pectate fraction of cell walls of tolerant plants contained five to six times as much zinc as in non-tolerant plants. The distribution of zinc in copper-tolerant plants was similar to its distribution in non-tolerant plants, confirming that the tolerance mechanism for the two metals is different. However, *Agrostis stolonifera* differed from *A. tenuis* by absorbing an appreciable quantity of zinc on the soluble RNA fraction, suggesting certain species differences as regards zinc distribution.

Various points are still not clear. It is not clear to what extent the cell wall is important in metal tolerance in organisms other than *Agrostis* spp. Evidence supporting the general importance of the cell wall in metal ion inactivation comes from the demonstration that plants from normal soils often contain large quantities of metals in the cell walls, and from work on tolerant micro-organisms (see Turner (1969) for review and references). There is also evidence from less rigorous work on metal-tolerant plants.

Ernst (1968c) showed that the zinc content of expressed sap from *Thlaspi alpestre* and *Minuartia verna* increased only slightly with large increases in total zinc concentration in the plant. There was more zinc in the sap of *Thlaspi alpestre* than *Minuartia verna*, suggesting species differences. Subsequently he showed (Ernst, 1969a) that the zinc concentration of cell sap from various organs of *Silene vulgaris* increased during the growing period, suggesting increased saturation of a complexing system in the cell walls. However, he also noted that a large proportion of the zinc was water extractable and probably found in the vacuole (e.g. 74% in *Cardaminopsis halleri*), and suggested that the vacuole was an important site of metal ion storage. Throughout his experiments Ernst did not, however, use non-tolerant material for comparison.

Gambi (1967) showed histochemically that nickel was concentrated in the epidermis and sclerenchymatous areas (cells with no living contents) between vascular bundles in *Alyssum bertoloni*, a nickel accumulator found on serpentine soils.

A cell wall complexing system suggests a mechanism with a finite capacity for absorbing metal. It is interesting that an apparent threshold nature of tolerance to increasing metal levels is observed in artificial selection for tolerance (see Section VA3) suggesting a "mop-up" system capable of being saturated.

There is evidence that tolerant plants are metabolically different from normal plants at low metal levels but it is difficult to know whether such differences are related to the tolerance mechanism or the result of independent selection for physiological adaptation to other aspects of the mine habitats (see Section VF). And at high metal levels other metabolic differences become apparent between tolerant and non-tolerant *Agrostis tenuis* (Gregory, 1965; Turner, 1967) but again it is not clear whether these are the result of differential sensitivity to metal ions or whether they are related to the mechanism of tolerance. Repp (1963) and Gries (1966) have both reported increased general protoplasmic resistance of tolerant strains and this is evidence that systems other than those in the cell walls may contribute to tolerance to heavy metals. However, the technique for testing protoplasmic resistance does not critically eliminate the possibility that the cell wall may be important.

Since differences in enzyme properties of tolerant strains of microorganisms have been reported, it is clearly worth while looking for similar differences in higher plant enzymes. The effect of lead ion concentration on the activity of certain root cell wall enzymes of *Agrostis tenuis* has been investigated by Woolhouse (1970). The rate of hydrolysis of *p*-nitrophenyl-phosphate by root tips of normal non-tolerant material was affected by concentrations of lead as low as

1 mM, whereas that of lead tolerant material was not affected until the concentration reached 10 mM. It is argued from this that there must be different forms of the phosphatic enzymes concerned. While this is possible, an alternative explanation is that the enzymes are the same but that in the tolerant material the heavy metal complexing mechanism in the cell wall is preventing the metal reaching the cell wall enzymes in the same way that it appears to protect other sensitive sites e.g. on mitochondria. Further investigation will be very rewarding.

A remarkable feature of metal tolerance is its specificity. Tolerance to one metal does not generally confer tolerance to other metals (see Gregory and Bradshaw, 1965; Turner, 1969, for discussion and review). The only observed instances of observed cross-tolerances are between zinc and nickel in *Agrostis tenuis* (Gregory and Bradshaw, 1965), and copper and chromium in desmids and higher plants from normal soils and copper-tolerant mosses (Url, 1955, 1956). Although many explanations of such specificity can be postulated, its precise nature remains unexplained.

3. *Other organisms*

The mechanism of tolerance in both yeasts and higher plants involves in part at least removal of toxic ions from general metabolism. Analogous mechanisms are known in other organisms. Rabanus (1931) and Shimazono (1951) both suggested that the metal tolerance of brown-rot fungi was the result of the copper ions being combined to produce insoluble oxalates. However, the earlier work of Richards (1925) demonstrated that copper tolerance did not confer zinc tolerance, yet a non-specific metal-complexing mechanism such as oxalate production would be expected to do so. An elegant example of a metal-complexing mechanism responsible for heavy metal tolerance has been shown for *Aspergillus niger* (Ashworth and Amin, 1964). Mercury tolerance in *A. niger* is due to a pool of non-protein sulphydryl groups that protects enzyme systems by forming complexes with the mercury as it enters the thallus. *Rhizoctinia solani* and *Pythium ultimum* do not have this pool of non-protein sulphydryl groups and are thus susceptible to mercury poisoning. These non-protein sulphydryl groups can combat zinc, nickel, lead, copper and silver poisoning (Ashworth, 1965).

Tonomura, Maeda and Futai (1968), Tonomura, Maeda, Futai, Nakagami and Yamada (1968) and Tonomura, Nakagami, Futai and Maeda (1968) have shown that mercury-resistant bacteria bind the components probably at the cell wall and convert them to a more volatile, but as yet unidentified, form so that mercury is lost from the medium. Rapid growth resumes as the mercury concentration of the medium falls.

Differential uptake may also at times be important: Earley (1943) found that zinc-tolerant soybeans did not take up as much zinc as did susceptible varieties. However, it is possible for tolerant organisms to have a low metal content when cultured at high metal levels which is not due to an active exclusion mechanism: an apparent lack of permeability may be the result of metal toxicity and injury which disrupts cell membranes. Terui *et al.* (1960) found less arsenic in tolerant hyphae of *Aspergillus oryzae* as the arsenite level was increased; however, increasing arsenite content of the medium was paralleled by increasing hyphal damage. Yamamoto (1963) found that in strontium-resistant yeast cells, the protein synthesis of the resistant cell is aided by the morphological stability of the ribosomal particles under conditions of hypertoxicity produced by excess strontium. Repp (1963) found that the protoplasm of *Silene vulgaris* was generally highly "shock resistant" when exposed to heavy metals. She also found that metal-tolerant races of *Tussilago* and *Taraxacum* had protoplasmic changes that allowed them to overcome the "shock" of excess heavy metal ions.

Increased concentrations of a metabolite may antagonize the effects of the metal. This mechanism is analogous to increases in a metal-binding agent as with sulphur in resistant yeast cells (Ashida and Nakamura, 1959). Selenium and sulphur have been shown to have competitive effects in metabolism: increases in sulphur nutrition antagonize selenium poisoning by competing for sites of reaction rather than by binding with the toxic metals (Rosenfeld and Beath, 1965). Lohrmann (1940) found magnesium interfered with boron and mercury poisoning in several species of fungi.

Wood and Sibley (1950) showed that dead tissues of oat plants always had higher concentrations of zinc than did normal photosynthesizing tissue, suggesting the possibility of "excretory" mechanisms being important in metal tolerance. However, there is no evidence for this in tolerant plants.

D. CONCLUSION

The mechanisms whereby plants combat toxic levels of heavy metals are very varied, and in yeasts in particular there is evidence that the whole metabolism is altered in copper-tolerant strains. In higher plants the tolerance mechanism appears to be designed to keep metal ions away from the active sites of metabolism by chelation in the cell wall. It is interesting that, in *Agrostis* at least, similar though independent complexing systems are present in the cell wall for both zinc and copper. This and the circumstantial evidence from other organisms argues that the cell wall may have a general function, hitherto unrecognized, in removing excess levels of toxic ions even in relatively normal situations.

A problem that remains to be solved is the reason for the high degree of specificity of metal tolerance. Even copper-tolerant yeasts which produce the relatively unspecific inactivating agent, hydrogen sulphide, do not show cross-tolerance to other metals. In higher plants the specific nature of the mechanism has to be reconciled with the fact that tolerance appears to be determined not by a single gene but polygenically (see Section VI). Genetic studies, particularly if combined with biochemical investigations, could help throw light on the tolerance mechanism and its specificity.

The various mechanisms of tolerance all suggest that special systems are developed by organisms to cope with excess metal ions: tolerance appears not to be achieved by producing excessive amounts of substrates (e.g. enzymes) that have been previously known to complex with metals in biological systems. This is understandable in the case of metals such as lead and mercury which are not normal components of metabolism, but more surprising with trace elements such as copper and zinc. However, the requirement for a special tolerance apparatus may explain why only a few organisms appear to be capable of evolving tolerance to any degree, and why none have been shown to possess an inherent metal tolerance (see Section VB). Nevertheless, the cell wall may have some general importance as regards metal binding.

The gross metabolic alterations that occur in tolerant races may in part explain their poorer competitive ability and changes that have been noticed in characters other than tolerance. However, the situation here needs clarifying since independent selection for other characters will explain both phenomena. Investigating the tolerance mechanism in artificially selected material and in segregating progenies should help to clarify these problems.

The nature of the tolerance mechanism, as well as being of intrinsic physiological and biochemical interest, clearly has considerable relevance to the evolutionary and ecological aspects of metal-tolerant plants. It is encouraging that an integrated, consistent picture is beginning to emerge, and further work should prove extremely rewarding.

VIII. PRACTICAL APPLICATIONS OF STUDIES OF METAL-TOLERANT ORGANISMS

A. BIOGEOCHEMICAL PROSPECTING

The role of plants as indicators of soils contaminated with heavy metals has already been discussed (Section IIA). The use of these plants to locate natural outcrops of metal ore is less well documented. They are frequently mentioned, but this is more often in terms of their curiosity value. Over the past twenty-five years the use of plants in prospecting has developed in more sophisticated ways. Valuable reviews are provided by Cannon (1960a), Malyuga (1964) and Cole (1965) so that only the general perspective will be given.

1. *Vegetation changes*

Many of the earlier prospectors relied on the look of the land and the plants growing there to tell them whether there was a chance of a strike. Old textbooks on mining recommended that attention should be paid to plants (Foster, 1894).

