EFFECT OF HEAVY METAL POLLUTION ON PLANTS

Volume 1

Effects of Trace Metals on Plant Function

POLLUTION MONITORING SERIES

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Monks Wood Experimental Station,
Abbots Ripton, Huntingdon

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Volume 1

Effects of Trace Metals on Plant Function

Edited by

N. W. LEPP

Department of Biology, Liverpool Polytechnic, Liverpool, UK

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Preface

Trace metals occur as natural constituents of the earth's crust, and are ever present constituents of soils, natural waters and living matter. The biological significance of this disparate assemblage of elements has gradually been uncovered during the twentieth century; the resultant picture is one of ever-increasing complexity. Several of these elements have been demonstrated to be essential to the functions of living organisms, others appear to only interact with living matter in a toxic manner, whilst an ever-decreasing number do not fall conveniently into either category.

When the interactions between trace metals and plants are considered, one must take full account of the known chemical properties of each element. Consideration must be given to differences in chemical reactivity, solubility and to interactions with other inorganic and organic molecules. A clear understanding of the basic chemical properties of an element of interest is an essential pre-requisite to any subsequent consideration of its biological significance. Due consideration to basic chemical considerations is a theme which runs through the collection of chapters in both volumes.

Perhaps the single most important stimulus to the rapid expansion of research in this field has been the great strides made in analytical techniques, particularly during the past decade. In many respects these advances have proceeded more rapidly than our ability to place the resultant data in its proper perspective; each increase in analytical sensitivity or reduction of detection limits has highlighted the inherent problems of sample contamination. In addition, the present dependence of most readily-available techniques on liquid samples results in the production of complex analytical matrices which present analytical problems for several elements. The need to overcome this problem is one area in which future developments will be keenly awaited. A further, more

disturbing aspect of basic analytical procedures is the lack of rigorous verification of individual methodology; many inter-laboratory comparisons often reveal significant discrepancies in analytical precision on the same prepared samples. As analytical data is the core of the majority of research in this field, all workers should pay more than lip service to the validity of their analytical techniques.

Having summarised the inherent problems in this field of study, one must now consider the best way in which the ever-expanding literature on this topic can be treated to produce a meaningful compendium of information. Any editor is faced with a difficult dichotomy in the organisation of the subject matter. It is now conclusively established that the impact of trace metals on plants, in real world situations, is never the result of the effect of a single element. Thus, it could be cogently argued that treatment of individual plant/element responses, relating, in the main, to laboratory or glasshouse studies, is not a particularly valid approach to the topic. Nevertheless, a great deal of valuable information can still be gathered from this approach; the study of elemental interactions is still in a formative stage, with some being well known and clearly understood, whereas others, only recently identified, are still under careful investigation. At present, therefore, the adoption of the 'element in isolation' approach to be found in Volume 1, has been taken as a matter of necessity. Hopefully, in the next decade, this will not be the case, and workers will lean much more towards an interactive approach; but for the present, this compromise situation is the only feasible approach.

The individual elements, explored in Volume 1, illustrate the wide diversity of plant response to trace metals. As technology continues to concentrate previously dispersed and rare elements, removing these from their original matrices and altering their chemical form, great care will have to be taken in interpreting the potential consequences of these steps to living organisms. The information presented here illustrates many interesting facets of the basic interaction. Elements such as copper and zinc, long known as essential to life, are now being joined in this category by nickel. The alarming mobility of cadmium in soils and crops is highlighted, but the other toxic element, lead, is demonstrated to have a much lesser potential impact on crop production. Many elements will need to be exhaustively appraised as new directions in manufacturing industry and energy generation provide the potential to liberate more exotic and little-considered elements into the biosphere. These should be considered now, before problems arise.

One certain aspect of this area is that the consequences of metal

contamination will be with us for a considerable period of time. Thus, the need for more critical identification of potential future problems continues to exist and such studies should place particular emphasis on agriculture and food production. Lessons learnt in the past must be rigorously applied to future research considerations, and areas where the magnitude of potential problems has been overstated must be carefully re-appraised.

The second volume presents a more integrated approach to plant/trace metal interactions, but the majority of evidence presented here requires the sound base of information from individual studies of the 'plant–element' type described in Volume 1.

It is essential that we understand the sources of trace metal additions to our environment, the reaction these have with soils, their biogeochemical cycles, and their consequences for natural and agricultural systems. All these topics have received detailed coverage, and the synthesis of ideas presented will be of considerable relevance to those concerned with the monitoring, regulation and amelioration of trace metal contamination. Practical aspects of reclamation are also emphasised, with full documentation of successful case histories.

Finally, two topics deserved special attention, the tolerance of plants to certain trace metals, and the interaction between trace metals and lower terrestrial plants. Metal tolerance is a phenomenon often noted, but the understanding of the physiological and biochemical aspects of this fascinating aspect of plant metabolism has not progressed apace. Recent advances in our understanding of this biological puzzle are carefully detailed. Lower plants have an interesting interrelationship with trace metals. The infinite capacity of some bryophytes and lichens to sorb trace metals has led to their use as biological monitors for atmospheric metal burdens; the unique lichen symbiosis presents a system in which basic effects of metals on biological systems can be monitored and probed.

In the compilation of a work of this nature, it is inevitable, and indeed desirable, that areas of overlap will occur. The juxtaposition of several points of view in relation to a topic is a valuable part of any critical appraisal; thus no attempt has been made to reconcile any differences of opinion. The authors have been selected for their specialised knowledge of particular fields, and all have contributed to produce an integrated synthesis of the current status of plant/trace metal interactions. I would like to record my pleasure in editing these volumes, and to praise the consistently high standard and informative nature of each contribution. I would also like to thank the authors for their time, effort and ready co-operation in the assembly of this work. It is they, not I, who have translated

an idea into reality. Finally, I would like to thank my colleagues at Liverpool Polytechnic for their encouragement and advice, and also the publishers for their full and friendly assistance in all stages of the production of these volumes.

NICHOLAS W. LEPP

Contents

Preface v

List of Contributors xi

1 CHEMISTRY AND BIOCHEMISTRY OF TRACE METALS IN BIOLOGICAL SYSTEMS 1
D. A. Phipps

2 LEAD: UNDERSTANDING THE MINIMAL TOXICITY OF LEAD IN PLANTS 55
David E. Koeppe

3 CADMIUM 77
A. L. Page, F. T. Bingham and A. C. Chang

4 COPPER 111
N. W. Lepp

5 ZINC 145
J. C. Collins

6 NICKEL 171
T. C. Hutchinson

7 OTHER TRACE METALS 213
P. J. Peterson and C. A. Girling

8 METALLOIDS 279
P. J. Peterson, L. M. Benson and R. Zieve

Index 343

List of Contributors

L. M. BENSON

Department of Botany and Biochemistry, Westfield College, University of London, Kidderpore Avenue, London NW3 7ST, UK

F. T. BINGHAM

Department of Soil and Environmental Sciences, University of California, Riverside, California 92521, USA

A. C. CHANG

Department of Soil and Environmental Sciences, University of California, Riverside, California 92521, USA

J. C. COLLINS

Department of Botany, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK

C. A. GIRLING

Chemistry Division, Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey KT22 7SF, UK

T. C. HUTCHINSON

Department of Botany, University of Toronto, Toronto, Ontario M5S 1A1, Canada

D. E. KOEPPE

Department of Agronomy, University of Illinois, Urbana, Illinois 61801, USA

N. W. LEPP

Department of Biology, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, UK

A. L. PAGE

Department of Soil and Environmental Sciences, University of California, Riverside, California 92521, USA

P. J. PETERSON

Department of Botany and Biochemistry, Westfield College, University of London, Kidderpore Avenue, London NW3 7ST, UK

D. A. PHIPPS

Department of Chemistry and Biochemistry, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, UK

R. ZIEVE

Department of Botany and Biochemistry, Westfield College, University of London, Kidderpore Avenue, London NW3 7ST, UK

CHAPTER 1

Chemistry and Biochemistry of Trace Metals in Biological Systems

D. A. PHIPPS

Department of Chemistry and Biochemistry, Liverpool Polytechnic, UK

1. INTRODUCTION

The past three decades have seen a remarkable advance in the appreciation of the significant role played by the so-called 'trace metals' in the health and productivity of plants. The effort has been spread widely, from the macro-ecological viewpoint at one extreme, down to the molecular viewpoint and, encouragingly, some highly successful attempts have been made to deal with the intimate details of the actions of metal ions, both 'essential' and 'toxic'. Whilst it is certainly true that real advances have been made over a broad front, it is also disturbingly true that the picture remains fragmented, and as with most areas of scientific research today, the sheer volume of experimental data now available makes it impossible to scrutinise more than a fraction of the existing material. The problem is aggravated by the constant necessity to make a critical re-appraisal of much published material, since it is sadly apparent that the quantity of data greatly outweighs its quality.

It does seem that there is a growing body of published material the value of which must be seriously questioned. It is not merely that newer discoveries have invalidated older conclusions, which is unfortunate but inevitable and acceptable, but rather (and this is much more distressing) that the validity of the results themselves is often suspect. This can be attributed to a number of causes, but commonly it is apparent that authors have either failed to understand or to apply those chemical principles which must govern the interaction of 'trace metals' and plants. More particularly,

Period	Group Ia	Group IIa	Group IIIa	Group IVa	Group Va	Group VIa	Group VIIa	Group VIII			Group Ib	Group IIb	Group IIIb	Group IVb	Group Vb	Group VIb	Group VIIb	Group O
1	1																1	2
1s	**H**																**H**	**He**
2	3	4											5	6	7	8	9	10
2s 2p	**Li**	**Be**											**B**	**C**	**N**	**O**	**F**	**Ne**
3	11	12											13	14	15	16	17	18
3s 3p	**Na**	**Mg**											**Al**	**Si**	**P**	**S**	**Cl**	**Ar**
4	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
4s 3d	**K**	**Ca**	**Sc**	**Ti**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Ga**	**Ge**	**As**	**Se**	**Br**	**Kr**
4p																		
5	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
5s 4d	**Rb**	**Sr**	**Y**	**Zr**	**Nb**	**Mo**	**Tc**	**Ru**	**Rh**	**Pd**	**Ag**	**Cd**	**In**	**Sn**	**Sb**	**Te**	**I**	**Xe**
5p																		
6	55	56	57*	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
6s	**Cs**	**Ba**	**La**	**Hf**	**Ta**	**W**	**Re**	**Os**	**Ir**	**Pt**	**Au**	**Hg**	**Tl**	**Pb**	**Bi**	**Po**	**At**	**Rn**
(4f)																		
5d																		
6p																		
7	87	88	89**															
7s	**Fr**	**Ra**	**Ac**															
(5f)																		
6d																		
*Lanthanide series 4f			58 **Ce**	59 **Pr**	60 **Nd**	61 **Pm**	62 **Sm**	63 **Eu**	64 **Gd**	65 **Tb**	66 **Dy**	67 **Ho**	68 **Er**	69 **Tm**	70 **Yb**	71 **Lu**		
**Actinide series 5f			90 **Th**	91 **Pa**	92 **U**	93 **Np**	94 **Pu**	95 **Am**	96 **Cm**	97 **Bk**	98 **Cf**	99 **Es**	100 **Fm**	101 **Md**	102 **No**	103 **Lr**		

FIG. 1.1 The Periodic Table of the Elements, showing five major groups of metals. s-block, [ ]; p-block, [ ]; d-block transition, [ ]; f-block lanthanon, [ ]; f-block actinon, [ ].

the validity of the chemical analysis, upon which so much of this work rests, leaves much to be desired.

Although it may be argued that this view is too extreme, it must be appreciated that chemical expertise, whether in designing experiments or evaluating analyses, is a central prerequisite to this whole area of study. Of course, it cannot sensibly be argued that a chemical or molecular viewpoint will solve all the problems currently under investigation, but it can and must be argued that without care for the chemical fundamentals, confusion is more likely than any further advance.

2. METALS AND THEIR CLASSIFICATION

2.1. Defining a Metal

Given the enormous amount of information now available concerning plant–metal interactions, and if it is accepted that a thorough understanding of chemical principles is an essential prerequisite to the orderly development of this subject, then it is natural to look at chemical classification via the Periodic Table (Fig. 1.1), as a basis for further discussions. Certainly, the classic definition of a metal, which refers almost exclusively to the physical properties of the elemental state (malleability, ductility, conductivity, etc.) is largely irrelevant for present purposes. If an alternative is required then it is certainly better to begin categorising elements in terms of their chemical reactivity. It has been suggested that the metals may be recognised as those elements which under biologically significant conditions tend to exist as cations (Phipps, 1976). Acting as Lewis acids (*vide infra*) the metal ions form complexes with a wide range of ligands, both organic and inorganic, and these complexes are then the biologically active species. Of course, such a sweeping definition does have exceptions, but it has the advantages of focusing attention directly on the metal ion, whilst eliminating elements such as boron, arsenic and selenium, which are often mistakenly included as trace metals by virtue of being 'trace elements'. Such elements are perhaps best considered as 'metalloids' and have somewhat different chemical characteristics. Happily, the definition of a metal advanced here coincides quite well with the classic physical definition, but unfortunately, well over two-thirds of all elements can be described as metals, so that it rapidly becomes clear that further refinement of the classification scheme is necessary if it is to prove at all useful.