The value of plants as indicators can be either general or particular. General changes in species occurrence is an indication of some sort of soil anomaly, and provides an initial sign that there is a soil and underlying rock change (Malyuga, 1964). There is no doubt that this technique has been widely used by prospectors who did not pay attention to individual species.

Changes in particular species can be more valuable, for these can indicate the presence of a particular metal. But whether or not they do is another matter as has already been seen in Section IIA1. The problem is that many of the species supposed to be associated with a particular metal are in fact associated with a particular rock or soil type. This is very clear in the case of serpentine which has a very characteristic depauperate appearance and flora associated with high magnesium level (Walker, 1954); it is a rock in which chromium and nickel is found. As a result of this chromium ores in serpentine were discovered in Maryland in 1818 (Singewald, 1928). But there are many other serpentine areas with an equally characteristic vegetation without metals. Another example is *Becium* (= *Ocimum*) *homblei* which has been reported to grow only on soil containing more than 100 ppm copper in Rhodesia (Anon., 1959). But it has recently been shown in an intensive investigation to grow widely on non-copper soil, on granite sandveld, being favoured by very poor soils and lack of competition from *Becium obovatum* (Howard-Williams, 1970). *Vellozia equisetoides* is similar to *B. homblei*; it is common on the skeletal soils of quartzite slopes yet is widespread and characteristic on copper soils (Wild, 1968).

If a species has a reasonable association with a particular mineral-bearing rock it can be a valuable indicator even if that association, as for *Vellozia equisetoides*, is not absolute and is due to other soil characteristics and absence of competition. The indicator plants that have, for instance, been most valuable for copper are *Elshotzia haichowensis* in China (Tsung-Shan, 1957), *Acrocephalus robertii* in Katanga (Duvigneaud, 1958), and *Becium homblei* in Rhodesia (Anon., 1959), all belonging to the Labiatae. In Australia in the nineteenth century, the pink *Polycarpea spirostylis* was recognized as a copper indicator by prospectors in Queensland (Cole, 1965). Areas of copper in Sweden have been located merely by examining the localities from which herbarium specimens of "copper mosses" had been collected (Persson, 1948). Uranium ores in the Colorado Plateau and in Peru have been detected by the occurrence of members of the genus *Astragalus* (Cannon, 1957, 1960b). But there are many other records in the literature: these are listed in Table Ib, and are discussed by Cannon (1960a) and Malyuga (1964). Indicator species pose very interesting problems: it is a pity no critical ecological and genetical investigations have been made of any except *Becium homblei* (Howard-Williams, 1970).

It is often better when reliance is not placed on the occurrence of a single species but on a group of commonly associated species—a lode assemblage. This is clearly indicated by the very extensive work recently carried out in Australia (Cole, 1965; Nicolls *et al.*, 1965; Cole *et al.*, 1968), and is supported by work in the U.S.A. (Cannon, 1960a), Russia (Malyuga, 1964; Chikishev, 1965) and Rhodesia (Wild, 1968, 1970). *Polycarpeae glabra, Eriachne mucronata, Bulbostylis barbata, Fimbristylis* sp. nov. and *Tephrosia* sp. nov. are all associated with lodes in the Dugald river area of Australia containing zinc, copper and lead, but they do not always all occur together. Their occurrence has revealed new areas for intensive prospecting. These species (particularly *Tephrosia* sp. nov.), and a few others, were found to be associated with similar mineralization in other areas of North Australia (Cole, 1965). But they may sometimes be found in areas where metals are not present but the soil is anomalous in other respects, and they may be absent on soils where the metal content is extremely high (Cole *et al.*, 1968). They may also be replaced by other species if the surrounding bedrock changes markedly (Nicolls *et al.*, 1965). As a result of the influence of climate and soil factors on the occurrence of indicator species, Wild (1968) advocates the need to become familiar with the assemblage of species on one copper outcrop in order even to recognize further outcrops in the immediate vicinity. This applies also to nickel (Wild, 1970).

Areas where the concentration of metal is very high may be completely bare of vegetation, or particular sensitive species may be absent. Bareness or lack of species can, therefore, be an important indicator of metal occurrence. This technique has been used in prospecting for copper in Armenia and Rhodesia. In the Congo high copper areas are recognized by the absence of trees in a predominantly forested area (Cannon, 1960a). Sometimes an indication of actual levels of metal in the soil is obtained by the appearance of particular plants. High zinc causes chlorosis in many species. Copper gradients in Katanga are recognized by the stunting of *Protea goetzeana* (Duvigneaud, 1958).

2. *The metal content of plant parts*

The value of plant analysis in prospecting stems from two characteristics of plants growing over contaminated soil. Firstly, as has been reviewed in Section VIIB, plants take up appreciable quantities of metal into their aerial parts. This is true of plants growing over non-toxic (but metal-containing) soils as well as toxic soils. Secondly, the roots of trees in particular penetrate to the lower depths of the soil, often to the bedrock. Sampling aerial parts of a plant may therefore supply information on metal content of the bedrock without recourse to drilling operations. But the disadvantage of the method in comparison with more normal geochemical techniques is the need for ashing the plant material, and lack of perfect correlation between plant and soil metal contents.

The method of prospecting has been examined, initially independently, in Canada, Scandinavia and Russia, and recently in Australia. It has been reviewed by Hawkes (1948, 1957).

In Canada, Warren and Howatson (1947) and Warren and Delavault (1948) made pioneer studies on copper and zinc contents of trees lying over known ore bodies and trees on normal soil. They concluded that newly grown twigs and leaves gave the most reliable results, and produced empirical data which, when exceeded, suggested abnormally high soil metal concentration. Absolute values and Zn/Cu ratios were both used. Even at this stage differences in uptake according to soil type were noted: considerably less was taken up on calcareous soils (see Section VIIB). Warren, Delavault and Irish (1949) extended these results and modified the empirical values. In the process of this work they discovered an unknown vein lying 15 ft below the surface. Warren and Delavault (1949) quoted a Cu/Zn ratio of 0·10–0·15 as about the normal range, and a value less or more than this as being indicative of zinc or copper anomalies. Warren and Delavault (1950a) showed that the technique could be applicable to the detection of gold. White (1950) made a more systematic study of metal contents of plants over ore deposits using mapping techniques. In this way he was able to detect the existence of metal shadows around ore deposits. Shallow deposits produced a small abrupt shadow whereas deeper deposits led to a shadow as much as fifty times as wide as the ore deposit itself. He concluded that the metal shadow can be detected with an overburden in excess of 30 ft. This is confirmed by Clarke (1953). The sampling technique has been further sophisticated by Warren, Delavault and Fortescue (1955).

In Norway Vogt (1939, 1942a, 1942b), Vogt and Braadlie (1942), Vogt and Bugge (1943), and Vogt *et al.* (1943) made a similar study in the Røros area of Norway, but beyond showing the higher level of metals, particularly copper and zinc, in plants from contaminated areas, they did not champion the value of this technique in prospecting. Rankama (1947) briefly reviewed some results of this type of study in Europe and quotes examples where it had been successful. Subsequently, he showed that nickel could only be detected if inorganic material covering nickel deposits was only a few metres thick (Rankama, 1954).

The Russian work on biogeochemical prospecting has been fully reviewed by Malyuga (1947, 1964). He considers some of the factors that affect the form of the metal shadow, (e.g. depth of ore body, aspect, root depth) and gives extensive details of examples where plant analyses have been of practical value, in outlining ore bodies and in making new discoveries. It is not possible to describe the wealth of information given by Malyuga. But it is clear that analyses of a wide range of plant material have been valuable in the U.S.S.R. in the detection of ore bodies of a wide variety of metals and can only cost one-tenth as much as normal exploration techniques involving drilling, trenching, etc. But care is necessary in the sampling processes because of the variation between species, between different parts of the plants, between seasons, and between different climatic regions.

The fact that the technique is not perfect and the correlation between soil and plant metal content can go astray was pointed out by Marmo (1953), who showed that Cu levels in soil are reflected by Cu levels in plants at low levels of soil copper but not when soil copper levels are high.

Recently, extensive analyses have been carried out on a wide range of Australian species (Nicolls *et al.*, 1965). The relationship, in all species analysed, for both copper and lead is one in which the plant metal content does not increase with increasing concentration of metal in the soil until a value of about 2000 ppm, above which there are only few records because the plants are eliminated. The same relationship for copper is described by Malyuga (1964). For zinc, however, the relationship is linear. These results suggest that the method may not be very useful for copper and lead although it is very satisfactory for zinc. But the evidence is confusing since copper levels in vegetation have been correlated with the presence of known and previously unknown ore bodies in the Gaspé peninsula (Riddell, 1952) and in British Columbia (Warren and Delavault, 1950b).

Cobalt, chromium, copper and nickel uptake by a New Zealand serpentine flora has been critically examined by Lyon *et al.* (1968). In the six species examined there was a reasonable correlation between metal levels in soil and plant for all the metals except copper, in *Cassinia vauvilliersii*, *Leptospermum scoparium* and *Hebe odora*. For the other species correlations for any metal were weak or absent. From this *Cassinia vauvilliersii* was suggested as a good species for biogeochemical prospecting, but it would not appear from the data to be useful for copper.

An extensive list of those cases where prospecting by plant analysis has been undertaken, and their success, is given by Cannon (1960a). The absorption of metals by plants is a complex phenomenon and not clearly understood (see Section VIIB). Part of the complexity is due to complexities of soil and climate, but part is due to the complexities of species. We are perhaps prepared to find that species differ in their patterns of uptake. But no one using plant analysis has considered the problem that the species being analysed may well consist of completely different populations with contrasting patterns of uptake.

3. *The metal content of the humus layer*

Goldschmidt (1954) showed that rare elements are 4–200 times more concentrated in coal than in the average rock. He concluded that humus layers tended to accumulate elements which were brought to the surface by deeply penetrating roots. This was independently realized by Vernadskii (see Malyuga, 1964 for full discussion) and has since been used in the Soviet Union in combination with plant analyses and detailed mapping techniques. It is an extension of the geochemical techniques which are very widely used. It appears to be of most value for copper which forms stable complexes with organic material.

4. *An integrated approach*

Recent studies (Malyuga, 1964; Cole, 1965; Nicolls *et al.*, 1965) have pointed the way towards a large-scale exploitation of these techniques. The first stage of such prospecting involves recognition of a suitable area for further study: this will be decided by geological studies and by associated general changes in vegetation. The next stage is a systematic study of the area: this may be by grid sampling or transect sampling, covering the whole or certain parts of the area. A survey is made of the vegetation and of individual species: and plants, humus and soil are analysed. In this way it is possible to locate metal shadows and specify the position and composition of ore bodies. More generally it can indicate promising areas for drilling operations.

This integrated method is almost certainly the most successful. Its value lies in the fact that useful information can be obtained without drilling and using relatively simple sampling techniques and simple analyses. It certainly provides information of considerable interest to the botanist: it is only a pity that parallel information is not being obtained on the population structure and physiology of the important species.

B. ORE EXTRACTION

Bryner *et al.* (1954) showed that micro-organisms isolated from leaching effluent promoted the solubilization of iron and copper sulphide ores by a factor of 10 to 20 over the rate of solubilization in sterile culture. Subsequent work confirmed these results and identified the active bacteria as *Thiobacillus ferro-oxidans*, *T. thio-oxidans* and *Ferrobacillus ferro-oxidans* (Beck and Elsden, 1958; Bryner and Jameson, 1958). Bryner and Anderson (1957) demonstrated that micro-organisms were also involved in the solubilization of sulphide ores of nickel, cobalt and molybdenum.

Recently work in this field had been reviewed by Razzell and Trussell (1963), Beck (1967) and Ehrlich and Fox (1967). The technique of extracting copper from low-grade ores involves three stages: the leaching process itself, precipitation of the copper by addition of iron, and oxidation of the ferrous sulphate to ferric resulting in precipitation of the iron with the recovery of water for the leaching process. Beck (1967) studied the role of *Thiobacillus ferro-oxidans* in each of these stages. He showed there was a decrease of bacteria in the precipitation plant, a large increase in the oxidation pond, and a slight decrease during passage through the ore dump. The latter surprising fact led Beck to speculate that large numbers of bacteria were filtered out of solution and that the leaching was as much a result of increased temperatures and (possibly triggered off by bacterial activity) chemical oxidation as biological oxidation. The role of bacteria in leaching operations has been further examined by Ehrlich and Fox (1967), who showed that bacteria did not just oxidize the sulphide ores, but helped extraction indirectly by releasing the ferric ion which then oxidized copper sulphide non-biologically (ferrous iron), and by depressing iron release from the ore in some unknown fashion. Ehrlich and Fox (1967) demonstrate that a wide variety of factors may affect ore extraction, including the interaction of bacteria with a wide range of micro-organisms (Ehrlich, 1963b).

It is clear from these studies that the role of bacteria and other micro-organisms in leach operations needs further definition particularly if any attempt is to be made to optimize the conditions for such leaching operations. The bacteria are likely to be present in most mine dumps and optimizing conditions for leaching may permit commercial exploitation and/or subsequent recolonization by higher plants of tips depleted of their metal content.

C. THE ESTABLISHMENT OF VEGETATION ON TOXIC WASTE MATERIALS

The toxic materials left by mining operations are unattractive to the eye and can be a considerable source of pollution of surrounding areas. The piles of country rock produced to get at the ore bodies are coarse, very low in major nutrients, and may contain small quantities of toxic metals. The tailings, produced by the dressing procedures which concentrate the ore, because of the economics of extraction procedures often contain as much as 1% of metal, although modern techniques may reduce this five-fold. The tailings contain not only the remains of the metal that has been extracted but also quantities of the other metals that were present in the ore and not extracted. These metals are released slowly as soluble compounds as the ore weathers, and may continue to be released for centuries.

As a result the waste heaps have very little plant growth upon them, owing to the combined effects of toxicity, lack of nutrients and physical conditions. The particle size of the tailings is often sufficiently small for it to blow in dry weather on to surrounding areas. Particles are nearly always fine enough to be able to be carried by run-off during storms into water courses, often for long distances, unless great care is taken in the establishment of dams and settling ponds. There will always be a certain amount of leaching of soluble salts from waste heaps, which will be enhanced if the heaps are unstable. Thus the pollution of streams by dissolved heavy metals in the neighbourhood of mine workings can be considerable: levels of 500 ppm can easily be found.

The establishment of a vegetation cover on toxic waste heaps will do a great deal to minimize pollution, particularly where the pollution is due to the movement of solid particles, and will also improve the appearance of the heaps. But the conditions of toxicity and poverty of nutrients, and the physical nature of the material make normal techniques of vegetation establishment impossible (Hooper and Newton, 1935).

The most obvious technique that will be effective is to cover the toxic material with a layer of normal soil and establish a herbage cover or trees in the normal manner. This is, however, exceedingly expensive, usually at least six times the normal for non-toxic areas.

As a result experiments have been carried out in several places to find ways in which the toxicity, nutrient poverty and physical conditions of the material can be ameliorated more simply by addition of less expensive materials. Physical and chemical methods are possible (Dean *et al.*, 1968) but the most promising additives are organic wastes such as sewage sludge and town refuse. With these, swards of normal herbage species have been established over highly toxic smelter waste in the lower Swansea Valley, so long as the ameliorating material is more than about ten centimetres thick (Weston *et al.*, 1965; Street and Goodman, 1967). Sewage sludge is particularly successful since its high organic matter content effectively ameliorates all three conditions. But the effect of these materials may be short term: when the organic matter decays the toxicity will return, and the vegetation will eventually suffer and die.

An alternative approach is to take advantage of the natural evolutionary processes that have already occurred and employ species and populations which are already tolerant to the toxicity present, together with fertilizer to ameliorate nutrient lack. Preliminary experiments with smelter waste in the lower Swansea Valley using vegetative material of various grass species showed that the performance of tolerant material was superior to that of non-tolerant (Gadgil, 1969). But, not unexpectedly, this superiority was not found when domestic refuse or sewage sludge was used, when all populations grew well.

This method has now been investigated using seed on a variety of mine tailings in Wales and Northern England, with the addition of different amounts of fertilizer (Smith and Bradshaw, 1970). The superiority of tolerant material was remarkable: non-tolerant material was almost dead in nine months while tolerant material grew almost as well as if on normal soil. The effect of fertilizer was very marked indeed, slow release forms giving best growth.

In both these experiments it appears that physical features of mine waste are not severe enough to prevent growth. However, in drier climates they may be important, but perhaps can be overcome by sowing in the wet season or by using mulching techniques (reviewed by Peterson and Monk, 1967).

The technique of using metal-tolerant populations has not been explored in a wide enough variety of sites. But initial results are promising and fit in with expectations. There is such a wide variety of species in all parts of the world in which tolerance is known or can be presumed to occur, that it seems a method capable of widespread use. Many of the species are vegetatively vigorous and perennial, and some are leguminous and therefore will perhaps be self-sufficient for nitrogen unless N fixation is precluded by toxic soils. The method should be investigated further.

IX. CONCLUSION

The plants growing in habitats contaminated with toxic levels of heavy metals have attracted interest for over a century. Since metal-contaminated areas are only a minor component of the environment, the subject of metal tolerance has on the whole been regarded as an area of marginal relevance to most other fields of investigation. The interest that has arisen has come from ecologists, evolutionary geneticists, physiologists, and applied biologists particularly in relation to prospecting for metal ores. The literature on such plants is therefore widely dispersed.

However, when this literature is collated it is seen that specialized habitats such as metal-contaminated areas are extremely valuable to evolutionary and ecological studies. Their value stems essentially from their simplicity and this is a consequence of three main features.

Firstly, the vegetation of such areas is influenced by one overriding factor, namely metal concentration. Undoubtedly other factors are also of importance but such factors and interactions are far more easily defined when the major determinant is clearly apparent. Secondly, the habitats are usually spatially distinct and clear cut. This is particularly true of areas resulting from mining activities. Thirdly, since the major factor involved is an edaphic one it is relatively constant and varies only slowly with time.

It is perhaps not surprising, therefore, that such areas have already proved invaluable in studies of evolution. The sequence and pattern of genetic change responsible for colonization of metal-contaminated areas is a unique record of natural selection in action. It is clear that studies of metal tolerance may be of equal value in clarifying ecological problems particularly when they impinge on other areas such as evolution, taxonomy, plant distribution and physiological processes.

A pointer towards these interactions has been made in this paper, mainly by reference to work which has been carried out without the specific aim of achieving an integrated view.

Certain problems of ecology can often be better analysed in environments that are highly specialized or even man-made, than in natural, more complex habitats.

ACKNOWLEDGEMENTS

Many workers have provided us with advanced information of their results, either by personal communication or by drawing our attention to unpublished theses. Their names appear in the text, but we would like to thank them for their help. We are also grateful to all those who have helped us at one time or another to locate or compile many of the references.

REFERENCES

Allen, R. and Sheppard, P. M. (1971). *Proc. R. Soc. B* **177**, 177–196. Copper tolerance in some Californian populations of the Monkey Flower, *Mimulus guttatus.*

Anonymous (1959). *Horizon* **1**, 35–39. A flower that led to a copper discovery.

Antoine, A. (1965). *Expl Cell Res.* **40**, 570–584. La resistance de la levure aux ions cuivriques. III. *Saccharomyces cerevisiae*, Yeast foam, nature de deux formes resistantes.

Antonovics, J. (1966). "The genetics and evolution of differences between closely adjacent plant populations with special reference to heavy metal tolerance." Ph.D. Thesis, University of Wales.

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

Antonovics, J. (1968b). *Heredity, Lond.* **23**, 507–524. Evolution in closely adjacent plant populations. VI. Manifold effects of gene flow.

Antonovics, J. and Bradshaw, A. D. (1970). *Heredity, Lond.* **25**, 349–362. Evolution in closely adjacent plant populations. VIII. Clinal patterns in *Anthoxanthum odoratum* across a mine boundary.

Antonovics, J., Lovett, J. and Bradshaw, A. D. (1967). *In* "Isotopes in Plant Nutrition and Physiology", pp. 549–567. Vienna I.A.E.A./F.A.O. Symposium. The evolution of adaptation to nutritional factors in populations of herbage plants.

Aomine, S., Kawasaki, H. and Inoue, K. (1967). *Soil Pl. Fd, Tokyo* **13**, 186–188. Retention of mercury by soils. I. Mercury residues of paddy and orchard soils.