2.2. Functional Classifications

One way in which the metals are commonly categorised is by a series of

TABLE 1.1
SOME COMMONLY ACCEPTED FUNCTIONALLY DESCRIPTIVE TERMS USED TO CLASSIFY METALS IN BIOENVIRONMENTAL STUDIES

Description	Comments
Metal	Metals may be defined by the physical properties of the elemental state, but are better identified by consideration of their chemical properties. The term is used indiscriminately to refer to both the element and compounds (e.g. 'the uptake of copper by . . .' does not distinguish the form in which the metal is absorbed).
Heavy Metal	A hopelessly imprecise and thoroughly objectionable term! It originates from a categorisation by density—hardly biologically significant. The term is now often used to mean any metal with atomic number >20, but there is no general concurrence.
Essential Metal	Broadly, one which is required for the complete life cycle of the organism and whose absence produces specific deficiency symptoms relieved only by that metal and whose effect is shown by a dose–response curve. The term is often used misleadingly since it should be accompanied by a statement of which organisms show a requirement for the element. New members, e.g. Ni, have recently been added to the group.
Beneficial Metal	An older term, now largely disused, which implied that a non-essential metal could improve vigour.
Toxic Metal	*All* metals are toxic, but the degree of toxicity varies greatly from metal to metal and from organism to organism. Toxicity, like essentiality, may be defined by a dose–response curve.
Abundant Metal	Usually refers to the proportion of the element in the earth's crust, though may be defined in terms of other regions, e.g. oceans, 'fresh water', etc.
Available Metal	One which is found in a form which is easily assimilated by living organisms.
Trace Metal	A metal found in low concentration, perhaps ppm or less, in some specified source, e.g. soil, plant, tissue, ground water, etc. Sometimes this term has confusing overtones of low nutritional requirement (by a specified organism).
Micro-nutrient	More recent term to describe the second of the meanings of trace metal, above.

functional terms, as outlined in Table 1.1, but the limitations of such terms are all too obvious. They are both arbitrary and imprecise in meaning. Several categories overlap heavily, which does not make for exact usage. Probably least useful is the term 'heavy metal', often used with perjorative connotations of pollution and toxicity; but surely any definition based on *density* (Lapades, 1974) has no place in a biological discussion, though undoubtedly the term will remain widely used as a convenient (but extremely misleading) shorthand despite efforts to oust it (Nieboer and Richardson, 1980). Similarly, *trace metal* is both widely used and poorly defined. Thus 'trace metal' may be taken by different readers to imply different descriptions; it may refer to geochemical abundance (which itself is subject to geographic variability) or the content of the metal in a living organism (which may in turn vary considerably from cell to cell or even within cellular boundaries). However, it is some comfort, at least, that the older meaning, reflecting nutritional content or requirement, has largely been replaced by the term 'micronutrient'. (For a general discussion of plant mineral nutrition see Hewitt and Smith (1974) or Sutcliffe and Baker (1974).) It is unlikely that such broad meanings, often defined by the context in which they are used, will be displaced; though it is to be hoped that whenever such a description is used it will be accompanied by the appropriate qualifications. It is therefore essential that whenever such categorisations are made they do not obscure basic differences within the group. Thus the 'trace metals', however envisaged, are an extremely disparate group with little in common except that they are broadly present in low concentration. This classification should be used with care since, all too often, it is taken to imply some functional similarity.

2.3. Classification via the Periodic Table

In an attempt to resolve this dilemma, and as an alternative to functional descriptions of the metals—essential, toxic micronutrient, etc.—it is possible to revert to a purely chemical classification. Neither route is entirely satisfactory by itself, but taken together they provide a sensible framework for discussion (Phipps, 1976; Nieboer and Richardson, 1980); it is certain, however, that in the majority of cases a chemical categorisation and explanation in terms of chemical properties underpin the functional description.

Any chemical classification for 'trace metals' must be related to bio-environmental processes and must seek to provide an informative basis for the discussion and solution of such problems as uptake selectivity (da Silva and Williams, 1976), functional role (Hughes, 1972; Hewitt and Smith,

TABLE 1.2
THE BIOLOGICAL SIGNIFICANCE OF BROAD CATEGORIES OF METALS

Grouping	Biologically significant chemical properties
s-block	The alkali-metal ions are highly mobile, normally forming only weak complexes, they act chiefly as bulk electrolytes. The alkaline earths form more stable complexes and therefore have more specialised functional roles as structure promoters and enzyme activators. Neither group has any significant redox chemistry *in vivo*.
p-block	Some, albeit limited, redox chemistry, e.g. Pb^{4+}/Pb^{2+} complicates the action of these metals. They generally form more stable complexes, the heavier elements being especially avid sulphur seekers which may account for some toxic activities.
d-block	These metals show an extremely wide range of both redox behaviour and complex formation. These properties fit them for their roles as 'biological catalysts'.
f-block	Though these metals show a wide range of redox behaviour and complex formation they are biologically insignificant under normal circumstances, but some (the actinons) may be especially important pollutants.

1974; Sutcliffe and Baker, 1974; Phipps, 1976), or toxicity pattern (Foy *et al.*, 1978). Within this restriction a number of alternatives are available.

The first, and clearly the most complete and orthodox classification, is the separation of the metals into almost 20 groups, as is done in the conventional Periodic Table (Fig. 1.1). Then, in each of the groups the members are related by (valence) electron configuration and hence by similarities in chemical reactivity. Certainly, such group classification which has long dominated inorganic chemistry, and is still regularly discussed (Puddephatt, 1972), must have its place in what is now conveniently called bio-inorganic chemistry, since it illustrates so well trends in behaviour, similarities and differences between elements, both within the groups and also between the groups themselves. However, such a complete separation probably produces far too many categories for the present use.

The second, and for many purposes, more convenient classification, is the separation of the metals into just five broad categories, but still based on the classic periodic system. Figure 1.1 shows that the metals are conveniently categorised as s-block, p-block, d-block transition, f-block lanthanon and f-block actinon, and Table 1.2 lists some pertinent comments about their biologically significant properties. However, even this scheme is not entirely successful, since it is based on consideration of

general reactivity and fails to emphasise sufficiently the broad differences between the metal ions in each of the different sections. However, when coupled with the next scheme it provides a powerful basis on which the treatment of behaviour of the metal ions can be based.

2.4. Lewis Acid Behaviour

Of great significance in the appreciation of the bio-environmental classification of the metals is the growing acceptance of the view that the interaction of metals with living systems is dominated by the properties of

TABLE 1.3
THE CLASSIFICATION OF SOME METAL ION ACCEPTOR PROPERTIES

Class *a*	Li^+, Na^+, K^+, Rb^+, Cs^+
	Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}
	Sc^{3+}, $La^{3+} \longrightarrow Lu^{3+}$
	$Ac^{4+} \longrightarrow$?
	Ti^{4+}, Zr^{4+}, Hf^{4+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{3+}
Class *b*	Cu^+, Ag^+, As^+, Cd^{2+}, Hg^+, Hg^{2+}, Tl^+
	Pd^{2+}, Pt^{2+}
Borderline	Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}
	Rh^{3+}, Ir^{3+}, Ru^{3+}, Os^{2+}
	Sb^{3+}, Bi^{3+}, Pb^{4+}

the metal ion as a Lewis acid (Lewis, 1923). Consequently, the problem of classification is reduced to assessing the behaviour of metal ions as electron acceptors, that is to say to appreciating their behaviour in complex formation. The currently accepted categorisation of metal ions in terms of differential Lewis acidity is well over 20 years old (Ahrland *et al.*, 1958). In the original scheme, *metal ions* were described as *class* a, *class* b or *borderline*, depending on their observed affinity for different ligands. (This should not be confused with the A,B sub-group classification of the conventional Periodic Table.)

Table 1.3 lists metal ions by class. In general, there is a relatively sharp separation between class *a* and borderline metal ions, but the difference between borderline and class *b* is less clearly defined. Although alternative descriptions have evolved, notably the use of the term *hard-acids* for class *a* ions and *soft-acids* for class *b* ions (Pearson, 1963, 1968*a*,*b*, 1969), the basic concept of the scheme remains unaltered.

The classification of metals by their Lewis acidity throws particular light

onto bonding in their complexes. Thus class *a* metal ions which are hard, or non-polarisable, preferentially form complexes with similar non-polarisable ligands, particularly oxygen donors, and the bonding in these complexes is mainly ionic. Conversely, class *b* or soft metal ions preferentially bind to polarisable, soft ligands to give rather more covalent bonding. In general, it is noticeable that such hard–hard or soft–soft combinations are preferred wherever possible.

The success of this scheme lies in the ability to predict both the preferred ligands and the general trend in the properties of the complexes. Thus the ultra-hard, s-block metals bind only poorly to soft ligands and form mainly ionically bound complexes with hard (oxygen) donor ligands, and as the bonding is mainly ionic, the metal ions are easily displaced and thus mobile. The p-block metal ions, in contrast, are generally softer, though Al^{3+} is much more like members of the s-block than its p-block congeners. The heavier p-block metals show strong affinity for soft ligands, such as sulphide or sulphur donors, and form highly covalent complexes from which they are difficult to displace, thus being relative immobile. It is interesting to note, in passing, that these two categories, *a* and *b*, have much in common with the older geochemical classification of metals (or rather their ions) as lithophile or chalcophile, and both rest on the same foundation. The borderline metal ions are much more difficult to treat. Such metals generally form relatively stable complexes with both hard and soft donor ligands; but the exact order of stability is not easily determined. First-row d-block transition metals fall mainly into this group, hence their widespread and variable coordination chemistry.

The success of this treatment belies its simplicity, but a certain amount of caution is advisable. It must be recognised that the classification refers in each case to a specific ion, so that in cases where the metal may exist in more than one oxidation state, each ionic form must be treated separately. In such cases, the ion with higher charge, which is therefore smaller and less polarisable, normally has considerable class *a* character (or at least less class *b* properties), whilst in the lower oxidation state the reverse is true. Thus Fe^{3+} is generally described as hard or class *a*, and, in keeping with this, binds preferentially to oxygen donor ligands such as phenolate or carboxylate groups in humic and fulvic acids, whilst Fe^{2+} is considered borderline and has a higher affinity for softer ligands. This can be extended to include the unsaturated nitrogen donors of the tetrapyrroles in heme, or the sulphide and thiolate groups in the ferredoxins. A further complicating factor can be seen in mixed ligand complexes, where the influence of one ligand affects the binding of the next, so that the class *b* character is

increased by binding a soft ligand, and mixed complexes with both hard and soft ligands are in general less stable.

Finally, it must be said that this classification scheme is not absolute (the very concept of borderline cases ensures this) and different authors may place the same metal ion into different classes. However, it is encouraging that in general, agreements far outweigh disagreement (Huheey, 1975; Nieboer and Richardson, 1980).

So far the *a* and *b* borderline classification has been treated empirically, by considering complex stability, but it is important to ask if their classification can be placed on a firmer footing. In general, this problem is not simple and requires quite extensive treatment (Pass, 1973; Burgess, 1978), but it is encouraging to note that it is receiving more attention, with some of the impetus arising from the desire to give chemical answers to biological questions (Williams, 1971; Hughes, 1972; Phipps, 1976; Nieboer and Richardson, 1980).

3. METAL ION CHEMISTRY IN AQUEOUS SOLUTION

3.1. Stability

Stability constants

The aqueous solution chemistry of metal ions plays a central and dominant role in determining their interaction and effect on plants, so it is fortunate that the fundamental principles of this area of coordination chemistry are well established, with numerous descriptions of aspects of this topic being available (Beck, 1970; Williams, 1971; Pass, 1973; Phipps, 1976; Purcell and Kotz, 1977; Burgess, 1978; Huheey, 1975). Nevertheless, for the sake of clarity and completeness, a brief account of the more important factors are given here, in order that the limitations of present treatments may be recognised and current problems delineated.

As has already been described, the metal ion as a Lewis acid will react with a wide variety of ligands and some factors relating to the stability of the complex have been outlined. Quantitatively, the tendency for metal–ligand interaction to occur is measured by the equilibrium constant for the reaction:

$$xM_{aq}^{n+} + yL^{m-} \rightarrow [M_xL_y]^{(nx-my)+}$$

$$K_{\text{stability}} = \frac{[M_xL_y]^{(nx-my)+}}{[M_{aq}^{n+}]_{\text{free}}^{x}[L^{m-}]_{\text{free}}^{y}}$$

where the terms in brackets refer to the equilibrium concentrations.

Also it is possible to define *conservation* equations such that:

$$[M^{n+}]_{total} = \sum [M^{n+}_{aq}]_{free} + [M_xL_y]^{(nx-my)+}$$

$$[L^{m-}]_{total} = \sum [L^{m-}]_{free} + [M_xL_y]^{(nx-my)+}$$

In many cases the complex formation can be considered to occur in a series of steps, so for example (omitting charges for the sake of simplicity)

$$M_{aq} + L \rightleftarrows ML_1 \qquad K_1 = \frac{[ML_1]}{[M][L]}$$

$$ML + L \rightleftarrows ML_2 \qquad K_2 = \frac{[ML_2]}{[ML_1][L]}$$

$$\vdots \qquad\qquad \vdots$$

$$ML_{n-1} + L \rightleftarrows ML_n \qquad K_n = \frac{[ML_n]}{[ML_{n-1}][L]}$$

where K_1 to K_n are described as stepwise stability constants, and the overall stability constant is merely the product of the microscopic stepwise constants

$$\beta_n = \prod_{i=1}^{n} K_i = \frac{[ML_n]}{[M][L]^n}$$

Again, conservation equations relating the total analytical concentration of the metal ion and the ligand to the sum of the individual species may be defined

$$[M]_{total} = [M]_{free} + \sum_{i=1}^{n} [ML_i] = \sum_{i=0}^{n} [ML_i]$$

$$[L]_{total} = [L]_{free} + \sum_{i=1}^{n} [ML_i]$$

Procedures for the determination of both the number of ligands and the appropriate formation constants are well known and have been described in detail elsewhere (Beck, 1970).

The proportions of individual complex species $ML_1, ML_2 \cdots ML_n$ will depend on the nature of the ligand and on the relative concentration of metal ion and ligand. Taking a unidentate donor species first, as the amount of ligand compared to metal ion increases, complexes with higher ratios of ligand to metal, i.e. higher n values, become increasingly significant, with the limit (commonly $n = 6$ for s, p and first row d-block metals but often

higher for second row d-block elements, lanthanons and actinons) being set by the fundamental properties of the metal ion itself. Clearly, if all the relevant stability constants are known, together with the analytical concentration for metal and ligand, it is possible to estimate the degree of formation (α_i) of each species.

$$\alpha_i = \frac{|ML_i|}{[M]_{total}}$$

$$\alpha_i = \frac{\beta_i [L]^i}{\sum_{i=0}^{n} \beta_i [L]^i}$$

There is then no difference in the treatment of polydentate ligands except that the maximum value of n will be related to the number of donor sites which can be used simultaneously (e.g. six for ligands such as EDTA, so that $n = 1$).

The use of stability constants can readily be extended to mixed metal ion/ligand systems. Thus in the simplest case of a direct substitution reaction

$$ML + L' \rightleftarrows ML' + L$$

$$K_{eq} = \frac{[ML'][L]}{[ML][L']} = \frac{K_{ML}}{K_{ML}}$$

and

$$ML + M' \rightleftarrows M'L + M$$

$$K_{eq} = \frac{[M'L][M]}{[ML][M']} = \frac{K_{M'L}}{K_{ML}}$$

This can obviously be extended to more complex reactions, though the algebraic solutions may not necessarily be so accessible. Alternatively, it is possible to consider the formation of mixed ligand complexes, with one of the most important cases being the formation of mixed ligand aquo complexes

$$M + L + L' + L'' \rightleftarrows MLL'L''$$

pH and hydrolysis reaction

One obvious extension of the treatment is to include the effect of pH on the system, considering reactions such as given below, where H^+ competes with the metal ion for the ligand (Morel *et al.*, 1973):

$$ML + H^+ \rightleftarrows M + LH$$

In this case the value of the acid-dissociation constant for the reaction

$$LH \rightleftarrows L^- + H^+$$

or rather the association constant, takes the place of the stability constant, and complex formation of any species capable of proton binding cannot be considered without accounting for the effect of H^+.