Archer, J. (1964). "Zinc tolerance in *Agrostis tenuis*, *Agrostis stolonifera*, and their hybrids." B.Sc. Dissertation, University College of North Wales.

Ashida, J. (1965). *A. Rev. Phytopathol.* **3**, 153–174. Adaptation of fungi to metal toxicants.

Ashida, J. and Nakamura, H. (1959). *Pl. Cell Physiol., Tokyo* **1**, 71–79. Role of sulphur metabolism in copper-resistance in yeast.

Ashworth, L. J. (1965). Personal communication. Agric. Res. Service, U.S. Cotton Res. Sta., Shafter, Calif.

Ashworth, L. J. and Amin, J. V. (1964). *Phytopathology* **54**, 1459–1463. A mechanism for mercury tolerance in fungi.

Auquier, P. (1964). *Bull. Soc. r. Bot. Belg.* **97**, 99–130. Les Festuca des terrains calaminaires de la Wallonie Septentrionale.

Bailey, F. J. (1889). "Queensland Flora." Melbourne.

Bailey, F. M. (1898). *Bot. Zbl.* **76**, 104. The copper plant.

Bainbridge, A. (1969). *Agriculture, Lond.* **76**, 97–98. Mercury tolerance in leaf spot of oats.

Barker, J. A. (1967). Personal communication. Department of Agricultural Botany, University College of North Wales, Bangor, Caernarvonshire, U.K.

Bateman, W. G. and Wells, L. S. (1917). *J. Am. chem. Soc.* **39**, 811–819. Copper in the flora of a copper tailing region.

Baumann, A. (1885). *Landwn VersStnen* **31**, 1–53. Das Verhalten von Zinksalzen gegen Pflanzen und im Boden.

Baumeister, W. (1954). *Ber. dt. bot. Ges.* **67**, 205–213. Uber den Einfluss des Zinks bei *Silene inflata* Smith. I.

Baumeister, W. (1967). *Angew. Bot.* **40**, 185–204. Schwermetall-Pflanzengesellschaften und Zinkresistenz einiger Schwermetallpflanzen.

Baumeister, W. and Burghardt, H. (1956). *Ber. dt. bot. Ges.* **69**, 161–168. Uber den Einfluss des Zinks bei *Silene inflata* Smith. II. CO_2 Assimilation und Pigmentgehalt.

Beck, J. V. (1967). *Biotechnol. Bioeng.* **9**, 487–497. The role of bacteria in copper mining operations.

Beck, J. V. and Elsden, S. R. (1958). *J. gen. Microbiol.* **19**, i. Isolation and some characteristics of an iron-oxidising bacterium.

Bertrand, G. and Andreitcheva, M. (1963). *C.R. hebd. Seanc. Acad. Sci., Paris* **197**, 1374–1376. Sur la teneur comparée en zinc des feuilles vertes et des feuilles étiolées.

Bhatia, C. R. and Narayanan, K. R. (1965). *Genetics, Princeton* **52**, 577–581. Genetic effects of ethyl methanesulfonate in combination with copper and zinc ions on *Arabidopsis thaliana.*

Biebl, R. (1947a). *Sber. Akad. Wiss. Wien, Abt. I.* **155**, 145–157. Die resistenz gegen Zink, Bor und Mangan als Mittel zur Kennzeichnung verschiedener pflanzlicher Plasmasorten.

Biebl, R. (1947b). *Ost. bot. Z.* **94**, 61–73. Uber die gegensatzliche Wirkung der Spurenelemente Zink und Bor auf die Blattzellen von *Mnium rostratum.*

Biebl, R. (1950). *Protoplasma* **39**, 18–259. Uber die Resistenz pflanzlicher Plasmen gegen Vanadium.

Birrell, K. S. and Wright, A. C. S. (1945). *New Zealand Journ. Sci. and Technol.*, **27A**, 72–76. A serpentine soil in New Caledonia.

Booth, G. H. and Mercer, S. J. (1963). *Nature, Lond.* **199**, 622. Resistance to copper of some oxidising and reducing bacteria.

Bradshaw, A. D. (1952). *Nature, Lond.* **169**, 1098. Populations of *Agrostis tenuis* resistant to lead and zinc poisoning.

Bradshaw, A. D. (1959). *New Phytol.* **58**, 208–227. Population differentiation in *Agrostis tenuis* Sibth. I. Morphological differentiation.

Bradshaw, A. D., McNeilly, T. S. and Gregory, R. P. G. (1965). *In* "Ecology and the Industrial Society", *Brit. Ecol. Soc. Symp.* **5**, 327–343. Industrialization, evolution and the development of heavy metal tolerance in plants.

Bradshaw, A. D., Antonovics, J., Khan, M. S. and Walley, K. (1969). *Int. Bot. Cong. XI. Seattle, Abstracts*, p. 21. The importance of extreme selection pressures in evolution.

Bradshaw, A. D., Chadwick, M. J., Jowett, D., Lodge, R. W. and Snaydon, R. W. (1960). *J. Ecol.* **48**, 631–637. Experimental investigations into the mineral nutrition of several grass species. III. Phosphate level.

Braun-Blanquet, J. (1951). "Pflanzensoziologie", 2nd Edit. Springer, Wien.

Brock, J. D. (1969). *In* "Microbial growth". *Symp. Soc. Gen. Microbiol. 19th*, pp. 15–41. Microbial growth under extreme conditions.

Bröker, W. (1963). *Flora, Jena*, B. **153**, 122–156. Genetisch-physiologische Untersuchungen uber die Zinkvertraglichkeit von *Silene inflata Sm.*

Bryner, L. C. and Anderson, R. (1957). *Ind. Engng. Chem. ind. (int.) Edn.* **49**, 1721–1724. Microorganisms in leaching sulphide materials.

Bryner, L. C. and Jameson, A. K. (1958). *Appl. Microbiol.* **6**, 281–287. Microorganisms in leaching sulfide minerals.

Bryner, L. C., Beck, J. V., Davis, D. B. and Wilson, D. G. (1954). *Ind. Engng. chem. ind. (int.) Edn.* **46**, 2587–2592. Microorganisms in leaching sulfide minerals.

Buck, L. J. (1949). *Jl N.Y. bot. Gdn.* **50**, 265–269. Association of plants and minerals.

Cannon, H. L. (1957). *Bull. U.S. geol. Surv.* No. 1030-M, 399–516. Description of Indicator Plants and Methods of Botanical Prospecting for Uranium Deposits on the Colorado Plateau.

Cannon, H. L. (1960a). *Science, N.Y.* **132**, 591–598. Botanical prospecting for ore deposits.

Cannon, H. L. (1960b). *Bull. U.S. geol. Surv.* No. 1085A. The development of botanical methods of prospecting for uranium ore in the Colorado Plateau.

Chatterjee, B. D., Barua, A. D. and Banerjee, P. L. (1968). *Indian J. med. Res.* **56**, 395–401. Mercury resistance of *Staph. pyogenes* isolated from different sources of a hospital: its relationship to phage types and antibiogram.

Chester, V. E. (1965). *Proc. R. Soc. Ser. B.* **162**, 555–566. The role of calcium in the increase of flocculence of yeast growing in the presence of copper.

Chikishev, A. G. (1965). "Plant indicators of soils, rocks and sub-surface water." Consultants Bureau, New York.

Cholak, J., Schaffer, L. J. and Yeager, D. (1968). *Am. ind. Hyg. Ass. J.* **29**, 562–568. The air transport of lead compounds in automobile exhaust gases.

Clarke, O. M. Jnr. (1953). *Econ. Geol.* **48**, 39–45. Geochemical prospecting for copper at Ray, Arizona.

Coackley, A. and Dawson, M. (1966). "Cross tolerance in *Rumex acetosa*". B.Sc. Dissertation, University College of North Wales.

Cole, M. M. (1965). *Proc. Commonw. Min. Metall. Congr. 8th,* **6**, 1429–1458. The use of vegetation in mineral exploration in Australia.

Cole, M. M., Provan, D. M. J. and Tooms, J. S. (1968). *Trans. Instn Min. Metall.* **77**, 81-104. Geobotany, biogeochemistry and geochemistry in mineral exploration in the Bulman-Waimura Springs area, Northern Territory, Australia.

Craig, G. C. (1970). Personal communication. Department of Biology, University of Stirling, Scotland, U.K.

Da Costa, E. W. B. and Kerruish, R. M. (1964). *Forest. Prod. J.* **14**, 106–112. Tolerance of *Poria* species to copper based wood preservatives.

Davis, B. D. (1958). *In* "Drug Resistance in Micro-organisms", Wolstenholme, G. E. W. and O'Connor, C. M. (Eds). Churchill, London. Physiological (phenotypic) mechanisms responsible for drug resistance.

Dean, K. C., Dolezal, H. and Havens, R. (1968). A Progress Report, U.S. Department of the Interior, Bureau of Mines, Pittsburgh, Pa. Utilisation and Stabilisation of Solid Mineral Wastes.

Delas, J. (1963). *Agrochimica* **7**, 258–280. Toxicity of copper accumulated in the soil.

Demoulin, V., Lambinon, J., Maquinay, A. and Ramaut, J. L. (1967). *Bull. Jard. bot. nat. Belg.* **37**, 305–308. Teneur en zinc et en plomb de quelques Gasteronycetes des terrains calaminaires belges.

Doksopulo, E. P. (1961). Tbilisi, Izd-vo Tbilisskogo Univ. Nickel in rocks, soils, waters and plants adjacent to the talc deposits of the Chorchanskaya group.

Dorn, Von P. (1937). *Biologie* **6,** 11–13. Pflanzen als Anzeichnen fur Erzlagerstatten.

Drouineau, G. and Mazoyer, R. (1956). *Int. Congr. Soil Sci. 6th. Reports D,* pp. 419–421. Copper toxicity in soils.

Duvigneaud, P. (1958). *Bull. Soc. r. Bot. Belg.* **90**, 127–286. La vegetation du Katanga et de ses sols metalliferes.

Duvigneaud, P. (1959). *Bull. Soc. r. Bot. Belg.* **91**, 111–134. Plant "cobaltophytes" dans le Haut Katanga.

Duvigneaud, P. and Denaeyer-de Smet, S. (1960). *In* "Rapports de sol et de la vegetation", pp. 121–139. Masson, Paris. Influence des sols toxiques sur la vegetation. Action de certains metaux lourds du sol (cuivre, cobalt, manganese, uranium) sur la vegetation dans le Haut Katanga.