Equally, it is totally impossible to discuss meaningfully the behaviour of metal ions in aqueous solution without considering hydrolysis reactions. Any complex which contains a water molecule as a ligand can undergo hydrolysis. This is the simplest case:

$$[ML_y(OH_2)_z]^{m+} \rightleftarrows [ML_y(OH_2)_{z-1}(OH)]^{(m-1)+} + H^+$$

A rather fuller treatment requires consideration of the ligand substitution reactions as shown below:

$$[ML_y] + :OH_2 \rightleftarrows [ML_{y-1}(OH_2)] + L$$

but this can be treated as the formation of a mixed ligand complex, and in many cases the appropriate stability constants are available from consideration of the stepwise formation of $[ML_n]$.

The binding of a water molecule to a metal ion will alter the strength of the O–H bond, generally weakening it and promoting the ionisation of H^+. In some cases the effect is quite marked, as for example with Fe^{3+} where the acid dissociation constant for the reaction

$$[Fe(III)(OH_2)_6]^{3+} \rightleftarrows [Fe(III)(OH_2)_5(OH)]^{2+} + H^+$$

is $10^{-3\cdot0}$ (i.e. $pK_a = 3$) and $[Fe(III)(OH_2)_6]^{3+}$ is a protonic acid of similar strength to acetic acid. This of course may be extended, and a simplified scheme for further hydrolysis reaction is presented below:

$$\begin{array}{ccccc} [M(OH_2)_m]^{n+} & \rightleftarrows & [M(OH_2)_{m-1}(OH)]^{(n-1)+} & \rightleftarrows & [M(OH)_m]^{(n-m)+} \\ & & \downarrow\uparrow & & \\ & & [M_2(OH_2)_{2m-4}(OH)_2]^{(2n-2)+} & & \\ & & \downarrow\uparrow & & \\ & & [M(OH)_q]^0_{\text{solid}} & & \end{array}$$

Such a scheme, which is not exhaustive, indicates that both polynuclear species and solid phases may be formed, with considerable complication in predicting metal ligand equilibria. Careful consideration of such reactions is essential, since the precipitation of the metal hydroxide as a solid phase

removes M^{n+} ions from solution, so that equilibria must readjust to take this into account. At this stage, further metal hydroxide is formed and the 'cascade' continues until the concentration of metal ions remaining in solution is less than that demanded by the solubility product. In this way the metal ion may be removed from quite stable complexes, especially if the solubility of the hydroxide is low. An important case is the precipitation of $[Fe(OH)_3]$ from $[Fe(III)\ EDTA]^-$ at a pH of just above neutrality.

Fortunately, such reactions have been well studied, and useful operational diagrams relating to metal concentration, pH and metal hydroxide formation are available (Kragten, 1977). The removal of the metal ion as an insoluble phase such as this can also be extended to absorption reactions and the like.

The effect of dissolved gases

A whole range of further factors must be included in any realistic computational scheme. Amongst these are the effect of CO_2, both by alteration of pH and formation of HCO_3^- and CO_3^{2-} ions, and it would be almost trivial to deal with the problem of 'hard water', i.e.

$$CaCO_3 + CO_2 + H_2O \rightleftarrows Ca(HCO_3)_2$$

but similar equilibria exist for other metal ions. Equally, O_2 (or its absence), can affect the reaction substantially by controlling redox reactions. Taking the case of lead:

$$(PbO_2)_{solid} + 4H^+ + 4e^- \rightleftarrows Pb^{2+} + 2H_2O$$

$$O_2 + 4H^+ + 4e^- \rightleftarrows 2H_2O$$

whence

$$Pb^{2+} + O_2 \rightleftarrows (PbO_2)_{solid}$$

Consideration of the appropriate redox potentials enables the equilibrium constant for the reaction to be estimated, and comparison with solubility products for hydroxide or carbonate precipitation shows that oxide formation is the *thermodynamically* favoured reaction.

One other restriction on the use of stability constants is highlighted in the last sentence. Stability constant data apply only to *equilibrium* situations and, in many cases, the concentrations of ions in solution may be controlled kinetically rather than thermodynamically so that rate constants for conversion processes must also be known (or assumed), in these cases.

3.2. Computational Speciation

Stability constants, then, if correctly and critically applied, are remarkably

useful in describing aqueous metal ion/ligand systems, since when used in combination with the appropriate conservation equations they allow, in principle at least, the calculation of a complete description of the distribution of complexes within the system. This is conveniently described as *computational speciation.* In general, with systems containing large numbers of metal ions and ligands, direct algebraic solutions are not attempted, since extended systems of non-linear equations would need to be solved. Instead, various iterative techniques are adopted to produce a description of the solution. The literature dealing with this problem is growing rapidly, as is the sophistication of the treatment, so that in less than a decade advances have been made from computer programs capable of dealing with three metals, 22 ligands and a total of 93 complex species (Cumme, 1973) to a program which it was claimed could calculate concentrations for up to 400 soluble complexes of 20 different metal ions and include corrections for up to 80 solid phases (Sposito and Bingham, 1981).

The use of stability constants in this fashion, to calculate the overall composition of the solution, is subject to a number of serious limitations, which must be recognised if the value of the method is not to be overstated. The method is not predictive. It can only compute concentrations of species defined initially. That is to say, unless all the possible species are considered, it is possible to obtain a result from the iterative procedure which satisfies whatever criteria have been set by the designers of the algorithm, yet is erroneous, since it takes no account of a particular species which may in fact be present.

Alternatively, it is possible to account for all species and yet still derive a result from iteration which is erroneous. It is well known that if the first estimates, or 'trial' values, for an iterative method are considerably different from the 'true' values, then it is possible to reach an apparently acceptable set of values, which are in fact in error. This is only one of the many problems which must be overcome in designing the procedure.

Another probably more serious difficulty is found in dealing with minor components, whose concentrations are rather smaller. In such cases, even small errors in the stability constants might have disastrous effects on the calculated value and much of the effort which goes into the design of the computational treatment has to be directed at this problem. The cumulative effect of even small errors, where large numbers of constants are involved, may become extremely serious, as comparisons between similar studies show for copper (Leckie and Davis, 1979).

The constants are usually described as stoichiometric stability constants,

since they are written in terms of concentrations, but it would of course be more correct to give the expression in terms of activities, where the molal activity of the ith component a_i is related to the measured concentration m_i and is given by

$$a_i = m_i \gamma_i$$

where γ_i is the activity coefficient. However, because of experimental difficulties, this is rarely done; instead, it is general practice to measure stability constants in the presence of a background electrolyte at high ionic strength which minimises any changes in ionic activities. It is generally assumed that stability constants of sufficient accuracy may be obtained by this means. This assumption certainly does not hold under biological conditions and it is essential to question the relevance of quoted stability constants and the validity of each example of computational speciation on this basis alone.

Nevertheless, speciation by computation is rapidly growing in popularity and, treated carefully, it can be extremely useful. Yet it suffers from a further major disadvantage which unhappily grows with the sophistication of the technique. As algorithms are evolved to handle increasing numbers of both metals and ligands, the number of complexes to be considered grows explosively, so that results are obtained predicting concentrations for a plethora of minor components. Sadly, there is as yet no way, however tedious or difficult, of measuring the true concentration of these components, so that the veracity of the results is undetermined. Nevertheless, despite the criticisms, computational speciation is valuable since it allows 'quantitative' estimates of the variation in the distribution of complex species caused by external changes (Morel and Morgan, 1972; McDuff and Morel, 1973; Truesdale and Jones, 1974; Morgan and Sibley, 1975), and it has been suggested that correlation between species predicted by computation and biological effects may play a key role in our understanding of the action of metal ions (Sposito and Bingham, 1981).

4. CHEMICAL ANALYSIS

If any one skill or area of expertise can be regarded as essential to the increased understanding of plant–metal interactions, it must surely be chemical analysis. It is not the purpose of this discussion to review analytical techniques or results in depth, nor would it be possible, since the volume of literature is truly enormous (one review on cadmium analysis

alone produced over 1000 references (Battelle Columbus Laboratories (1977)), but it is hoped to present a *critical* assessment of the current state of analytical methodology, thereby illustrating both the limitations and the current pattern of developments. Certainly, there seems to be a growing awareness of the need to evaluate current problem trends in analysis (Kopp, 1975; Baudin, 1977; Crosby, 1977; Eller and Haartz, 1977; Ewing, 1977; Harrison and Laxen, 1977; Morgan and Bretthauer, 1977).

4.1. An Approach to Quantitative Analysis

Any survey of the literature will show that, until recently, analysis has really meant quantitative analysis, and that it has often been restricted to the determination of the amount and concentration of a single element (or at best a very limited range of elements) without regard for the chemical form in which it is present. Since much of the attention which has been focused on the impact of trace metals on plants has been pollution oriented, often directed at measuring the movement of materials through the biosphere, it is not surprising that this type of quantitative analysis has played such a central role in delineating these processes. Analysis has been required firstly to determine the amount of material in any 'pool' or reservoir (and these may be of any predetermined size, from an entire atmosphere or a watershed, down to discrete sub-cellular components) and then, by repetition, to determine the rate of transfer between the various pools. Therefore, *accurate* and *precise* quantitative analysis has been one of the most basic information-gathering procedures in this type of research. Yet despite this, and at the risk of being repetitive, it must be emphasised that much of the published data is of dubious provenance. These comments are not merely prejudice, but are supported by a range of trials which show that very disturbing discrepancies can occur between results obtained in different laboratories. This point will be examined in more detail later.

The details of analytical methods are extremely varied, and the choice of analysis is often determined not by chemical considerations but by the availability of facilities in the particular laboratory. Nevertheless, general principles can be recognised, and if the standard of analytical measurement is to be raised and the validity of data improved, these must be considered for every analysis.

4.2. Sample Collection

Sampling statistics

Analysis proper begins with sampling, which like all other stages of the

analytical procedure may be subject to both random and systematic errors. Although the theory of sampling is well developed, and it might be thought that problems with experimental design should be minimal, this is not always the case, as untoward effects often intrude. For example, many studies are concerned with the determination of mean levels of elemental concentration in a population of plants, and hence comparisons between populations. Apart from such well-known problems as 'edge-effects' in block sampling, other difficulties have also been revealed. Wallace *et al.* (1977) have reported that the concentration of some metals measured in corn plants grown on uniformly contaminated soil showed log-normal rather than normal distribution and, additionally, some of the set (Zn, Fe, Mn, Ni, Li, Cd) showed negative skewness. Alternatively, Garten *et al.* (1977) showed that frequency distributions of many elements present in the vegetation of a flood plain community were positively skewed. Similarly, Beckett (1980) has shown a skewed, log-normal distribution for trace metals in sewage sludge. Clearly, such observations mean that statistical treatments and inferences based on normal distributions will be invalid unless the data is appropriately transformed.

General procedures

Accepting that these factors are recognised and experimental design is sound, the actual process of sampling also presents numerous pitfalls. Trace-element cycles encompass all the phases of chemical and physical structure, each with its own problems. Broadly, sampling may be divided into four: airborne material, material in ground and natural waters, material in rocks and soil, and material contained in the plant; clearly there is extensive overlap between the areas.

Sampling atmospheric metal burdens

Atmospheric metal burdens, which are vitally important in considering the transfer of pollutants such as lead or mercury, are extremely difficult to measure meaningfully. Even the simplest question—what is the total amount of the element in the atmosphere?—may be difficult to answer accurately. Primary sampling of atmospheric metal burdens has been carried out in a variety of ways, including liquid scrubbers, microporous filters, precipitators (electrical or mechanical), interceptors and deposit gauges (moss bags, filter paper, grass swards, etc.), each with its own limitations.

Restricting the discussion in the main to lead (though similar comments

apply to other elements), some of these comments are briefly outlined here, since they serve to illustrate the many pitfalls in this area.

'Absolute' removal of a metal from the atmosphere is usually attempted by use of a 'liquid scrubber', yet for lead it is questionable as to whether this is totally efficient. Certainly, the problem seems to have been attacked (Purdue *et al.*, 1973), but earlier reports (Snyder and Henderson, 1962) must all put into question the value of results obtained during that period.

The disadvantage of such methods is that they provide little chemical, or indeed physical, information on the sample, and also that the basis of trapping is chemical reaction. However, as particulate materials are of primary importance in the distribution of many metals via the atmosphere, much effort has been expended on the examination of this fraction in order to answer such questions as:

(a) What is the source of the input?
(b) Does the airborne material represent depletion or enrichment with respect to the source?
(c) What is the mobility of the material in the atmosphere and its residence time?
(d) What is the chemical form of the element within the sample?
(e) What is its physical distribution?
(f) Hence, what is its likely reactivity in prevailing environmental conditions?

Primary sampling for particulates may involve filtration of large volumes of air, but numerous problems abound. For example, it has been shown that filters exhibit abrupt discontinuities (depending on the physical parameters of the collection system) since at supra-critical velocities, large particles may be reflected (Goodman *et al.*, 1975) and small particles may not be trapped (Skogerboe, 1974). It has been suggested (Lee *et al.*, 1972) that for lead, over half the atmospheric burden may be included in size ranges for which filters are least efficient, so that up to one-third of the total particle mass would not be collected.

Another popular method of sampling for airborne material is the use of 'moss bags' which are claimed to produce a realistic estimate of the interception of airborne metals by plants (Goodman and Roberts, 1971; Goodman and Smith, 1975; Cameron and Nickless, 1977; Goodman *et al.*, 1975; Tan and Lepp, 1977) but the comparability of data collected by use of one or other of these methods is not easy. These are merely some of the problems associated with sampling for aerial pollutants, and more detailed discussions are available (Perry and Young, 1977).

Sampling aqueous phases

Analysis of aqueous phases can range from studies on large bodies of water, lakes, rivers, etc., down to experiments on plant fluids such as xylem sap or guttation fluid. On this general area two major problems can be discerned. First is the necessity for careful sampling to obtain an overall representative view of the material, and second is the preservation of the samples until analysis can take place in order that they are representative of the source. Whilst the first is partly a problem in statistical design and has been outlined earlier, the second is much more a problem of chemical expertise. As has been pointed out recently (Hughes *et al.*, 1980), natural water, even of droplet size, can contain a considerable amount of inorganic and organic material and in many cases may also be host to a variety of living organisms. Clearly, such a system is unlikely to remain constant for any length of time and changes, as for example in dissolved gases pO_2 and pCO_2, can have substantial effects on the form in which the 'trace metals' are to be found.