Duvigneaud, P. and Denaeyer-de Smet, S. (1963). *Bull. Soc. r. Bot. Belg.* **93**, 93–231. Cuivre et vegetation au Katanga.

Duvigneaud, P. and Timperman, J. (1959). *Bull. Soc. r. Bot. Belg.* **91**, 135–176. Etudes sur le genre *Crotalaria.*

D'Yakov, Y. T. (1963a). *Dokl. mosk. sel.-khoz. Akad. K.A. Timiryazeva* **83**, 267–272. Some physiological changes associated with the adaptation of *P. infestans* to fungicides.

D'Yakov, Y. T. (1963b). *Izv. timiryazev. sel. -khoz. Akad.* **2**, 39–46. Adaptation of the pathogen of potato late blight to copper acetate.

Dykeman, W. R. and de Sousa, A. S. (1966). *Can. J. Bot.* **44**, 871-878. Natural mechanisms of copper tolerance in a copper swamp forest.

Earley, E. B. (1943). *J. Am. Soc. Agron.* **35**, 1012–1023. Minor element studies with Soybeans. I. Varietal reactions to concentrations of zinc in excess of the nutritional requirement.

Ehrlich, H. L. (1962). *In* "Biogeochemistry of Sulphur Isotopes", M. L. Jenson (Ed.). National Science Foundation Symposium. Yale Univ., New Haven. pp. 153–168.

Ehrlich, H. L. (1963a). *Appl. Microbiol.* **11**, 15–19. Bacteriology of manganese nodules. I. Bacterial action on manganese on nodule enrichments.

Ehrlich, H. L. (1963b). *J. Bact.* **86**, 350–352. Micro-organisms in acid drainage from a copper mine.

Ehrlich, H. L. (1965). Personal communication. Dept. Biology, Rensselaer Polytechn. Inst., Troy, New York.

Ehrlich, H. L. and Fox, S. I. (1967). *Biotechnol. Bioeng.* **9**, 471–485. Environmental effects on bacterial copper extraction from low-grade copper sulfide ores.

Ernst, W. (1965a). *Abh. Landesmus. Naturk. Munster* **27**, (1), 54 pp. Okologische-Soziologische Untersuchungen der Schwermetall-Pflanzengesellschaften Mitteleuropas unter Einschluss der Alpen.

Ernst, W. (1965b). *Ber. dt. bot. Ges.* **78**, 205–212. Uber den Einfluss des Zinks auf die Keimung von Schwermetallpflanzen und auf die Entwicklung der Schwermetallpflanzengesellschaft.

Ernst, W. (1966). *Flora, Jena, B.* **156**, 301–318. Okologisch-soziologische Untersuchungen auf Schwermetallpflanzengesellschaften Südfrankreiches und des östlichen Harzvorlandes.

Ernst, W. (1967). *Excerpta bot., Sectio B.* **8**, 50–61. Bibliographie der Arbeiten uber Pflanzengesellschaften auf schwermetallhaltigen Boden mit Ausnahme des Serpentins.

Ernst, W. (1968a). *Mitt. flor.-soz. Arbgemein.* **13**, 263-268. Das Violetum calaminariae westfalicum, eine Schwermetallpflanzengesellschaft bein Blankenrode in Westfalen.

Ernst, W. (1968b). *Flora, Jena, B.* **158**, 95–106. Okologische Untersuchungen auf Pflanzengesellschaften unterschiedlich gestorter schwermetallreicher Boden in Grossbritannien.

Ernst, W. (1968c). *Ber. dt. bot. Ges.* **81**, 116–124. Zur Kenntnis der Soziologie und Okologie der Schwermetallvegetation Grossbritanniens.

Ernst, W. (1968d). *Physiologia Pl.* **21**, 323–333. Der Einfluss der Phosphatversorgung sowie die Wirkung von ionogenem und chelatisiertem Zink auf die Zink-und Phosphataufnahme einiger Schwermetallpflanzen.

Ernst, W. (1969a). *Ber.dt.bot. Ges.* **82**, 161–164. Zur Physiologie der Schwermetallpflanzensubselluläre Speicherungsorte des Zinks.

Ernst, W. (1969b). *Vegetatio* **18**, 393–400. Pollenanalytischer Nachweis eines Schwermetallrasens in Wales.

Foster, C. le N. (1894). "Ore and Stone Mining." Griffin, London.

Gadgil, R. L. (1969). *J. appl. Ecol.* **6**, 247–259. Tolerance of heavy metals and the reclamation of industrial waste.

Gambi, O. V. (1967). *G. Bot. Ital.* **101**, 59-60. First data on the histological localization of nickel in *Alyssum bertolonii* Desv.

Gibson, I. A. S. (1958). *E. Afr. agric. J.* **24**, 125–127. Phytotoxic effects of copper fungicides on acid soils.

Goldschmidt, U. M. (1954). "Geochemistry." Clarendon Press, Oxford.

Godwin, H. (1956). "The History of the British Flora." Cambridge University Press, London and New York.

Gorsline, G. W., Thomas, W. I. and Baker, D. E. (1964). *Crop Sci.* **4**, 207–210. Inheritance of P, K, Mg, Cu, B, Zn, Mn, Al and Fe concentrations by corn (*Zea Mays* L.) leaves and grain.

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

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

Greenaway, W. and Cowan, J. W. (1970). *Trans. Br. mycol. Soc.* **54**, 127–138. The stability of mercury resistance in *Pyrenophora avenae.*

Gries, B. (1966). *Flora, Jena*, B. **156**, 271–290. Zellphysiologische Untersuchungen uber die Zinkresistenz bei Galmeiokotypen und Normalformen von *Silene cucubalus* Wib.

Griffith, J. W. (1919). *J. agric. Sci., Camb.* **9**, 366–395. Influence of mines upon land and livestock in Cardiganshire.

Harris, T. M. (1946). *New Phytol.* **45**, 50–55. Zinc poisoning of wild plants from wire netting.

Hassall, K. A. (1962). *Nature, Lond.* **193**, 90. A specific effect of copper on the respiration of *Chlorella vulgaris.*

Hawkes, H. E. (1948). *Circular No. 28. U.S. Department of Interior.* Annotated bibliography on papers on geochemical prospecting for ores.

Hawkes, H. E. (1957). *Bull. U.S. geol. Surv.* No. 1000-F, 225-352. Principles of geochemical prospecting.

Heimans, J. (1936). *Ned. kruidk. Archf.* **46**, 778-897. De Merkomst van de Zinkflora aan de Geul.

Heimans, J. (1960). *Publicaties van het natuurhistorisch Genootschap in Limburg.* **12**, (1960–61), 55–71. Taxonomic, phytogeographical and ecological problems round *Viola calaminaria* Lej.

Heimans, J. (1966). *Levende Nat.* **69**, 265–270. Het blauwe Zinkviooltje van Westfallen.

Henwood, W. J. (1857). *Edin. New Phil. J.* **5**, 61–63. Notice of the copper turf of Merioneth.

Hilton, K. J. (1967). "The Lower Swansea Valley Project." Longmans, Green, London.

Hodgson, J. F., Lindsay, W. L. and Frierweiler, J. F. (1966). *Proc. Soil. Sci. Soc.*

Am. **30**, 723–726. Micronutrient cation complexing in soil solution. II. Complexing of zinc and copper in displaced solution from calcareous soils.

Hodgson, M. B. (1969). "Mercury resistance in ship-borne Enteromorpha." Hons. Thesis, Dept. Botany, Univ. Liverpool.

Hoekstra, W. G. (1964). *Fedn. Am. Socs. exp. Biol.* **23**, 1068–1076. Recent observations on mineral inter-relationships.

Hofsten, B. von (1962). *Expl. Cell. Res.* **26**, 606–667. The effect of copper on the growth of *Escherichia coli.*

Holmes, E. A. De B. (1965). "Root culture and measurement of lead tolerance in *Festuca ovina.*" M.Sc. Thesis, University of Birmingham.

Holmes, R. S. (1964). Research report No. 23, Division of Soil and Fertiliser Investigations, Bureau of Plant Industry, Soils and Agricultural Engineering, U.S. Dept. Agriculture, Beltsville. The effect of liming on the availability to plants of zinc and copper.

Hooper, M. C. and Newton, L. (1935). *Minist. Agric. Fish. Rep.* **519**, (723). The effect of lead on plants with some suggestions for colonisation of Goginan mine.

Horn, P. and Wilkie, D. (1966). *Heredity, Lond.* **21**, 625-635. Selective advantage of the cytoplasmic respiratory mutant of *Saccharomyces cerevisiae* in a cobalt medium.

Horne, M. (1967). Personal communication. Department of Agricultural Botany, University College of North Wales, Bangor, Caerns. U.K.

Horsfall, J. G. (1956). "Principals of Fungicidal Action." Waltham, Massachusetts: Chronica Botanica.

Howard-Williams, C. (1969). "The ecology of *Becium homblei* Duvign. and Plancke." M. Phil. Thesis, University of London.

Howard-Williams, C. (1970). *J. Ecol.* **58**, 745–763. The ecology of *Becium homblei* in central Africa with special reference to metalliferous soils.

Humphreys, D. W. and Farnworth, J. (1964). "An investigation of the growth of *Minuartia verna* (a zinc tolerant species) on various soil types." B.Sc. Dissertation, University College of North Wales.

Jacobsen, W. B. G. (1967). *Kirkia* **6**, 63–80. The influence of the copper content of the soil on trees and shrubs of Molly South Hill, Mangula.

Jacobsen, W. B. G. (1968). *Kirkia* **6**, 259-277. The influence of the copper content of the soil on the vegetation at Silverside North, Mangula Area.

Jain, S. K. and Bradshaw, A. D. (1966). *Heredity, Lond.* **21**, 407–441. Evolutionary divergence among adjacent plant populations. I. The evidence and its theoretical analysis.

Jefferies, R. L., Laycock, D., Stewart, G. R. and Sims, A. P. (1969). *Brit. Ecol. Soc. Symp.* **9**, 281–308. *In* I. H. Rorison (Ed.) "Ecological aspects of the mineral nutrition of plants". The properties of mechanisms involved in the uptake and utilisation of calcium and potassium by plants in relation to an understanding of plant distribution.

Jenkins, A. E. and Winfield, R. J. (1964). "Zinc tolerance in *Holcus lanatus.*" B.Sc. Dissertation, University College of North Wales.

Jensch, E. (1894). *Z. angew. Chem.* **7**, 14–15. Beitrage zur Galmeiflora von Oberschlesien.

Jones, O. T. (1922). *Mem. geol. Surv. spec. Rep. Miner. Resour. Gt. Br.* **XX**. 207 pp. Lead and zinc: the mining district of North Cardiganshire and West Montgomeryshire.

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

Jowett, D. (1959a). "Genecology of Heavy Metal Tolerance in Agrostis." Ph.D. Thesis, University of Wales.

Jowett, D. (1959b). *Nature, Lond.* **184**, 43. Adaptation of a lead tolerant population of *Agrostis tenuis* to low soil fertility.

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

Kerin, Z. (1968). *Qualitas Pl. Mater. veg.* **15**, 372-379. Verunreinigungen von Gemuse aus Emissionen einer Bleihutte.

Khan, M. S. I. (1969). "The process of evolution of heavy metal tolerance in *Agrostis tenuis* and other grasses." M.Sc. Thesis, University of Wales.

Koch, K. (1932). *Wissenschaftliche Arbeiten des Bezirkskomitees fur Naturdenkmalpflege und Heimatschutz in Osnabruk* **1**, 91–115. Die Vegetationsverhaltnisse des Silberberges im Huggelgebiet bei Osnabruck.

Kruckeberg, A. R. (1954). *Ecology* **35**, 267–274. The ecology of serpentine soils III. Plant species in relation to serpentine soils.

Kruckeberg, A. R. (1964). *Am. Fern J.* **54**, 113–126. Ferns associated with ultramafic rocks in the pacific northwest.

Lackey, J. B. (1938). *Publ. Hlth Rep., Wash.* **53**, 1499–1507. The flora and fauna of surface waters polluted by acid mine drainage.

Lambinon, J. (1964). *Lejeunia,* **27**, 1–8. *Stereocaulon nanodes* Tuck. en Wallonie et en Rhenanie.

Lambinon, J. and Auquier, P. (1964). *Natura mosana* **16**, (4). La vegetation des terrains calaminaires de la Wallonie Septrentrionale et de la Rhenanie Aixoise. Types chorologiques et groupes ecologiques.

Lambinon, J., Maquinay, A. and Ramaut, J. L. (1964). *Bull. Jard. bot. Etat. Brux.* **34**, 273–282. La teneur en zinc de quelques lichens des terrains calaminaires Belges.

Lampe, W. and Klement, O. (1958). *Z. Mus. Hildesheim* **12**. Die Flechtenvegetation zwischen Oker und Leine in Raume von Hildesheim biz zum Harzrand.

Lange, O. L. and Ziegler, H. (1963). *Mitt. flor.-soz. ArbGemein.* **10**, 156–183. Der Schwermetallgehalt von Flechten aus dem *Acarosporetum sinopicae* auf Erzschalckenhalden des Harzes.

Lawrence, A. W. and McCarty, P. L. (1965). *J. Wat. Pollut. control Fed.* **37**, 392–406. The role of sulphide in preventing heavy metal toxicity in anaerobic treatment.

Lebrun, J., Noirfalaise, A., Heinemann, P. and Van Den Berghen, C. (1949). *Bull. Soc. r. Bot. Belg.* **82**, 105-207. Les Associations vegetales de Belgiques.

Lefebvre, C. (1967). *Bull. Soc. r. Bot. Belg.* **100**, 213–229. Etude de la position des populations d'*Armeria* calaminaires de Belgique et des environs d' Aix-la-Chappelle par rapport a des types alpines et maritimes d'*Armeria maritima* (Mill.) Willd.

Lefebvre, C. (1968). *Bull. Soc. r. Bot. Belg.* **102**, 5–11. Note sur un indice de tolerance au zinc chez des populations d'*Armeria maritima* (Mill.) Willd.

Lefebvre, C. (1969). Personal communication. Laboratoire de Génétique des Plantes Supérieures, Université Libre de Bruxelles, Belgium.

Lefebvre, C. (1970a). Personal communication. Laboratoire de Génétique des Plantes Supérieures, Université Libre de Bruxelles, Belgium.

Lefebvre, C. (1970b). *Evolution, Lancaster, Pa.* **24** (in press). Self-fertility in maritime and zinc mine populations of *Armeria maritima* (Mill.) Willd.

Le Riche, H. H. (1968). *J. agric. Sci., Camb.* **71**, 205–208. Metal contamination of soil in the Woburn market-garden experiment resulting from the application of sewage sludge.

Levin, D. A. and Kerster, H. W. (1967). *Evolution*, **21**, 679–687. Natural selection for reproductive isolation in *Phlox*.

Lewis, H. (1962). *Evolution, Lancaster, Pa.* **16**, 257–271. Catastrophic selection as a factor in speciation.

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

Lohrmann, W. (1940). *Arch. Mikrobiol.* **11**, 329–367. Untersuchungen uber die antagonistische Wirkung von Magnesium gegenüber Bor und Quecksilber bei eingen Pilzen.

Lovering, T. S., Huff, L. C. and Almond, H. (1950). *Econ. Geol.* **45**, 493–514. Dispersion of copper from the San Manuel copper deposit, Pinal County, Arizona.

Lyon, G. L., Brooks, R. R., Peterson, P. J. and Butler, G. W. (1968). *Plant and Soil*, **29**, 225–240. Trace elements in a New Zealand serpentine flora.

McAllister, H. (1965). *Biol. J. Univ. St. Andrews* **10-11**. The ecology of an old rifle range target area with lead poisoned soil.

McBrian, D. C. H. and Hassall, K. A. (1965). *Physiologia Pl.* **18**, 1059–1065. Loss of cell potassium by *Chlorella vulgaris* after contact with toxic amounts of copper sulphate.

McHargue, J. S. and Roy, W. R. (1932). *Bot. Gaz.* **94**, 381–393. Mineral and nitrogen content of leaves of some forest trees at different times of the growing season.

McNeilly, T. S. (1965). "The evolution of copper tolerance in *Agrostis tenuis* (Sibth)." Ph.D. Thesis, University of Wales.

McNeilly, T. (1968). *Heredity, Lond.* **23**, 99–108. Evolution in closely adjacent plant populations. III. *Agrostis tenuis* on a small copper mine.

McNeilly, T. (1970). Personal communication. Department of Botany, University of Liverpool, Liverpool, U.K.

McNeilly, T. and Antonovics, J. (1968). *Heredity, Lond.* **23**, 205–218. Evolution in closely adjacent plant populations. IV. Barriers to gene flow.

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

Malone, J. P. (1968). *Pl. Path.* **17**, 41–45. Mercury-resistant *Pyrenophora avenae* in Northern Ireland seed oats.

Malyuga, D. P. (1947). *Izvest. Akad. Nauk SSSR, Ser. Geograf. i. Geofiz.* **11**, 135–138. Chemical composition of soils and plants as indicators in prospecting for metals.

Malyuga, D. P. (1950). In "Report at the Conference on Trace Elements in the U.S.S.R." (*Ref. dokl. na. konf. po mikroelementam SSSR) Moscow.* On endemic plant diseases in districts of nickel ore deposits in the southern Urals.

Malyuga, D. P. (1964). "Biogeochemical methods of prospecting." (Authorised translation.) Consultants Bureau, New York.

Malyuga, D. P., Makashkina, N. S. and Makarova, A. I. (1959). *Geokhimiya* **5**. Biogeochemical investigations at Kadzharan, Armenian S.S.R.

Maquinay, A. and Ramaut, J. L. (1960). *Naturalistes Belg.* **41**, 265–273. La teneur en zinc des plantes du *Violetum calaminariae*.

Maquinay, A., Lamb, I. M., Lambinon, J. and Ramaut, J. L. (1961). *Physiologia Pl.* **14**, 284–289. Dosage du zinc chez un lichen calaminaire belge; *Stereocaulon nanodes* Tuck f. *tyroliense* (Nyl.) M. Lamb.

Marmo, V. (1953). *Econ. Geol.* **48**, 211–224. Biogeochemical investigations in Finland.

Minguzzi, C. and Vergano, O. e. (1948). *Atti. Soc. Tosc. Sci. Nat.* **55**. Il contennto di nichel nelle ceneri di *Alyssum bertoloni* Desv.

Murayama, T. (1961). *Mem. Ehime Univ.* IIB, **4**, 53–66. Studies on the metabolic pattern of yeast with reference to its copper resistance. IV. Characteristics in the tricarboxylic acid cycle.

Nestvetaylova, N. G. (1955). *Trudy Vses. aerogeol. Tresta* **1**, 146–152. Geobotanical investigations in the search for ore deposits.

Nicolls, O. W., Provan, D. M. J., Cole, M. M. and Tooms, J. S. (1965). *Trans. Instn. Min. Metall.* **74**, 695–799. Geobotany and geochemistry in mineral exploration in the Dugald River Area, Cloncurry District, Australia.

Noble, M., MacGarvie, Q. D., Hams, A. F. and Leafe, E. L. (1966). *Pl. Path.* **15**, 23–28. Resistance to mercury of *Pyrenophora avenae* in Scottish seed oats.

Noguchi, A. (1956). *Kumamoto J. Sci. Ser. B.* **2**, 239–257. On some mosses of *Merceya*, with special reference to the variation and ecology.

Noguchi, A. and Furuta, H. (1956). *J. Hattori bot. Lab.* **17**, 32–44. Germination of spores and regeneration of leaves of *Merceya ligulata* and *M. gedeana*.

Novick, R. P. and Roth, C. (1968). *J. Bact.* **95**, 1335–1342. Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*.

Old, K. M. (1968). *Trans. Br. mycol. Soc.* **51**, 525–534. Mercury tolerant *Pyrenophora avenae* in seed oats.

Passow, H., Rothstein, A. and Clarkson, T. W. (1961). *Pharmac. Rev.* **13**, 185–224. The general pharmacology of heavy metals.

Persson, H. (1948). *Revue bryol. lichen* **17**, 75–78. On the discovery of *Merceya ligulata* in the Azores with a discussion of the so-called "copper mosses".