Certainly, if the identification of metal species is the main objective, considerable difficulties exist in certifying that the sample analysed is truly representative of the source, both spatially and temporally. Even if the aim is quantitation, considerable difficulties still exist in preserving the sample for accurate analysis. Many difficulties arise, but perhaps the best known is loss of solute onto walls of the container; a variety of studies continue to appear reporting detailed loss profiles and endorsing particular preservation regimes. For example one study (Subramanian *et al.*, 1978) reported the effect of pH and container material on the loss of eleven 'trace metals' for stored samples of natural and synthetic water samples. These authors recommended acidification with nitric acid to $pH \leq 1{\cdot}5$ as a suitable means of storage, noting also that different metals showed different losses on different container materials. A large number of similar studies are available (e.g. Moore and Meredith, 1977). Such studies should be considered carefully by anyone attempting trace or ultra-trace metal analysis, together with problems such as matrix interference (Thompson *et al.*, 1977) which is not constant but variable, and may alter on storage as the processes of aggregation, precipitation, etc. occur.

Certainly what is often and widely ignored is the fact that aqueous samples derived directly from plants, especially cell sap or other similar plant fluids are quite *unlikely* to be representative of the source unless rather careful precautions are taken. For example, any technique which involves a step requiring cellular disruption must significantly change the environment from which the metals are to be extracted. In disrupting the cell, extracellular sites, previously unavailable to the metal ion, will become available,

with a consequent exposure to new ion-exchange processes, not to mention the availability of new ligands, which must surely lead to a readjustment of equilibria involving metal binding. Consequently, there is no guarantee that the aqueous sample obtained is truly representative of the intra-cellular composition. Rather, the opposite is likely to be true, and it is remarkable that this problem has generated so little discussion, since at worst it clearly invalidates many experimental findings, and at best it calls for considerable re-interpretation of the results.

Sampling solid phases

As for any other phase, an accurate determination of the 'trace metal' status of a solid sample requires that the sample be representative. For example, in soil sampling, the number of cores taken, together with their micro-geographical distribution must be considered (Fitts and Nelson, 1950), yet little research seems to have been conducted on soil sampling in order to quantify these and other effects. In any case, the majority of the work reported relates to macronutrients, notably P and K, and the use of these studies as a basis for later discussion has been criticised (Cox and Kamprath, 1972). In general it is agreed that a larger number of samples will always be preferable to a smaller one, allowing only for the limits of the actual analytical process. Simple statistical theory may readily be applied to predict the number of samples in relation to the specified degree of certainty, but with the caveat that the appropriate form of distribution must be employed. Unhappily, it is not possible to gather any considerable number of studies dealing with 'trace metal' distributions, but the heterogeneity of their distribution on all scales is generally accepted (Peck and Melsted, 1967).

Sampling plant tissues presents different problems, since it must be generally agreed that a 'representative' plant is an improbability, particularly amongst the larger higher plants, but fortunately in many cases there is a sufficiently large population for sophisticated statistical comparison. Some of the more recalcitrant problems arise from separation of different plant parts and sample contamination. Soil–root conjugates are difficult or even impossible to separate, and it is certainly impossible to rule out contamination.

Equally, but less spectacularly, aerial parts are subject to contamination (unless carefully grown in a controlled environment) and this may be one of the major impacts of trace metals on plants (Hughes *et al.*, 1980). In general, one problem in pollution studies, often conveniently ignored, is the difficulty of finding an 'uncontaminated' material as a reference.

4.3. Sample Preparation

Preconcentration

Preconcentration of the sample prior to analysis is becoming increasingly common and a variety of methods have been proposed. Probably the best-recognised method is solvent extraction, in which the metal ion is transformed into a complex which is selectively soluble in a solvent which is immiscible with the sample. Solvent extraction, which has been extensively studied, received an early impetus from studies on the separation of rare-earths and, latterly, radiogenic nuclides. Nevertheless, it is still important and continues to receive much attention (Leszko and Zaborska, 1977; Danielsson *et al.*, 1978; Bone and Hibbert, 1979; Wilson, 1979), particularly since careful choice of the extracting ligand and solvent may impart considerable selectivity, so that solvent extraction, like some other preconcentration techniques, may also yield some information relevant to speciation.

Allied to solvent extraction is the use of ion-exchange chromatography (Wilson, 1979), in which the metal is selectively complexed with binding sites on a macromolecular matrix. This may occasionally be liquid, and the use of a foam-supported liquid anion-exchange resin has been described (Maloney *et al.*, 1977), but more commonly the resin is a solid. In attempts to gain both selectivity and increased efficiency, a number of variants of this process have been reported. The most important would seem to be the use of a chelating resin, to give more stable metal–resin complexes (Kingston *et al.*, 1978; Lamathe, 1979) which has been extended to the use of resins with specifically tailored functional groups for the capture of groups of metals; for example, the use of a thioglycollate chelating resin for Zn^{2+}, Cd^{2+} and Pb^{2+} (Phillips and Fritz, 1978).

Each advance brings its own problems, for example, it has been reported that surfactants may interfere with preconcentration by capture on an ion-exchange resin (Pakalns and Farrar, 1977; Pakalns *et al.*, 1978). In general, the selectivity of ion-exchange methods is low, but may often be improved by changing the form in which the metal is absorbed. Thus the addition of a complexing agent, whether a simple inorganic ion or a large organic molecule, can give other structures and charges on the species to be absorbed (Strelow, 1978), and certainly enables differentiation between naturally complexed forms (Shuman and Dempsey, 1977).

Other methods of preconcentration are described from time to time, including: non-boiling evaporation (Boutron and Martin, 1979), absorption on chemically modified filter paper (Gendre *et al.*, 1977), co-precipitation (Hudnik *et al.*, 1978; Welch and Ure, 1980) which is

particularly interesting since one process involves the use of iron(III) hydroxide mimicking partition processes in natural waters, and electrochemical deposition, either on a graphite tube (Batley and Matousek, 1977) followed by direct analysis via atomic absorption, or on a platinum wire electrode (Lund *et al.*, 1977). The variety and frequency of such reports clearly demonstrates the growing importance of this technique.

Digestion

If a representative sample can be obtained, the next stage in the analytical procedure is often further sample preparation. Only in a very limited number of cases can samples be analysed directly. It is usually more convenient, at least for the purposes of quantitative analysis, to convert the primary sample into a standardised form, usually an aqueous solution of reasonably defined composition. For example, a CsCl/HCl solution has been recommended for analysis by atomic absorption (Hansen and Hall, 1977). In this way, matrix effects from solid samples were minimised and standards could be prepared which were as closely representative of the sample as possible. However, there is a growing interest in the use of techniques such as X-ray emission, which, like the better established neutron activation analysis, can be used on solid samples. This clearly has advantages for certain topics, notably in the characterisation of particulate matter, whether airborne or colloidal, or alternatively to describe the surface composition of soil matrices.

Nevertheless, for many techniques (atomic absorption and emission, mass spectrometry, and electrochemical methods such as anode-stripping voltammetry or polarography) it is more usual to convert the sample to an aqueous solution. Properly controlled, such methods are entirely acceptable, but loss can occur (Menden and Brockman, 1977), particularly for the more volatile metals. Generally, dry-ashing is more likely to give rise to higher losses, whilst wet-ashing is more likely to lead to contamination, unless special care is taken with choice of reagents, since relatively large amounts of digestant (usually a mineral acid mixture) are used (Friend *et al.*, 1977).

4.4. Analytical Techniques

Classical v. instrumental analysis

Modern quantitative analysis leans increasingly towards sophisticated instrumental methods. Although classical gravimetric or titrimetric methods may sometimes still be applicable, these are generally rejected since they usually require large samples (by biochemical standards at least),

have poor limits of detection, are tedious and require a high degree of operator skill. Nevertheless, in some cases, classical methods or well-established simple instrumental methods, often based on spectrophotometry, can still be used to great effect (Ministry of Agriculture, Fisheries and Food, 1973).

In general, chemically based analyses rely heavily on complex formation, the aim being to provide reagents which give maximum sensitivity and selectivity. Typical of such methods are the colorimetric analyses using dithizone which are still widely employed (Ministry of Agriculture, Fisheries and Food, 1973), but even where complexometric methods are not used directly, complex formation may still play an essential role, as in preconcentration steps discussed earlier. Much can be learnt, especially about selectivity, from consideration of more traditional methods.

Quantitative analysis of very small amounts of a trace metal, whether in a very dilute original sample or because of a very small sample size, represents a considerable problem. This can be attacked in several ways, often by increased analytical sensitivity through new or improved techniques to give lower absolute limits of detection. Alternatively, preconcentration techniques can be employed.

Atomic absorption spectrometry

Atomic absorption spectrometry (AA) is extensively used for trace metal analyses and the limitations and advantages of this technique have been widely discussed (Van Loon, 1975; Willis, 1975, 1979; Mertz, 1979; Ottaway, 1979). Numerous modifications, improvements and refinements have been reported and specific reference to experimental difficulties are only selectively quoted here, to illustrate the difficulties of what is commonly regarded as a 'standard' technique. The sensitivity of AA can be defined in several ways (Rowe and Routh, 1977) but the trend is towards lower detection limits and smaller sample sizes (Price, 1978, 1979), though whether results are meaningful is often doubtful.

Perhaps the most important development in AA is the introduction of non-flame atomisation procedures—principally electrothermal atomisation from a graphite substrate; though a tungsten tube atomiser has been reported (Sychra *et al.*, 1979). The advantages of this modification are substantial increases in sensitivity and the possibility of direct atomisation from solid samples. The disadvantages are numerous and are still being discovered. Amongst the problems to be treated are background reduction (Harnly and O'Haver, 1977; Nichols *et al.*, 1978), including a novel form of instrumentation based on the Zeeman effect (Koizumi *et al.*, 1977); solid

matrix interferences, which have been treated chemically (Thompson *et al.*, 1977) and by redesigning the atomiser (Manning and Slavin, 1979); interferences in solution (Oelschläger and Lautenschläger, 1977; Czobik and Matousek, 1978); the mechanism of atom loss (Gregoire *et al.*, 1978); the effect of heating rate (Sturgeon and Chakrabarti, 1977) and the doubling of peaks (in lead analysis) (McLaren and Wheeler, 1977).

Nevertheless, flameless AA does hold out distinct advantages if the difficulties can be overcome. Illustratively, it is possible to quote a whole range of studies in which direct atomisation has been used. This is increasingly common in studies of airborne trace metals, where samples collected on interceptors such as filter paper may be analysed without further treatment (Thomassen *et al.*, 1977; Geladi and Adams, 1978). Alternatively, the graphite atomiser can be used directly as the collector (Noller and Bloom, 1977; Siemer, 1978).

In addition to advances in atomisation techniques, sample handling methods have become more sophisticated so that automated trace analysis of small samples ($< 100\,\mu$ litres) by both flameless and flame AA has been described (Berndt and Slavin, 1978) with obvious implications for analysis of plant exudates or leachates, soil solution, etc. Detection limits for lead are quoted as being as low as 1 ng for 40 μ litres injection (i.e. approximately 0·025 ppm). Modern instruments can therefore analyse small samples with great throughput, accuracy and precision, but only if the limitations of the technique are well understood.

The major drawback of AA is that it is essentially a single-element technique, though various attempts have been made to find means of simultaneous analysis of several elements. For example, the use of a multiplexed slit scheme has been described (Salin and Ingle, 1978) which allows simultaneous determination of any four of ten stated elements. However, the trend to multi-element analyses is being satisfied by other techniques, notably emission spectroscopy, X-ray fluorescence and induced emission, mass spectrometry or anode-stripping voltametry.

Modern emission techniques

Unlike AA, optical emission spectrometry is potentially capable of determining the presence of almost all elements in a sample, though the technique is not simple in practice.

Optical emission spectroscopy has a long and varied history. Emission spectrograph analysis using carbon-arc or spark sources and a photographic plate detector has been widely used for qualitative analysis of trace metals in metallurgical samples. For quantitative analysis this technique

has been relatively neglected because of problems with low sensitivity, though reports of its use have continued to appear (Sugimae and Skogerboe, 1978), and a double-arc technique has been developed which is more sensitive, giving a detection limit for lead of 5 $\mu g/dm^3$ (Cecchetti *et al.*, 1977). However, with the advent of inductively coupled plasma (ICP) discharges giving much more efficient atomisation and excitation processes, emission spectroscopy has again become fashionable (Ward and Sobel, 1977).

As with many techniques, ICP-emission analysis has rapidly grown in popularity since commercially produced instruments became available and has been reviewed recently (Abercrombie *et al.*, 1979; Alexander and McAnulty, 1981; Boumans, 1979; Haas *et al.*, 1979). It appears to be a remarkably promising technique with good detection limits. Boumans (1979), for example, quotes limits for Cd, Zn and Pb as 0·3, 0·3 and 10 ng/cm^3, respectively, but most authors agree that the technique demands a high degree of operator skill; considerable effort must be expended in setting up and calibrating the method. Once operational, however, it can simultaneously produce quantitative elemental distributions from a variety of samples for a wide range of elements. Moreover, it is clear that the method has not yet reached its full potential and variants are already beginning to appear, including a microwave resonant cavity for plasma production (Zander and Hieftje, 1978) and, quite remarkably, the use of exploding films of aluminium as a free-atom generator and excitation source (Duchane and Sachs, 1978).

A recent example of the never-ending variety of new techniques is flame-resonance spectrometry, a cross between emission and AA methods, which has recently been advocated as possessing multi-element capability (Larkins and Walsh, 1975; Van Loon and Radziuk, 1977*a*,*b*).

Mass spectrometry

Mass spectrometry (usually by spark-source, SSMS) is a late-comer to the field of multi-element analysis (Bingham and Vossen, 1975). Whilst organic mass spectrometry is highly developed, the use of this technique for determination of the *concentration* of trace-elements, as opposed to structural elucidation and qualitative identification, is relatively new but nevertheless shows promise. In one report, digested sludge was analysed for no less than 73 elements by SSMS, emission spectroscopy and other methods, and the mass spectrometric method was found to be promising (Beckett, 1978), whilst in another series of experiments SSMS has been successfully applied to the quantitative analysis of rocks and soils (Ure and

Bacon, 1978). As an alternative to SSMS, ion-microprobe spectrometry has been used in the determination of the surface distribu of elements in airborne particles (Linton *et al.*, 1977).

Anode-stripping voltametry

Another recently developed technique with multi-element capabilit anode-stripping voltametry. The basic technique is sensitive but su from the triple disadvantage of relatively long plating time (so tha analysis of Cd, Pb, Cu and Zn in rain and snow required 75 min (Nguy *al.*, 1979)), large sample volumes (Ryan and Siemer, 1977) and sophisticated instrumentation. Simplified (and inexpensive) variants been proposed (Jagner, 1978; Poldoski and Glass, 1978) and conti improvements to the technique are described, with tabular or rotating electrodes (Schieffer and Blaedel, 1977; Wang and Ariel, 1978) witl latter including a flow system for multi-sample analysis, or even the u computer averaging of differential voltamagrams to give reduced times (Brown and Kowalski, 1979). It seems, therefore, that this techn will continue to find favour in the analysis of trace metals, since direct simultaneous determination of several elements, e.g. Zn, Cd, Pb, Cu and Bi by a differential technique, has been reported (Gillain *et al.*, 1

X-ray fluorescence

X-ray fluorescence is another method being rapidly developed for m component trace element analysis. It may be applied to a specific elem as with analysis of lead in air (Djemkova and Schiller, 1978), or a m element survey. In one case, 40 elements were determined in fly-ash sam although the method is not especially sensitive, with a quoted accurac $\pm 5\%$ (Giauque *et al.*, 1977). Typical of the sort of problems to be so are the removal of matrix effects (Nielson, 1977), preconcentra techniques (Panayappan *et al.*, 1978) and the introduction of autom methods (Van Espen *et al.*, 1977). Advances in this field have recently l reviewed (Campbell, 1979).

Radiochemical methods

Radio-analytical methods are well established, whether as 'tra experiments, as for example in a typical study on the behaviour of t metals in natural waters (Benes and Steinnes, 1976), or by activa techniques, of which there are many variants. The application of neu activation in environmental research (Filby and Shah, 1974) specifically the determination of trace elements in natural waters (Li

and Calmano, 1977) have both been reviewed, whilst comparisons have been made of proton and neutron activation (Chattopadhay, 1977; Zikovsky and Schweiker, 1977) and found to be of similar utility. Similarly, gamma activation analysis has also been appraised (Hislop, 1978). Neutron activation does seem to offer certain advantages in that it is both highly sensitive and has multi-element capability, often being used as a reference method (Mavrodineau, 1977). Advances in instrumentation (Egan and Spyrou, 1977; Guinn and Miller, 1977; Wainerdi *et al.*, 1977; Ryan *et al.*, 1978; Schutyser *et al.*, 1978) and techniques such as preconcentration (Bergerioux *et al.*, 1977; Jewett *et al.*, 1977) and separation (Smet *et al.*, 1978) have all been recently described.

4.5. The Prospects for Quantitative Analysis

The trend towards multi-element profiles has the major advantage that element ratios may be computed, and that such calculations may in time lead to data concerning pollution sources (Wesolowski *et al.*, 1973; Zoller *et al.*, 1973). If element ratios are unavailable, insufficient or inappropriate, isotope ratios may be used for the same purpose (Chow *et al.*, 1974). However, it is possible to be over-enthusiastic in the pursuit of such data and it has been suggested that the relationship between metals should be examined via multivariate statistics (Garten *et al.*, 1977) since there is little to be gained in making many measurements, some of marginal reliability, if they are highly correlated to a few indicators, and if additionally, some of these metal concentrations are those for which sound measurements can be made.

Quantitative analysis is not easy, despite a popular assumption to the contrary. If further evidence of this is required it is only necessary to look at some of the inter-laboratory comparisons which have been conducted in the past few years. The general failure to produce good correlation for a variety of metals and sample types (Uthe *et al.*, 1971; Brown *et al.*, 1974; Lauwerys *et al.*, 1975; Dybczynski *et al.*, 1978*a*,*b*; Long *et al.*, 1979) must be of serious concern to anyone who wishes to rely on analytical data for studies of trace-element distribution, and although reviews of methodology continue to appear (Sawicki, 1975; Eller and Haartz, 1977; Fuwa, 1977; Kirchoff, 1977; Mertz, 1979) there does not appear to be a corresponding increase in reliability.

What then can be done? In order to improve the quality of analytical data a number of suggestions should be considered:

(a) more extensive inter-laboratory trials and the certification of participating laboratories,

(b) more use of standard reference materials (Alvarez, 1981) and agreement on standard methods (Mavrodineau, 1977),

(c) better presentation of data on publication, with comments on such factors as within-run and between-run precision and test analysis of certified standards, etc., as tests of reliability.

Until these or similar proposals are adopted, then analysis must continue to be suspect.

4.6. Speciation

If the problems associated with quantitative analysis are serious, then those involved in the determination of the chemical form of the metal in the sample, i.e. that aspect of qualitative analysis which has become known as 'speciation', seem almost insuperable. Yet answers to this problem must be seen to be as vitally important, and fortunately attention is now being directed to this problem. Mass-flow budgets are undoubtedly important, but speciation profiles are surely necessary for their conversion to environmental impact statements (Hodgson, 1963; 1969; Norvell, 1972; Lindsay, 1974; Cantillo and Segar, 1975; Florence, 1977; Florence and Batley, 1977). It is doubly unfortunate that most of the major methods of chemical analysis referred to so far yield little information about chemical form. Indeed, as with digestion, many sample preparation techniques are specifically aimed at converting the metal to a standard form which bears no relationship to its original speciation, and even where sample preparation is non-destructive, answers are chiefly related to total elemental concentration and not to chemical form.

Speciation requires a substantially different approach, since any technique which disturbs the chemical distribution within the sample is forbidden. Taken to its extreme, speciation can be defined as the determination of *all* the physico-chemical parameters which define the form, and to some extent distribution, of the metal in the sample.

Research efforts in speciation can be conveniently classified as analytical or computational. Computational speciation depends on a thorough understanding of the principles of coordination chemistry and other aspects of the behaviour of metal ions, notably the properties of inorganic colloidals. The principles have been outlined elsewhere in this chapter and are not discussed further here. Analysis may require different methods than for quantitation, or alternatively the same methods used in different ways. Certainly, this is one area where classical analysis ought not to be neglected since the presence of inorganic ions such as chloride, phosphate or cyanide

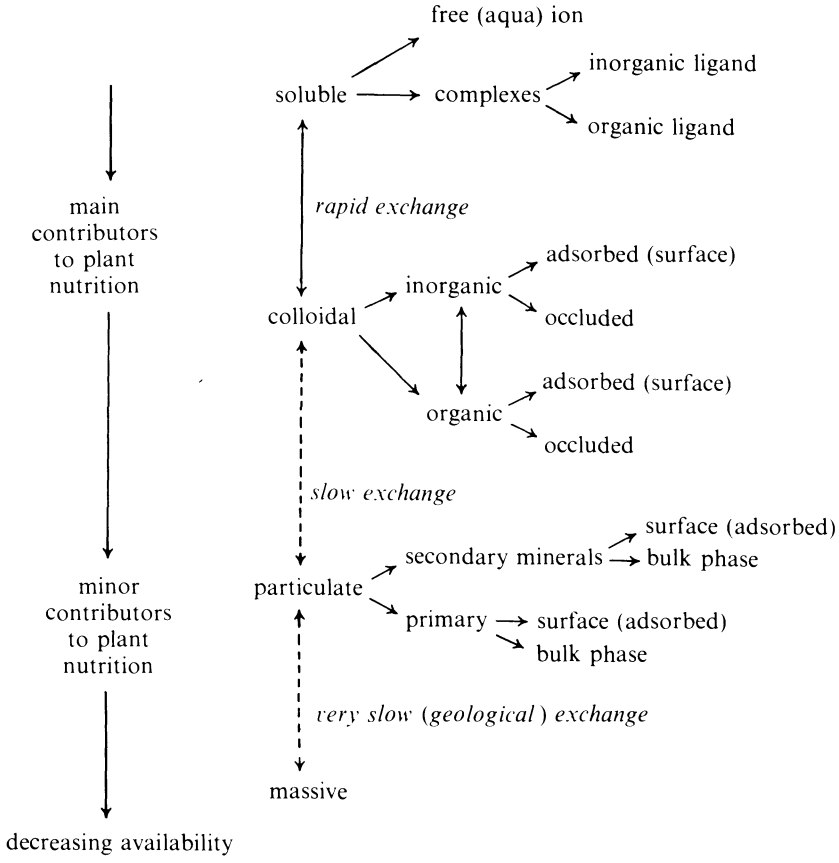

FIG. 1.2 A fraction scheme for soil-metal content.

may go far to determining the behaviour of the metal ion in aqueous solution (Wagner, 1977).

The total speciation of a natural mixture of metal ions and ligands by analysis is unlikely, but the metals may be classified into sensible functional groups. In an aqueous specimen, this may be done as shown in Fig. 1.2 though this is by no means the only way. Different techniques may then be applied to determine the fractionation, and this has been discussed on several occasions (Hodgson *et al.*, 1965, 1966; Lindsay, 1972; Guy and Chakrabarti, 1975; Chau and Wong, 1977; Florence, 1977; Florence and Batley, 1977).

If different forms of the metal react differently to any analytical method

then obviously this method may be used in some form of fractionation. Typical examples are the use of polarography (Nuernberg *et al.*, 1976) and ion-selective electrodes (Buffle *et al.*, 1977) in which different complexed forms react differently. Alternatively, the differing forms of the metal may be selectively extracted with a variety of reagents (making use of their different reactivities) including ion-exchange resins (Batley and Florence, 1976; Hackett and Sigsia, 1977; Phillips and Fritz, 1978), a variant of the problem of preconcentration described earlier. Alternatively, selective extractions have been described for sediments (Agemian and Chau, 1977; Engler *et al.*, 1977; Malo, 1977) or airborne particulates (Snyder, 1977). Such procedures are routine in all forms of soil analysis and the extractability of Cd, Pb and Zn has recently been reviewed (Låg and Elsokkary, 1978).

An interesting view of the determination of the analyst to exploit every technique to the full is seen in the use of conventional quantitative techniques in a modified form for speciation. Typical is the use of AA, both flame and flameless, as a *detector* for a separation technique such as gas chromatography (Fernandez, 1977; Guy and Chakrabarti, 1977), with particular application in the determination of organo-lead compounds (Robinson *et al.*, 1977; Bye *et al.*, 1978). It seems certain that such novelties will become more and more an accepted part of the analytical chemist's repertoire for speciation, as indeed they must if the problem is to be attacked at all seriously.

5. THE UPTAKE OF METAL IONS BY BIOLOGICAL SYSTEMS

5.1. General Principles

The uptake and subsequent transport of metals, whether they are acting as essential micronutrients or toxic pollutants, can be treated in a similar fashion, though the level of treatment may vary enormously from the pathological approach, to metal ion functionality and toxicity, to a detailed treatment of the mechanism of uptake at the molecular level. Yet at any level, chemical principles, whether stated explicitly or used implicitly are of great importance—as recent reviews have shown (da Silva and Williams, 1976). In all except the smallest minority of cases, where volatile metal compounds are concerned, entry into the plant must involve transmission through an aqueous phase, so that the 'mobility' of the metal ion in its various forms in aqueous solution is dominated by the complex formation,

precipitation reaction, etc. For example, it has recently been emphasised that the uptake of copper by excised roots is markedly affected by the form in which the copper is presented, with

$$[Cu(II)(OH_2)]^{2+} \simeq [Cu(II)(en)_2]^{2+} > [Cu(II)(gly)_2]^0 \gg [Cu(II)EDTA]^-$$

so that the primary determinant here was suggested to be the overall charge on the complex (Coombes *et al.*, 1977). The relative scarcity of studies such as this leaves a huge gap in the picture of metal uptake, and it is not possible to emphasise too strongly that the uptake of metals by plants, whatever the route, is dominated by the principles of coordination and redox chemistry outlined previously in this chapter. These must be used to account for the *availability* of the metal at the plant surface, whether it be root, stem or leaf, the *primary capture* of the metal by the plant and the subsequent *mechanism of uptake*, which may be described as active, facilitated or passive.

5.2. The Availability of Metals

Soil–metal plant system

Plant trace element nutrition owes much to early work on field problems of manganese deficiency in oats, as has been re-emphasised recently (Cox and Kamprath, 1972). It was shown that pathological symptoms were not caused by *absolute deficiency* of manganese in the soil, but by its *unavailability* or functional deficiency caused by the insolubility of manganese oxides. Similar problems have emerged for other trace metals, notably iron, and whilst many studies have attempted to quantify 'unavailability', or, more realistically 'availability' (Fitts and Nelson, 1950), no single acceptable definition has yet emerged. In practice, the best approach appears to be an attempt to estimate the amount of metal extracted from the sample by various procedures, and to correlate this with the amount taken up by the plant, an approach which can be extended from soil to ground waters or to airborne particulate material. This approach is preferable to the alternative concept of linking 'availability' to plant growth, since growth is a multi-faceted problem affected by so many uncontrollable variables, particularly in field trials, yet micronutrient soil tests generally remain related to growth effects (Cox and Kamprath, 1972).

It has been suggested (Viets, 1962) that the metal content of soils could be classified into five pools, namely:

(i) water soluble,
(ii) exchangeable,
(iii) absorbed, complexed and chelated,

(iv) secondary clay minerals and insoluble metal oxides,
(v) primary minerals, with the first three fractions being in equilibrium and only these being important in plant mineral nutrition.

However, this approach is open to some criticism since it is apparent that there is both overlap between the defined classes and an apparent misconception of the fundamental terminology of coordination chemistry. Thus the water-soluble fraction (i) will also contain material described under (iii) since it will include material that is complexed by relatively low molecular weight soluble ligands. Also it is nonsensical to use both the terms 'complexed' and 'chelated', since the latter is merely a special case of the former; chelating ligands form complexes like any other and have no special attributes beyond the fact that the ligand occupies several coordination sites simultaneously to form especially stable complexes. However, if the ligands are in the appropriate fraction of soil organic matter then the 'chelated' fraction can be soluble, indeed the use of smaller polydentate ligands in this way may account for some of the solubilisation of metals in the rhizosphere, with the best understood example being iron.

Nevertheless, this and similar schemes are important since they do point out that the total metal content of the soil is not available to the plant, and that the fraction loosely described as 'available' may be sub-divided into components of differing mobility and accessibility.

A more logical approach is to attempt to define the available fractions in terms of their extractability or ease of exchange, in other words to related availability to the processes of ligand exchange or metal substitution. Extractability may be treated in outline as shown in Fig. 1.2.

In the case of extraction, the degree of extraction is essentially a problem in applied coordination chemistry or speciation. Thus in the simplest case, if the concentration of the metal ion is known, together with its affinity or stability constant for the various binding sites and their concentrations, then the equilibrium distribution of the metal over the sites can be calculated as described before. If the extractant which is an additional ligand (L_{Ex}) of known metal-binding power (stability constant) is included at known concentration, the new equilibrium concentrations may be estimated, and hence the concentration of ML_{Ex}, the *proportion* available to this extractant, can be calculated. It would be interesting to compare such predictions with the experimentally determined extractable pool as measured by the particular extractant.