Persson, H. (1956). *J. Hattori Bot. Lab.* **17**, 1–18. Studies in "copper mosses".

Peterson, H. B. and Monk, R. (1967). Vegetation and metal toxicity in relation to mine and mill wastes—an annotated bibliography. *Utah State Univ. Agr. Exp. Sta., Circ. No. 148.*

Peterson, P. J. (1969). *J. exp. Bot.* **20**, 863–875. The distribution of zinc-65 in *Agrostis tenuis* Sibth. and *A. stolonifera* L. tissues.

Pigott, C. D. and Walters, S. M. (1954). *J. Ecol.* **42**, 95–116. On the interpretation of the discontinuous distributions shown by certain British species of open habitats.

Poelt, J. (1955). *Verh. zool.-bot. Ges. Wien* **95**, 107–113. Flechten der Schwarzen Wand in der Grossarl.

Poore, M. E. D. (1955a). *J. Ecol.* **43**, 226–244. The use of phytosociological methods in ecological investigations. I. The Braun-Blanquet system.

Poore, M. E. D. (1955b). *J. Ecol.* **43**, 245–269. The use of phytosociological methods in ecological investigations. II. Practical issues involved in an attempt to apply the Braun-Blanquet system.

Poore, M. E. D. (1955c). *J. Ecol.* **43**, 606–651. The use of phytosociological methods in ecological investigations. III. Practical applications.

Poore, M. E. D. (1956). *J. Ecol.* **44**, 28–50. The use of phytosociological methods in ecological investigations. IV. General discussion of phytosociological problems.

Prat, S. (1934). *Ber. dt. bot. Ges.* **52**, 65–67. Die Erblichkeit der Resistenz gegen Kupfer.

Prat, S. and Komarek, K. (1934). *Zvlastni otisk ze sbornicku map.* VII, **8**, 1–16. Vegetace u medenych dolu.

Putwain, P. D. (1963). "Zinc tolerance in *Anthoxanthum odoratum.*" B.Sc. dissertation, University College of North Wales.

Rabanus, A. (1931). *Angew. Bot.* **13**, 352–357. The toximetric testing of wood preservatives.

Ramaut, J. L. (1964). *Naturalistes belg.* **45**, 133–145. Un aspect de la pollution atmospherique: l'action des poussieres de zinc sur les soles et les vegetaux dans la region de Prayon.

Rankama, K. (1947). *Min. Metall., N.Y.* **28**, 282–284. Some recent trends in prospecting.

Rankama, K. (1954). In "Geochemical Methods for Exploration of Ore Deposits". Moscow, II. The uses of trace elements in the solution of certain problems in applied geology.

Razzell, W. E. and Trussell, P. C. (1963). *Appl. Microbiol.* **11**, 105–110. Microbiological leaching of metallic sulfides.

Repp, G. (1963). *Protoplasma* **57**, 643–659. Die Kupferresistenz des Protoplasmas hoherer Pflanzen auf Kupfererzboden.

Richards, C. A. (1925). *Proc. Am. Wood-Preservers Assoc.* 1925, 18–22. The comparative resistance of eighteen species of wood destroying fungi to zinc chloride.

Riddell, J. E. (1952). *Prelim. Rep. Dep. Mines, Queb.* **269**, 1–15. Anomalous copper and zinc values in trees in Holland Township, Gaspe Peninsula, North County.

Robinson, W. O., Lakin, H. W. and Reichen, L. E. (1947). *Econ. Geol.* **43**, 572–583. The zinc content of plants on the Friedensville zinc slime ponds in *relation to biochemical* prospecting.

Rosenfeld, I. and Beath, O. A. (1965). "Selenium-Geobotany, biochemistry, toxicity and nutrition." Academic Press, New York and London.

Ruhling, A. and Tyler, G. (1968). *Bot. Notiser* **121**, 321–342. An ecological approach to the lead problem.

Ruhling, A. and Tyler, G. (1969). *Bot. Notiser* **122**, 248–259. Ecology of heavy metals—a regional and historical study.

Rune, O. (1953). *Acta phytogeogr. suec.* **31**, 1–139. Plant life on serpentine and related rocks in the North of Sweden.

Russell, G. and Morris, O. P. (1970). *Nature, Lond.*, **228**, 288–289. Copper tolerance in the marine fouling alga, *Ectocarpus siliculosus.*

Russell, P. (1955). *Nature, Lond.* **176**, 1123–1124. Inactivation of phenyl mercuric acetate in groundwood pulp by a mercury resistant strain of *Penicillium roqueforti* Thom.

Schacklette, H. T. (1965). *Geol. Survey. Bull. 1198C. Washington.* Bryophytes associated with mineral deposits and solutions in Alaska.

Schatz, A. (1955). *Bryologist* **58**, 113–120. Speculations on the ecology and photosynthesis of "copper mosses".

Schmidt, W. E., Haag, H. P. and Epstein, E. (1965). *Physiol. Pl.* **18**, 860-869. Absorption of zinc by excised barley roots.

Schubert, R. (1952). "Die Pflanzengesellschaften der schwermetallhaltigen Boden des Ostlichen Harzvorlandes." Diss. Halle/S.

Schubert, R. (1953). *Wiss. Z. Martin-Luther-Univ. Halle-Wittenb.* **3**, 51–70. Die Schwermetallpflanzengesellschaften des ostlichen Harzorlandes.

Schubert, R. (1954a). *Wiss. Z. Martin-Luther-Univ. Halle-Wittenb.* **3**, 863-882. Zur Systematik und Pflanzengeographie der charakterpflanzen der Mitteldeutschen Schwernetallpflanzengesellschaften.

Schubert, R. (1954b). *Wiss. Z. Martin-Luther-Univ. Halle-Wittenb.* **4**, 99-120. Die Pflanzengesellschaften der Bottendorfer Hohen.

Schultz, A. (1912). *Jber. westf. ProvVer. Wiss. Kunst.* **40**, 209-227. Uber die auf schwermetallhaltigen Boden wachsenden Phanerogamen Deutschlands.

Schwanitz, F. and Hahn, H. (1954a). *Z. Bot.* **42**, 179–190. Genetischentwicklungsphysiologische Untersuchungen an Galmei pflanzen. I. Pflanzengrosse und Resistenz gegen Zinksulfat bei *Viola lutea* Hudson, *Alsine verna* L., und *Silene inflata* Sm.

Schwanitz, F. and Hahn H. (1954b). *Z. Bot.* **42**, 459–471. Genetischentwicklungsphysiologische Untersuchungen an Galmeipflanzen. II. Uber Galmeibiotypen bei *Linum catharticum* L., *Campanula rotundifolia* L., *Plantago lanceolata* L. und *Rumex acetosa* L.

Schwickerath, M. (1931). *Beitr. NatDenkmPflage.* **14**, 463–503. Das Violetum calaminariae der Zinkboden in der Umgebund Aachens. Eine Pflanzensoziologische Studie.

Seal, K. (1970). "The isolation and preliminary investigation of copper tolerance shown by strains of *Aspergillus serreus* and *Penicillium notatum.*" M.Sc. Thesis, University of Wales.

Seno, T. (1962). *Japan. J. Genet.* **37**, 207–217. Genetical studies on copper resistance of *Saccharomyces cerevisiae.*

Sheridan, J. E., Tickle, J. H. and Chin, Y. S. (1968). *N.Z. J. Agric. Res.* **11**, 601–605. Resistance to mercury of *Pyrenophora avenae* (condial state *Helminthosporium avenae*) in New Zealand seed oats.

Shimazono, H. (1951). *J. Jap. For. Soc.* **33**, 393–397. The biochemistry of wood rotting fungi: the accumulation of oxalic acid.

Shimwell, D. (1967). (Manuscript.) Heavy metal plant communities in the Pennines.

Singewald, J. T. (1928). *Maryland Geol. Survey Repts.* **12**, 158–191. The chrome industry in Maryland.

Smith, J. M. (1966). *Am. Nat.* **100**, 637–650. Sympatric speciation.

Smith, R. A. H. and Bradshaw, A. D. (1970). *Nature, Lond.* **227**, 376–377. The reclamation of toxic metalliferous wastes.

Soane, B. D. and Saunder, D. H. (1959). *Soil Sci.* **88**, 322–330. Nickel and chromium toxicity of serpentine soils in Southern Rhodesia.

Somers, E. (1963). *Ann. appl. Biol.* **51**, 425–437. The uptake of copper by fungal cells.

Spanis, W. C., Munnecke, D. E. and Solberg, R. A. (1962). *Phytopathology* **52**, 455–462. Biological breakdown of two organic mercurial fungicides.

Spence, D. (1970). (Manuscript.) Scottish serpentine vegetation.

Spilling, C. and Thomas, D. (1964). "Zinc tolerance in *Rumex acetosa.*" B.Sc. Dissertation, University College of North Wales.

Starkey, R. L. (1964). *In* "Principles and Applications in Aquatic Microbiology" (Henkelekian and Dondero, eds.). Discussion on paper by Ehrlich, H. L. (1964). John Wiley & Sons, Inc., New York.

Starkey, R. L. and Waksman, S. A. (1943). *J. Bact.* **45**, 509. Fungi tolerant to extreme acidity and high concentrations of copper sulfate.

Stebbins, G. L. (1942). *Madroño* **6**, 241–272. The genetic approach to problems of rare and endemic species.

Street, H. E. and Goodman, G. T. (1967). *In* "The Lower Swansea Valley Project", K. J. Hilton (Ed.), pp. 71–110. Longmans, Green, London. Revegetation techniques in the lower Swansea Valley.

Suchodoller, A. (1967). *Ber. schweitz. bot. Ges.* **77**, 266--308. Untersuchungen uber den Bleigehalt von Pflanzen in der Nahe von Strassen und uber die Aufnahme und Translokation von Blei durch Pflanzen.

Sudzuki-Hills, F. (1963). *Agr. Tec. Chile* **23/24**, 15–62. Relave de cobra y aquas de riego del Rio Cachapoal.

Swedish Royal Commission on Natural Resources (1967). *Oikos* Suppl. 9, 9–51. The Mercury Problem: Symposium concerning Mercury in the Environment, Stockholm, Sweden, 1966.

Taschenberg, E. F., Mack, G. L. and Gambrell, F. L. (1961). *J. agric. Fd. Chem.* **9**, 207–209. DDT and copper residues in a vineyard soil.