However, some caution is needed since a number of factors must be considered which may affect the outcome of the experimentally determined

availability. Firstly, it must be recognised that other metal ions are present, so that there will be competition between different ions for the extractant ligand. This may largely be overcome by adding the extractant at sufficiently high concentration to complex all metal species, subject to stability constraints. Secondly, the extraction procedure must allow sufficient time for 'equilibrium' to be attained. This must be coupled with the constraint that excessive extraction times may lead to leaching from pools of lower availability. Thirdly, the cut-off between fractions is not absolute, as simple calculations demonstrate.

Consideration of equilibria for ligand substitution described earlier can be applied to show that for the reaction

$$ML + L_{Ex} \rightleftarrows ML_{Ex} + L$$

the equilibrium constant could be expressed as a function of the individual stability constants for the two metal–ligand species:

$$K_{eq}(\text{extraction}) = \frac{K_{stab}(ML_{Ex})}{K_{stab}(ML)} = \frac{[ML_{Ex}][L]}{[ML][L_{Ex}]}$$

As Fig. 1.3 shows, three regions may be defined where

(a) essentially no metal is extracted,
(b) some metal is extracted, the amount depending critically on the ratio of $L_{Ex}:L$,
(c) all metal is extracted provided $[L_{Ex}] \geq [M]$.

Whilst (a) and (c) are clearly distinguished by the extractant and may be described as *available* and *unavailable* to this extractant, respectively, (b) may overlap with (a) or (c) depending on the actual experimental conditions.

It is therefore possible to propose a multiple extraction sequence using a series of ligands of gradually increasing metal-binding power (Fig. 1.3), which may separate the metal burden into extractively available fractions of decreasing availability.

Such experiments, whilst simple in principle are complex in practice, but simplified versions are used implicitly by workers who described 'new' extractants and their correlation with plant-metal uptake, as in micronutrient soil tests (Cox and Kamprath, 1972).

Metal displacement by exchange is essentially the mirror image process of ligand extraction, thus:

$$ML + M_{Ex} \rightleftarrows M_{Ex}L + M$$

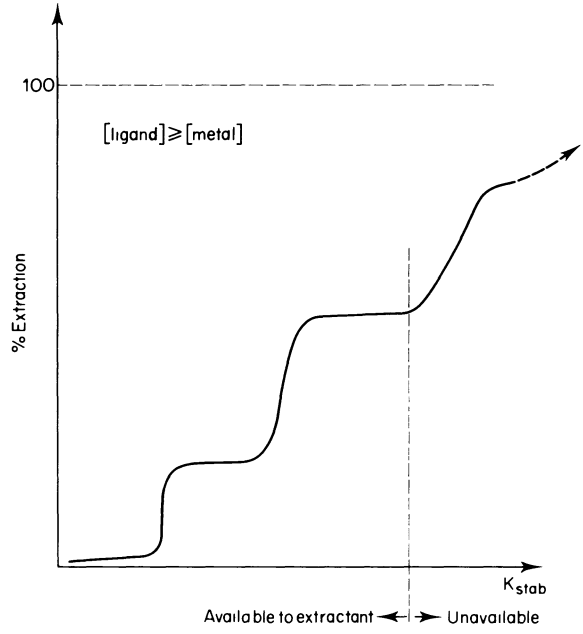

FIG. 1.3 Extractive capability related to metal-binding power (stability constant).

or the special case

$$ML + H^+ \rightleftarrows HL + M$$

Hence, they may be treated in the same fashion though with the disadvantage that the range of stability difference is much smaller.

What is clear, however, is that both ligand extraction and metal exchange estimates of availability cut across the boundaries of other classification schemes for soil-metal contents as, for example, outlined in Fig. 1.2.

Airborne metal burdens

Airborne metal-containing material intercepted by the plant may be treated in much the same fashion as soil-metal burdens, though both the physical and chemical classifications are much simpler. Physically, the material can be described as dissolved or particulate and the particulate fraction then sub-divided according to the ease with which the metal is extractable, i.e. whether it is readily leached by water or very dilute acid, or whether it is insoluble and is likely to remain in the particle till it is physically removed from the plant. Nevertheless, the same principles must be seen to apply.

5.3. The Primary Capture of Metal-ions

Trace metal capture via aerial parts

The atmospheric trace metal pool may be conveniently divided into dissolved and particulate fractions as described previously, and these are brought into the trapping zone by rain-out, wash-out and gravitational precipitation. However, in all cases, primary capture of the metal will be regulated by reactions in solutions at the leaf surface. The metal will be found at various sites on the leaf surface depending on:

(a) the chemical form in which the metal is presented,
(b) the nature of competing ligands,
(c) the pH of the leaf surface,
(d) the nature of binding sites,
(e) the nature of competing metal ions.

It may appear surprising at first sight, but this problem is essentially the same as the question of metal ion availability, since it can be reduced to a variant of the speciation exercise. In this case the interest centres round the proportion of the metal ion attached to specific leaf sites where the immobile sites can be treated as a competing ligand. Under these conditions, surface-capture may be considered to be analogous to equilibrium ion exchange and treated from the same theoretical standpoint. Effectively, the leaf-surface solution is a milieu of metals and ligands normally at moderately acid pH, with fixed negatively charged sites in the surface to act as adsorbers. In such a case, computational speciation should be relatively straightforward, if the appropriate data is available, though a considerable number of competing reactions must be considered as shown below:

$$ML_{solution} + L' + M' \rightleftarrows ML' + M + L$$

$M_{aq} + L \rightleftarrows ML$	Complexing of 'free' or aquo ion by available ligands
$H^+ + L \rightleftarrows HL$ $H^+ + L' \rightleftarrows HL'$	Competition for ligand by H^+
$ML + L' \rightleftarrows ML' + L$	Ligand substitution to give more stable complex species
$ML + M' \rightleftarrows M'L + M_{aq}$	Metal ion substitution to give more stable complex species. Increase in M_{aq} probably aids absorption

$$\left.\begin{aligned} M_{aq} + \text{Site} &\rightleftarrows \text{M-Site} \\ ML + \text{Site} &\rightleftarrows \text{M-Site} \\ ML' + \text{Site} &\rightleftarrows \text{M-Site} + L' \end{aligned}\right\}$$

Binding of metal ion to site, the more stable ML and ML′, the less easily the metal is bound to the site

$$\left.\begin{aligned} \text{M-Site} + M' &\rightleftarrows M + \text{M}'\text{-Site} \\ H^+ + \text{Site} &\rightleftarrows \text{H-Site} \end{aligned}\right\}$$

Competition between ions for a site; if M′-Site $\gg$ M-Site or H-Site > M-Site the bound metal may be displaced

Nevertheless, if metal ion and ligand concentrations are known, together with estimates for ion-exchange capacity and pK_a of ion-binding sites on the leaf surface, then estimates of primary ion-binding may be obtained.

Adsorption will depend on the chemical form of the metal species and obvious conclusions may be drawn. Notably, anionic complex forms are only poorly adsorbed since the preponderance of leaf sites are negatively charged. In turn, chemical form will be affected by pH, ligand substitution and metal exchange which may combine to give metal complexes in solution which have different adsorption characteristics to the primary source. Additionally, conversion of ML to ML′ (where L′ is more lipid soluble) may then affect uptake by increasing the ease of penetration of waxy leaves. Whatever the complications, the principles established earlier should be a reasonable guide to this process.

The binding of the metal ion to the leaf surface should follow a simple adsorption 'isotherm', which may be described by the classical treatment of Langmuir or treated similarly to the Michaelis–Menten explanation of enzyme kinetics, though the two approaches are essentially identical. In both cases the surface sites have a finite binding capacity and therefore the affinity of these sites for metal ion, K_m, may be expressed in the conventional way as the concentration of metal required to saturate half the available sites (Fig. 1.4).

Consequently, it is possible to write:

$$\text{amount bound} \propto [M] \times \log K_m$$

In general, estimates of K_m show that they do not differ greatly from metal to metal, so that leaf-surface binding is probably controlled by concentration effects rather than specific affinity; the primary capture will probably be a function of the available concentrations and may well be unselective (da Silva and Williams, 1976).

Root capture of metal ions

The broad basis of root capture by metal ions can be treated in largely the

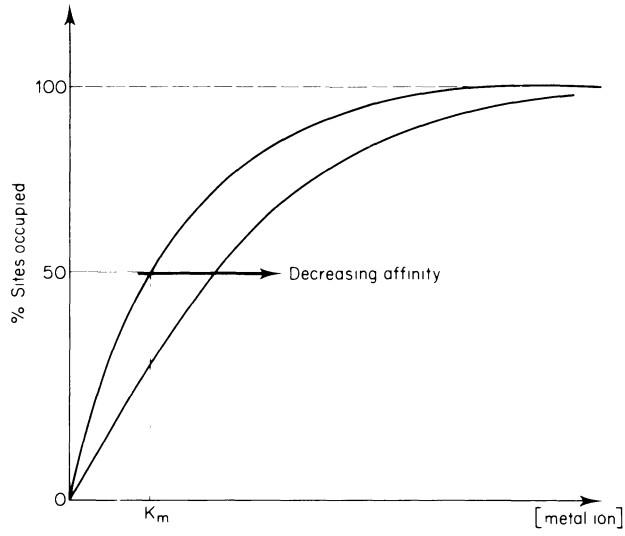

FIG. 1.4 Absorption 'isotherm' for metal ions on fixed sites. The ratio of 'free' to 'bound' metal is given by the equation $[M^{n+}]_{free}/[M^{n+}]_{bound} = [M]_{total}/(K_m + [M_{total}])$.

same fashion as for leaf-surface capture, provided that due note is taken of the *availability* of the metal (and its fraction into various pools under this heading) and the problem of diffusion through the soil solution and into the water free space of the root as a limiting factor in the replacement of metal ions. However, the total soil–metal–plant system is rather more complex than the leaf surface and especially is more subject to the influence of biological activity. It is increasingly evident that the root itself provides a variety of modifying factors, including secretions of H^+, chelating agents, reducing compounds and, equally significantly, secretion of substrates which encourage high concentrations of micro-organisms which in turn secrete further agents to modify the whole chemistry of the rhizosphere (Lindsay, 1974).

5.4. The Uptake of Metals by Plants

Passive, facilitated and active uptake

The processes of uptake can be described as passive, facilitated or active. Passive uptake depends effectively upon concentration differences and is based on diffusion processes, requiring no specific carrier. Thus the primary capture of an ion by a root or leaf is preceded by diffusion of the ion through

the bulk phase of solution to the solution–membrane interface. In the case of the root, diffusion can carry the ion into the water free space, i.e. into the volume which is bounded by the root but which is contiguous with the external aqueous phase. Passive uptake can only continue if the membrane is freely permeable to the ion, which implies that there is a continuous extension from the free space into and through the cells which form the boundary of root or leaf, otherwise some specific mechanism must exist for the transfer of the ion across the selectively permeable membrane.

Broadly speaking, passive uptake tends to reach saturation only at relatively high external levels of the ions in question and is often subject to severe competition between ions of the same charge and/or ionic radius. This competition is often used in *desorption* processes where ions in the root water free space may be removed by washing with a solution of a competing ion, so separating them from ions which have actually penetrated the membrane barrier. In some cases ion interchange is used with radioactive and stable isotopes of the same metal, whilst in other cases different metals have been used, e.g. as in the desorption of copper by lead (Harrison *et al.*, 1979). Clearly, however, the most pertinent test for passive uptake is still that it is unaffected by metabolic inhibitors or temperature effects.

Facilitated uptake requires metabolic activity but is not directly linked to a specific energetic process. Typically, uptake is facilitated by the secretions from the cell either of H^+ or a metal-binding agent, so that the chemical form of the metal may be altered, possibly by a ligand replacement reaction, thus giving a more favourable structure for uptake. It is interesting to note that the rhizosphere is now recognised to be substantially different from the bulk soil phase and root secretion is known to be quite extensive. Facilitated uptake will be affected by metabolic changes, but not immediately, since some time must elapse before the rhizosphere is significantly altered. Again, the principles of this process may be treated by speciation of the rhizosphere constituents with a kinetic component to allow for rates of secretion and diffusion. At the present time, a detailed treatment is still probably beyond the capability of current speciation schemes, though it is to be hoped that this problem will be attacked, if not solved, in the near future. Finally, it must be remarked that since trace metals, because of their chemical nature, are readily complexed, it is not possible to treat this process solely by recourse to well-established examples of ion diffusion, as say for the alkali metals.

Active uptake, which usually shows the highest degree of specificity, requires the direct intervention of a metabolic process, often, though not always, by use of a carrier molecule. It is therefore controlled directly by

applying metabolic inhibitors or by altering the temperature to alter the rate of metabolic activity. Additionally, active uptake can sometimes be recognised by the ability to *accumulate* the element in question. That is to say, the organism may continue to take up the nutrient even when the internal concentration exceeds the external (available) concentration. Clearly, in such cases, active processes require both high affinity and high specificity, thus enabling the organism to counter the twin problems of low availability and competition from other metals; as a result, active processes are often easily saturated. Conversely, in cases of low nutrient availability, growth may become self-limiting as the plant efficiently scavenges the nutrient from the regions accessible to the root at a faster rate than it can be replaced.

Clearly, of all the processes of uptake, active transport is least amenable to treatment via the simple coordination chemistry approach, and normally a much fuller appreciation of biochemical factors is required. Nevertheless, complex formation must be considered, as is shown in the following section, as one way at least in which selectivity may be generated. Finally, it must also be made clear that the three processes outlined here are in no way exclusive and there is no reason to suppose that they cannot operate either in series or in parallel, and as for iron (probably the best studied of all micronutrients with respect to root uptake) it is almost certain that both active and passive routes operate concurrently (Brown, 1979; Pandey and Kannan, 1979).

The generation of selectivity

If a metal is essential, then it is imperative that the plant be able to select it for uptake from a milieu containing a wide variety of metals, some with perhaps quite similar chemistry, and conversely to simultaneously exclude unwanted metal species by virtue of the same selectivity. The problem is not simple, and recourse to classical analytical chemistry soon shows the extent of the difficulty. In conventional complexometric analysis, much attention has been paid to synthesising ligands which have just the high degree of specificity which seems to be required by any living system (West, 1979). Analytically, the problem is to form a specific complex between the reagent ligand and only one particular metal ion. Generally, the degree of success is not encouraging, and the chemist has to resort to various secondary techniques such as control of pH, masking, etc., which are generally not employed biologically. Yet it is undoubtedly true that plants and other living organisms do show a remarkable degree of selectivity towards metal ion uptake. One brief, and most pertinent observation, is that the ligands

used are generally much more sophisticated than those employed by the analytical chemist, but it is also true that the same principles outlined and employed all through this chapter may be applied here.

Selectivity is a demonstration of either thermodynamic or kinetic effects. The treatment of thermodynamic selectivity is rather more formalised than examples of kinetic selectivity, which are treated on a much more empirical basis. If a metal ion has a choice of sites onto which it may bind, then it may be expected that binding will be controlled by the appropriate free energy changes. Thus if site (1) is preferred to site (2) the condition may be set as

$$G^{\ominus}_{\text{site (1)}} - G^{\ominus}_{\text{solution}} < G^{\ominus}_{\text{site (2)}} - G^{\ominus}_{\text{solution}}$$

Alternatively if two ions (A, B) are competing for a single site and A is preferred to B:

$$G^{\ominus}_{\text{site (A)}} - G^{\ominus}_{\text{solution (A)}} < G^{\ominus}_{\text{site (B)}} - G^{\ominus}_{\text{solution (B)}}$$

(where the free energies are referred to some constant, but arbitrary, standard state).

Clearly the first case is relatively trivial and almost tautologous since it merely predicts that binding of the ion must be stronger to site (1) than to site (2).

However, the argument can be extended if it is assumed that ion binding is dominated by predominantly Coulombic forces. In this case the *smaller site* should produce the highest ion–site forces and hence produce the most energetically favourable binding situation, with the proviso that the ion itself must actually be small enough to enter the site.

In contrast, the second case is more illuminating, since two extreme cases can be derived. If ion–site forces are large, then solution energy terms may be neglected so that the equation reduces to:

$$G^{\ominus}_{\text{site (A)}} < G^{\ominus}_{\text{site (B)}}$$

which is clearly the counterpart of the previous case. In this example a *smaller ion* will be bound in preference to a larger ion since in this way ion–site forces will be maximised. Conversely, if ion–site forces are weak, as with relatively large sites, then solution forces may dominate the selectivity pattern so that the equation reduces to:

$$G^{\ominus}_{\text{solution (B)}} < G^{\ominus}_{\text{solution (A)}}$$

In the case of selectivity between simple aquo ions, the solution energy term may be equated with the hydration energy of the ions, whereupon it is possible to make reasoned predictions, since tabulations of single ion hydration energies are readily available (Huheey, 1975; Phipps, 1976; Purcell and Kotz, 1977). Of course, such predictions assume that ion–site

forces in particular are principally ionic and are dominated by a Coulombic attraction term. Whilst this may be true for binding of s-block metals (especially the alkali metals) to oxygen donor ligands (i.e. hard–hard acid–base combinations) it is certainly not the case for other combinations and the deviations from these simple predictions will become larger as covalent bonding becomes more significant. This is apparent as ionic charges increase or as p- and d-block metals are considered. In general then, selectivity between metal ions may arise from differences in size (ionic radii), charge, Lewis acidity or redox behaviour, or a partial combination of several of these factors.

Selectivity by size and stereochemistry

Perhaps one of the more remarkable developments in 'bio-inorganic' chemistry in recent years has been the recognition of a whole new area of alkali-metal coordination chemistry in which the ligands are either natural macrocycles such as the peptide antibiotics or the totally synthetic 'crown polyethers'. In either case, it has been possible to demonstrate that a remarkable degree of selectivity is engendered by the construction of an appropriately sized 'hole' into which a specified metal ion will have a precise fit. Ions smaller than this will bind only poorly since their contact with the ligand donor groups will be restricted, whilst ions larger than the 'hole' will clearly be excluded. Of course, these specific ligands can only be invoked to explain restricted examples of selectivity (Na^+/K^+ for example, which is not of immediate interest here) but the principle is equally valid for other ions and it is possible to recognise other bio-molecular species, notably proteins, which can be used to produce ion-binding sites of specific size. However, it is unlikely that there are many cases in which selectivity is generated solely by size effects, and the contribution of ligand geometry cannot be ignored. This is particularly true of p-block metals and for the d-block transition elements where binding in the complex can be considered to have a much greater covalent contribution, with a concomitant directional nature for the metal–ligand bonds, rather than the less directional ionic nature of the bonding in the s-block (or even f-block) metals. Thus bonding requirements for Zn^{2+} will ideally demand a tetrahedral geometry for the donor groups in the binding site, whilst Cu^{2+} would clearly require a square-planar geometry. Such requirements must then be superimposed on the necessity for appropriate overall dimensions to the binding site.

Selectivity by charge

From the foregoing discussion, it might be expected that a divalent ion,

M^{2+}, would always tend to displace a monovalent ion, M^{+}, provided both ions could bind satisfactorily to the same site, since ion–site forces will increase rapidly with increasing charges on either the cation or the anion. Surprisingly, the anticipated effect is not always observed, and in some cases monovalent ions may actually be bound in preference to divalent species. The critical factor in generating selectivity in this situation is the relative spacing of the binding sites (Diamond and Wright, 1969). It has been shown that in zeolites, widely spaced sites exhibit a preference for M^{+} cations but as the site density increases, the binding of M^{2+} ions becomes preferred. Clearly then, although the observations relate critically to an inorganic medium, there is no reason why this argument should not be extended to biological membranes and the like, where a multiplicity of binding sites are available.

Within a given charge type, selectivity may be predicted as outlined previously by considering site and solution energies; though for highly charged or covalently bonded ions deviations are substantial.

Selectivity by Lewis acidity

Little need be said to expand previous discussion, since to the inorganic chemist at least, the concept of Lewis acidity coupled with the division of electron pair donors and acceptors into 'hard' and 'soft' types is sufficient in itself to rationalise many of the observed biological selectivity patterns.

Selectivity by redox behaviour

Whilst s-block and some p-block metals do not exhibit any redox chemistry under biological conditions, the majority of what are generally considered to be 'trace elements' do have significant opportunity for variation in oxidation state. In many cases the change in oxidation state may be the single most important effect in controlling biological counteractions. For example, Cr(VI) and Cr(III) are both readily available oxidation states for the metal under biochemically significant conditions, and the chemistry of the metal in these two states is substantially different. Similar observations apply in other cases where a choice of oxidation states is available, for example Pb(IV)/Pb(II).

6. CONCLUSION

It has been the aim of this chapter to point to the relevance of chemical principles in understanding biological actions. Clearly, in a limited space

many topics have necessarily been treated cursorily, or even omitted, but it is to be hoped that the major areas of contact between chemist and biologist, relevant to the understanding of metal ion behaviour, especially as to their entry into plants, have been covered. One major limitation has been the omission of almost all discussion on functionality for metals in plants, but specific aspects of this topic are covered by other authors. In any case, the principles hopefully established in this chapter apply whether uptake or functional role is being considered.

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CHAPTER 2

Lead: Understanding the Minimal Toxicity of Lead in Plants

DAVID E. KOEPPE

Department of Agronomy, University of Illinois, USA

1. INTRODUCTION

The presence of indigenous lead in the earth's crust at concentrations averaging 16 μg/g soil (De Treville, 1964), its recent deposition near highways and other pollutant sources (Koeppe, 1977), and the knowledge that lead is highly toxic to various physiological processes have brought into focus over the last 15–20 years the question of the effects of lead on higher plants. This has seemed an especially relevant question when consideration has been given to the fact that certain higher plants probably suffer a lead insult that is greater than that imposed on any other living organism. Recent man-effected lead deposits have been near highways through the burning of leaded gasolines by automobiles (Cannon and Bowles, 1962), in soils near buildings painted with leaded paints (Getz *et al.*, 1977), on agricultural lands fertilized with sewage sludge (Hinesly *et al.*, 1972) and in various localized situations near metal ore deposits, mining, smelting and other industrial operations (Antonovics *et al.*, 1971). Pesticides containing lead have, dependent on usage, left substantial lead residues in orchards and other agricultural areas where they have been sprayed (Schuck and Locke, 1970).

2. UPTAKE OF LEAD BY PLANTS

Lead is available to plants from soil and aerosol sources. The actual uptake and association of lead with plants is affected (to varying degrees) by almost all environmental factors (Zimdahl and Koeppe, 1977). In a very general way it has been observed that the lead content (in and/or on) of plants

generally reflects the extent of lead insult, or what can be termed biologically-available lead. The chemical form of lead as it impacts plants is of critical importance, however, as this is a factor in movements into plants, in translocation and in the toxic effectiveness of lead within the plant.

Plants growing near highways are usually exposed to more lead than most other locations (Zimdahl and Hassett, 1977). The source of this lead is primarily from aerosol automobile exhaust particulates as lead halogens which are not water soluble (Rolfe and Reinbold, 1977). Even lead incorporated into soils is nearly always tightly bound to organic or colloidal materials, or in a precipitated form; all of which serve to reduce the uptake of lead into plant roots by conventional processes involving soluble ionic movement (Zimdahl and Koeppe, 1977). These observations are supported by experiments which show that increased cation-exchange capacity (Baumhardt and Welch, 1972; Miller *et al.*, 1975*a*; Miller *et al.*, 1975*b*; Motto *et al.*, 1970), lowered pH (Cox and Rains, 1972; Lagerwerff *et al.*, 1973; Miller *et al.*, 1975*a*; Miller *et al.*, 1975*b*; Zimdahl and Foster, 1976; John and Van Laerhoven, 1972), organic matter (Liebhardt and Koske, 1974), phosphorus (Hassett *et al.*, 1976; Miller and Koeppe, 1971; Rolfe, 1973; Zimdahl and Foster, 1976; Sung and Jeong, 1977) and other lead-precipitating anions (Rolfe and Reinbold, 1977; Karamanos *et al.*, 1976; Sung, 1976) reduced the uptake of lead into plant roots.

Aside from the general low availability to plants of soil lead, the movement or translocation of lead from absorbing roots, or root hairs, is apparently impeded by a number of biochemical and/or physical processes involving lead binding, inactivation and/or precipitation. Early work by Hammett (1928) showed lead to be localized in the cell walls and nuclei of absorbing roots. Tandler and Solari (1969) found that lead was bound to orthophosphate ions within the nucleolus of onion root tips fixed in a lead solution. Other studies have shown that lead is often found in the cytoplasm of cells associated with electron-dense precipitates localized in membranous inclusions, vesicles or organelles. Malone *et al.* (1974) reported that the roots of corn plants exposed to lead in hydroponic solution accumulated a surface lead precipitate and lead crystals in the cell walls. They hypothesized that dictyosome vesicles were responsible for an active extrusion of apparently soluble lead from root cells. In corn, an encased deposit of lead surrounded by the vesicular membrane was observed to migrate toward the outside of the cell where the membrane surrounding the deposit fused with the plasmalemma. The material surrounding the deposit then fused with the cell wall, resulting in a concentration of lead deposits in the cell wall outside the plasmalemma. Similar deposits were also observed

in stems and leaves which would support the hypothesis that once translocated, lead could be 'extruded' from cells throughout the plant.

The impaction on plant leaves of aerosol lead particles from automobile exhaust has been well documented (see Zimdahl and Koeppe, 1977 for review). The uptake into plant cells of such lead has been a source of much controversy. Zimdahl and Koeppe (1977) concluded from a literature review that little, if any, aerosol-deposited lead in nature is accumulated through the cuticular barrier of higher plant leaves. The bryophyte *Rhytidiadelphus squarrosus* did take up lead from traffic exhaust, as electron-dense precipitates were recognized within cytoplasmic vesicles or vacuoles, chloroplasts, mitochondria, microbodies and plasmodesmata (Ophus and Gullvag, 1974).

Cannon and Bowles (1962) reported that lead was associated with the above-ground portions of grass grown close to roads in concentrations as high as 3000 μg/g dry weight of plant matter. Schuck and Locke (1970) and Wedding *et al.* (1975, 1976) determined that substantial lead particulate matter was deposited on plant surfaces. Such deposition was dependent upon the characteristics of the leaf surface as well as the wind speed, and, to a lesser extent, other environmental conditions. Seven times more lead was deposited on pubescent sunflower leaves than on glabrous tulip poplar leaves (Wedding *et al.*, 1975). These, and additional experiments (Wedding *et al.*, 1975; Carlson *et al.*, 1976) found no re-entrainment of monodispersed lead particulates from a leaf surface at wind speeds up to 6·7 m/s, even though these winds caused a rapid fluttering of the test leaves. While topically applied lead was minimally removed by wind, it was readily removed by simulated rain conditions. A falling mist was more effective than large raindrops in lead removal (Carlson *et al.*, 1976). The smaller drops of mist coalesced into larger drops, ran toward the leaf edge and fell off; while the larger, less effective drops splashed off at impact.

It is almost certain that plants growing near highways possess elevated lead levels, probably in a particulate form, associated with leaves and stems. Even if this lead is not absorbed into the plant cells, its presence as a topical coating on leaf surfaces must be considered. Does it have effects on plant processes or those of the microorganisms co-inhabiting these plant surfaces?

3. EFFECTS

The ubiquitous presence of lead in soils, complicated and accentuated now by man-effected additions, makes questions of lead effect on plants of

critical importance in formulating both short- and long-term environmental policy. The importance of these questions is amplified by two possibly conflicting observations that have been in the literature for many years. The first is that lead is highly toxic to many organisms under certain conditions. Secondly, even though very large concentrations of lead are present in localized plant environments, and even associated in or on plants, there are few reports of lead-induced toxic effects on plants grown in natural ecosystems that have been severely impacted with lead.

This section will consider the effects of lead on plants when it is available from rooting media or atmospheric sources. It will also discuss some of the studies which specifically ask questions of the effects of lead on *in vitro* physiological processes. Finally, the association of lead with plants will be summarized from the larger ecosystem–food chain vantage point.

3.1. Distribution

When an analysis is made of how lead may influence plant growth, questions relating to uptake patterns and mechanisms of deposition (localization) within the plant are often the first to be considered. All studies show that most lead impacted at the root or leaf surface remains at that site without movement. When roots are treated, very little lead is translocated into edible fruits (Baumhardt and Welch, 1972; Ter Haar, 1970; Zimdahl and Koeppe, 1977). Early studies, including those by MacLean *et al.* (1969), Page and Ganje (1970), Aarkrog and Lippert (1971), and many others, have found the lead content of fruits, vegetables and grain to be less than in other vegetative plant parts. In plants exposed to lead-contaminated rooting media, the roots always contain lead at concentrations considerably greater than other above-ground tissues. Hevesy (1923), in one of the earliest studies of this problem, concluded that lead is bound in the roots subsequent to root treatment, and that such binding serves to protect the remaining plant parts from injury. Recent studies utilizing various types of microscopical and histochemical techniques support the concept that large amounts of lead are bound to the outside of roots, the 'free space', as well as cell walls throughout the roots (Malone *et al.*, 1974). Studies where root metabolism has been controlled, suggest that the outside and free-space binding is passive (Goren and Wanner, 1971). Less is known about the energetics of the intracellular lead deposits associated with organelles and vesicles. It seems unlikely that the dictyosome vesicle lead deposits in corn reported by Malone *et al.* (1974) are passive, however, their studies did not seek to answer this question directly. Sharpe and Denny (1976) observed that the plasmalemma acted as a barrier to lead in *Potamogeton pectinatas*.