Taylor, J. (1953). *Phytopathology* **43**, 268–270. The effect of continual use of certain fungicides on *Phylaspora obtusa.*

Temple, K. L. and Le Roux, N. W. (1964). *Econ. Geol.* **59**, 647–655. Syngenesis of sulphide ores: desorption of adsorbed metal ions and their precipitation as sulphides.

Terui, G., Mochizuki, T., Irie, R. and Takano, M. (1960). *Technol. Rept. Osaka Univ.* **10**, 279–290. Mass adaptation of mold spores to acquire drug resistance in the course of germination.

Thyssen, S. von. (1942). *Beitr. angew. Geophys.* **10**, 35–84. Geochemical and botanical relationships in the light of applied geophysics.

Tonomura, K., Maeda, F. and Futai, F. (1968). *J. Ferment. Technol., Osaka* **46**, 685–692. Studies on the action of mercury-resistant micro-organisms on mercurials.

Tonomura, K., Nakagami, T., Futai, F. and Maeda, K. (1968). *J. Ferment. Technol., Osaka* **46**, 506–512. Studies on the action of mercury-resistant micro-organisms on mercurials.

Tonomura, K., Maeda, K., Futai, F., Nakagami, T. and Yamada, M. (1968). *Nature, Lond.* **217**, 644. Stimulative vaporization of phenylmercuric acetate by mercury-resistant bacteria.

Tooms, J. S. and Jay, J. R. (1964). *Econ. Geol.* **59**, 826–834. The role of the biochemical cycle in the development of copper/cobalt anomalies in the freely drained soils of the Northern Rhodesian Copperbelt.

Tsung-Shan, K. (1957). *Scientia sin.* **6**, 1105–1119. Skarn type of metamorphic copper ore deposits in Lower Yangtse.

Turner, R. G. (1967). "Experimental Studies on Heavy Metal Tolerance." Ph.D. Thesis, University of Wales.

Turner, R. G. (1969). *In* "Ecological Aspects of the Mineral Nutrition of Plants". (I. H. Rorison, ed.). *Brit. Ecol. Soc. Symp.* **9**, pp. 399–410. Heavy metal tolerance in plants.

Turner, R. G. (1970). *New Phytol.* **69**, 725–731. The subcellular distribution of zinc and copper within the roots of metal tolerant clones of *Agrostis tenuis* Sibth.

Turner, R. G. and Gregory, R. P. G. (1967). *In* "Isotopes in Plant Nutrition and Physiology", pp. 493–509. Vienna: I.A.E.A./F.A.O. Symposium. The use of radioisotopes to investigate heavy metal tolerances in plants.

Turner, R. G. and Marshall, C. (1971). *New Phytol.* **70**, 539–545. The accumulation of ^{65}Zn by root homogenates of zinc tolerant and non-tolerant clones of *Agrostis tenuis* Sibth.

Tuxen, R. (1937). *Mitt. flor.-soz. ArbGemein.* **3**. Die Pflanzengesellschaften Nordwestdeutschlands.

Url, W. (1955). *Sber. Akad. Wiss. Wien. Mathem.-naturw. Klasse Abt. I.* **164**, 207–230. Resistenz von Desmidiaceen gegen Schwermetallsake.

Url, W. (1956). *Protoplasma* **46**, 768–693. Über Schwermetall-, zumal Kupferresistenz einiger Moose.

Urquhart, C. (1971). *Heredity* **26**, 19–33. Genetics of lead tolerance in *Festuca ovina*.

Vernon, L. P., Magnum, J. H., Beck, J. V. and Shafia, F. M. (1960). *Archs. Biochem. Biophys.* **88**, 227–231. Studies on a ferrous-ion-oxidising bacterium. II. Cytochrome composition.

Vinogradov, A. P. (1955). *Tr. Biogeokhim. Lab. Akad. Nauk SSSR.* No. **3**, 5–31. Elemental composition of organisms and D. I. Mendelleev's Periodic System.

Vogt, T. (1939). *K. norske Vidensk. Selsk. Skr.* **12**, 81–84. Chemical and botanical ore prospecting in the Røros area.

Vogt, T. (1942a). *K. norske Vidensk. Selsk. Skr.* **15**, 5–8. Geochemical and geobotanical ore prospecting. II. *Viscaria alpina* (L) G. Don.

Vogt, T. (1942b). *K. norske Vidensk. Selsk. Skr.* **15**, 21–24. Geochemical and geobotanical ore prospecting. III. Some notes on the vegetation at the ore deposits at Røros.

Vogt, T. and Braadlie, O. (1942). *K. norske Vidensk. Selsk. Skr.* **15**, 25-28. Geochemical and geobotanical ore prospecting. IV. Vegetation and soil at the ore deposits at Røros.

Vogt, T. and Bugge, J. (1943). *K. norske Vidensk. Selsk. Skr.* **16**, 51–54. Geochemical and geobotanical ore prospecting. VIII. Determination of copper in plants from the Røros area by quantitative X-ray analysis.

Vogt, T., Braadlie, O. and Bergh, H. (1943). *K. norske Vidensk. Selsk. Skr.* **16**, 55–58. Geochemical and geobotanical ore prospecting. IX. Determination of Cu, Zn, Pb, Mn and Fe in plants from the Røros area.

Von Rosen, G. (1964). *Hereditas* **51**, 89–134. Mutations induced by the action of metal ions in Pisum. II. Further investigations on the mutagenic action of metal ions and comparison with the activity of ionizing radiation.

Vose, P. B. and Randall, P. J. (1962). *Nature, Lond.* **196**, 85–86. Resistance to aluminium and manganese toxicities in plants related to variety and cation exchange capacity.

Wachsmann, C. (1961). *Dissertation en der Mathematisch-Naturwissenschaftlichen Fakultät der Westfalischen Wilhelms-Universität zu Munster in Referaten* **19**, 35–46. Wasserkulturversuche zur Wirkung von Blei, Kupfer und Zink auf die Gartenform und Schwermetallbiotypen von *Silene inflata* Sm.

Walker, R. B. (1954). *Ecology* **35**, 259–266. The ecology of serpentine soils. II. Factors affecting plant growth on serpentine soils.

Walley, K. A. (1967). "A study of gene flow in populations of *Agrostis tenuis*." B.Sc. Dissertation, University College of North Wales.

Warncke, E. (1968). *Bot. Tidsskr.* **63**, 358–368. *Marchantia alpestris* in Denmark.

Warren, H. V. and Delavault, R. E. (1948). *Geophysics*, **48**, 609–624. Biogeochemical investigations in British Columbia.

Warren, H. V. and Delavault, R. E. (1949). *Bull. geol. Soc. Am.* **60**, 531–560. Further studies in biogeochemistry.

Warren, H. V. and Delavault, R. E. (1950a). *Bull. geol. Soc. Am.* **61**, 123–128. Gold and silver content of some trees and horsetails in British Columbia.

Warren, H. V. and Delavault, R. E. (1950b). *Trans. Can. Inst. Min. Metall.* **53**, 236–270. A history of biochemical investigation in British Columbia.

Warren, H. V. and Delavault, R. E. (1960). *Trans. R. Soc. Can. Sect. IV* **54**, 11–20. Observations on the biogeochemistry of lead in Canada.

Warren, H. V. and Delavault, R. E. (1962). *J. Sci. Fd Agric.* **2**, 96–98. Lead in some food crops and trees.

Warren, H. V. and Howatson, C. H. (1947). *Bull. geol. Soc. Am.* **58**, 803–820. Biogeochemical prospecting for copper and zinc.

Warren, H. V., Delavault, R. E. and Fortescue, J. A. C. (1955). *Bull. geol. Soc. Am.* **66**, 229–238. Sampling in biogeochemistry.

Warren, H. V., Delavault, R. E. and Irish, R. I. (1949). *Proc. Trans. R. Soc. Can. 3rd Ser. Sect. IV* **43**, 119–138. Biogeochemical researches on copper in British Columbia.

Weston, R. L., Gadgil, P. D., Salter, B. R. and Goodman, G. T. (1965). *In* "Ecology and the Industrial Society". *Brit. Ecol. Soc. Symp.* **5**, 297–325. Problems of revegetation in the lower Swansea Valley, an area of extensive dereliction.

White, W. H. (1950). *Trans. Can. Inst. Min. Metall.* **53**, 243–246. Plant anomalies related to some British Columbia ore deposits.

Wild, H. (1968). *Kirkia* **7**, 1–71. Geobotanical anomalies in Rhodesia. I. The vegetation of copper-bearing soils.

Wild, H. (1970). *Kirkia* **7** supplement, 1–62. Geobotanical anomalies in Rhodesia. 3. The vegetation of nickel-bearing soils.

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

Wilkins, D. A. (1960). *Rep. Scott. Pl. Breed. Stn.* 1960, 85–98. The measurement and genetic analysis of lead tolerance in *Festuca ovina.*

Williams, J. (1830). "Faunula Grustensis, being an outline of the natural contents of the Parish of Llanrwst." (See Mem. Geol. Survey Sp. Rept. Min. Res. Gt. Britain **23**, 59).

Williams, D. and Morgan, E. (1964). "Zinc tolerance in *Plantago lanceolata.*" B.Sc. Dissertation, University College of North Wales.

Williams, R. F. (1967). *J. Hyg. Camb.* **65**, 299–309. Mercury resistance and tetracycline resistance in *Staphylococcus aureus.*

Williams, S. T. (1970). Personal Communication. Department of Botany. University of Liverpool, Liverpool, U.K.

Wood, J. G. and Sibley, P. M. (1950). *Aust. J. scient. Res. Ser. B. Biol. Sci.* **3**, 14–27. The distribution of zinc in oat plants.

Woolhouse, H. W. (1970). *In* "Phytochemical Phylogeny", Phytochemical Soc. (Ed.) Academic Press, London. pp. 207-231. Environment and enzyme evolution in plants.

Yamamoto, T. (1963). *J. Inst. Polytech. Osaka Ag. Univ. Ser. D. Biol.* **14**, 159–165. Resistance of yeast (Saccharomyces cerevisiae) to high concentrations of strontium chloride.

Young, G. Y. (1961). *U.S. Dep. Agr. Forest Serv., Forest Prod. Lab. Rept. No. 2223*, 7 pp. Copper tolerance of some wood rotting fungi.