The movement of lead once it is clearly within the functional metabolic regions of the roots has been hard to evaluate. Whether lead moves as an ion, or as a chelated complex, is a reasonable question but not easily answered. Chelation of lead with EDTA does facilitate lead movement. Wallace *et al.* (1976) and Patel *et al.* (1977) observed that chelating agents modified the uptake of heavy metals from the soil, and Marten and Hammond (1966) found that the presence of EDTA increased lead uptake by plants.

Tanton and Crowdy (1971) reported that lead–EDTA entered roots only in the short root-hair zone, and that the root endoderms appears to limit the movement of the lead chelate into the transpiration stream. Once in the transpiration stream, however, they suggested that lead moved passively, and that it accumulated at the evaporative surfaces of the leaf. The possibility that lead moves into and within plants in a naturally chelated form is suggested from work at the University of Illinois where naturally exuded compounds were found which chelated lead (Gadde and Laitinen, 1972).

Only a few reports are available which assess the question of the chemical identity of lead in plants. One compound, lead pyrophosphate, has been identified in the roots of pinto bean where the lead concentration was 32 400 μg/g dry weight (Zimdahl, 1972). There has also been a report that lead orthophosphate ($Pb_3(PO_4)_2$) was present in soybean roots (see Zimdahl and Koeppe, 1977). Other studies by Brown and Slingsby (1972) with the lichen *Cladonia rangiformis* detected lead bound to insoluble anionic sites in exchangeable form, but external to the cell membranes.

The potentially tight and nearly irreversible binding of lead to most exchangeable surfaces reported by soil scientists, and those reports referred to here with plant roots, adequately set the stage and predict, *a priori*, why plants show so little response to potentially toxic concentrations of lead in the plant environment. Numerous experiments have been conducted, however, which have quantitated the effects of lead on both general yield parameters (including gross vegetative yield and grain yield) and more specific or integrated whole-plant physiological processes. These studies will be considered in the next section of this chapter.

3.2. Aerosol Effects

Lead particles deposited on leaf surfaces could be hypothesized to affect plant processes in two general modes. First, intracellular physiological processes could be affected if lead is solubilized and moves into the cells of the leaf. In Section 2 the possibility of this seems to have been

experimentally discounted. Carlson *et al.* (1976) made measurements of lead movement from leaf surfaces (fumigated with 1–3-μm diameter $PbCl_2$ aerosol), to non-fumigated plant tissues. In two experiments at least, several fully developed leaves of soybean plants were selectively fumigated. Following a post-fumigation period, the lead associated with the fumigated leaves was determined, as was the lead concentration of several non-fumigated sink tissues, including newly developed leaves and developing bean pods. In both the above experiments and a third in which the lead concentration of fumigation was very high and conditions were optimized for the movement of lead from the source leaf, negligible movement into and/or within the plant was observed.

In corollary experiments Carlson *et al.* (1977) measured the rate of photosynthesis of leaves after topical application of $PbCl_2$ particles up to concentrations of 700 μg/g fresh leaf weight. Photosynthesis was not reduced significantly with this extent of leaf contamination over a three week post-fumigation period.

The question next arises as to whether lead particles may accumulate in the stomata of leaves, thus affecting the ability of the stomata to open and close diurnally and with environmental stress, such as that imposed by water deficits. Flückiger *et al.* (1979) reported that the diffusive resistance of aspen leaves was reduced when the surface was impacted with chemically inert 5-μm diameter silica gel particles. Their interpretation of this situation was that particles of a proper size will affect plant–water relations by blocking stomata, thus preventing their closure during hot and dry periods that would be required to prevent drought injury. Carlson *et al.* (1976, 1977), however, argue that 1–3-μm diameter $PbCl_2$ particles on soybean leaf surfaces are not likely to physically block stomata. They point out that to physically block only 10 % of the stomatal pore area of a soybean leaf with 1-μm diameter $PbCl_2$ particles would require about 7000 μg $PbCl_2$/g of leaf. In using this reasoning, and their photosynthesis rate studies, they conclude that stomatal blockage will not effect decreased rates of photosynthesis unless particulate deposition reaches extremely high values.

Other studies of physiological processes of plants exposed to air pollution (including lead) have been conducted with plants grown at varying distances from highways. A recent experiment by Flückiger *et al.* (1978) has correlated Pb collected in filters at varying distances from a highway with growth, peroxidase activity and ATP formation in chloroplasts isolated from the exposed plants. Concentrations of lead decreased rapidly from the highway, being reduced to 33 % at a distance of 10 m from the highway border. Peroxidase activity was increased in plants

at 2 m and 12 m distance when compared to activity in plants at 42 m and 186 m distance. The formation of ATP in isolated chloroplasts and plant growth exhibited an increasing inhibition with decreasing distance from the highway; plants at 2 m distance were the most inhibited. Unfortunately, these authors were not able to specifically isolate the effects of lead from other pollution sources arising in the vicinity of highways. And while it was inferred that dust and exhaust gases were responsible for the observed effects, the authors suggested that the exhaust gases exerted a predominant influence.

Steenken (1973) directly fumigated, with automobile exhaust, a number of cultivated plants in greenhouses, seeing in certain situations lethal effects on older leaves, but usually only slight effects on younger leaves. Interestingly, in complementary experiments where fumigation was with Pb-free automobile exhaust, the effects were even more severe at most exposure levels. Although Steenken did find test plants with Pb concentrations of 350 mg Pb/kg dry matter, he was unable to show any statistically significant depressions of yield and growth due solely to lead.

Smith (1976) has found that the lead burden of plants due largely to surface deposition was as high as 200 × the baseline levels for unwashed plants. He was unable to find, however, any acute and direct effects of this lead on the roadside biota. His work does point in another direction that we will consider later, that being the potential of a topical lead coating to exert a subtle impact on the microflora on the plant surface and to have food-chain effects amplified through foraging insects and animals.

3.3. Whole Plant Effects (Lead in the Rooting Medium)

Reports on the effects of lead on selected aspects of plant growth have appeared in the literature for over 50 years. Both growth stimulations and reductions have been reported to result from varying lead concentrations in the rooting media. Growth stimulations, mostly at low lead concentrations, have been reported for several different plants by Keaton (1937), Berry (1923), and others (see Tornabene *et al.*, 1977 for review). Since most of these reports came before the definition of optimal conditions for hydroponic growth of plants, it might be suspected that the growth stimulation itself was not due to lead, but possibly to other ion(s) made available as a result of lead salt treatments to the rooting media. Numerous other studies utilizing lead-amended hydroponic culture have shown a significant reduction of growth with increasing lead concentrations. Evidence has also been presented that the magnitude of the lead effect various inversely with the phosphate concentration of the nutrient solution

and with the phosphate status of the plant itself (Miller and Koeppe, 1971; Rolfe, 1973).

Other examples in which general decreases in growth parameters have been determined after lead treatment in hydroponic culture include studies with red maple (Davis and Barnes, 1973), oats (Fiussello and Molinari, 1973), soybeans (Huang *et al.*, 1974), corn (Carlson *et al.*, 1975), sunflower (Carlson *et al.*, 1975), rice (Fiussello and Molinari, 1973; Mukherji and Maitra, 1976, 1977), loblolly pine (Davis and Barnes, 1973), and groundsel (Briggs, 1976). In most of these situations the time of treatment was a significant variable in the extent of the lead-induced toxicity.

In a comparative hydroponic study to determine the relative toxicity of lead to a number of different plants, Fiussello and Molinari (1973) determined the following sequence of decreasing sensitivity to lead:

Capsicum annuum > *Beta vulgaris* > *Phalaris canariensis* > *Vicia sativa* > *Helianthus annuus* > *Oryza sativa* > *Triticum vulgare* > *Avena sativa* > *Pisum sativum*

An example of the extent of the lead toxicity is that *A. sativa*, one of the least sensitive species, showed a 34% decrease in fresh weight, a 23% decrease in dry weight and a 26% decrease in chlorophyll content when grown for 21 days in nutrient solution amended with 10^{-4} M $Pb(NO_3)_2$.

Merakchiiska *et al.* (1976) reported that *Phaseolus vulgaris* (bean) exhibited reductions in whole plant, leaf and root fresh weight after 10 days' treatment with 10^{-5} M $PbCl_2$ in hydroponic solution. The reductions were about twice as great when the $PbCl_2$ concentration was increased to 10^{-3} M. With the 10^{-5} M $PbCl_2$ treatment, the length of the whole plant was reduced by 34% and the length of the root by 70%. Leaf surface area was reduced 26%, but measurements of the length of the aerial part of the plant and the hypocotyl node each showed increases of 16%.

With rice (*Oryza sativa*) root growth was completely inhibited by 2×10^{-2} M lead acetate and germination by 10^{-1} M lead acetate (Mukherji and Maitra, 1976). At 10^{-3} M lead acetate, shoot growth was inhibited by 9%, whereas the inhibition of root growth was 46%.

Broyer *et al.* (1972) in experiments with *Phaseolus vulgaris* (bean, var. bush blue lake), *Hordeum vulgare* (barley) and *Lycopersicon esculentum* (tomato) observed only slight reduction in plant top and root dry weight following treatments of up to 50 μg atom/litre initial lead supply. Possible reasons for the lack of observable lead effect may have resulted from shorter treatment times and much lower lead concentrations over the entire

duration of the treatment period. The authors did find most of the lead associated with the roots and little with the plant tops. This lead sorption to the roots of barley was very rapid and not influenced by temperature. It is often very difficult to compare the total amount of lead treatment with the roots of plants, and herein often resides the probable quantitative differences in lead-effected plant response.

In other experiments, lead salts have been incorporated into a number of different soils. Neither alfalfa nor corn were affected by lead concentrations up to 212 μg/g in Chester silt loam soil (Lagerwerff *et al.*, 1973). Soybean (*Glycine max.* L.) and corn (*Zea mays* L.) were sown in a sandy-clay soil and watered with fixed concentrations of lead and various anions (Sung and Jeong, 1977). When the plants were harvested before flowering they showed no signs of lead toxicity in spite of 3773 μg Pb/g soil. Total lead concentrations in the plants did not exceed 3·64 μg/g dry weight. In another study with 500 μg/g Pb in the soil, radish, carrot and spinach showed considerable uptake of lead but without reducing yield or toxicity symptoms (Judel and Stelte, 1977). Other work has revealed no yield reduction in alfalfa and bromegrass grown on soils amended with up to 100 μg Pb/g soil (Karamanos *et al.*, 1976). In many of the more recent studies the defined presence of anions such as phosphate and sulfate has reduced lead uptake and/or effect (see Section 2).

One of the more significant applications of lead to agricultural soils has been with numerous other heavy metals and other major and minor micronutrients in sewage sludge. With the ever-present possibility that a number of these metals besides lead might be toxic to plants, it has not been possible to isolate any distinct lead effects from such sludge applications. In actuality, the presence of so many other competing ions probably serves to reduce the uptake and any effect of lead. Several studies have questioned, however, whether combinations of heavy metals that are known plant toxins might at certain concentrations act synergistically (or antagonistically). Two reports from a trace element research project at the University of Illinois serve to illustrate this point. In a study by Miller *et al.* (1977) varying concentrations of lead and cadmium were incorporated into a sandy loam soil with a relatively low cation-exchange capacity. In measurements of 10–31 days old corn shoots there was a tendency for soil lead to increase the plant cadmium concentration and the total cadmium uptake. Conversely, soil cadmium reduced the total lead uptake and in some cases lead concentration of the shoots. Statistical analyses show that both lead and cadmium individually reduced corn shoot growth, and that there was a positive interaction of the two metals on growth. In another

study by Carlson and Bazzaz (1977), seedlings of American sycamore (*Plantanus occidentalis* L.) were grown in Drummer silty clay loam soil treated with various concentrations of lead, cadmium or lead plus cadmium. Plant growth and heavy metal content were measured after 90 days of treatment. Concentrations of both cadmium and lead were increased in the plant tissue, and root growth, woody stem growth, new stem growth and foliage growth were synergistically affected by the lead–cadmium treatment. That is, the effect of the two heavy metals together was greater than the sum of the effects of the individual heavy metal treatments.

Research with several plants including *Lactuca sativa* L., *Raphanus sativus*, *Oleifera* sp., *Setaria italica* L., *Raphanus sativus radicula* L. and *Tagetes* sp. by Krause and Kaiser (1977) assessed the interaction of lead and sulfur dioxide fumigation. In their studies, neither the uptake and translocation of lead, nor its effects on yield were influenced by the sulfur dioxide treatment.

As a result of observations of the uptake and effect of lead on the general growth of plants in the field and the literature cited here, along with many other studies too numerous to mention, the following concepts have emerged. First, that lead is taken up by plants with the magnitude of uptake being correlated inversely to soil or other artificial rooting-media conditions that bind lead. Such binding of lead reduces its activity (in a physical chemistry sense) in the soil or rooting-medium solution. In other words, good agricultural practice in monocultural or natural systems should virtually negate the uptake and effect of lead under field conditions. In the absence of agricultural disturbance, it seems that Pb uptake from the soil would be found only under conditions where the soils were light and had low fertility levels, low organic matter levels and/or a low cation-exchange capacity. Even here, where some lead uptake is likely, studies have shown that 90 % or more of the lead taken into roots is tightly bound there, not available for translocation to other parts of the plant, and not an effective toxin. The chances that enough lead can be taken up by any higher plant under natural conditions to effect a toxic response seem remote. Under controlled lead-treatment conditions, toxic responses influencing physiological parameters other than general growth have been quantitated, and are the subject of the next section of this chapter.

3.4. Specific *In Vivo* Lead Effects (Lead in the Rooting Medium)

This section will review studies in which plants were exposed to high lead concentrations in the rooting medium, and in which the response to lead of

processes other than general growth were measured. These effects might be considered *in vivo* as compared to *in vitro* studies where Pb was applied directly in the 'test tube' to enzymes, organelles or other subfractionated cellular components.

Gas exchange, particularly the uptake of CO_2 in photosynthesis, has been determined for several different plant species treated with different concentrations of lead for increasing periods of time. Among the plants tested in these experiments, soybean (*Glycine max.* L.) (Bazzaz *et al.*, 1974*b*), sunflower (*Helianthus annuus*) (Bazzaz *et al.*, 1975; Carlson *et al.*, 1975; Bazzaz *et al.*, 1974*a*), American sycamore (*Plantanus occidentalis* L.) (Carlson and Bazzaz, 1977), corn (*Zea mays* L.) (Bazzaz *et al.*, 1975; Carlson *et al.*, 1975), loblolly pine (Rolfe and Bazzaz, 1975), autumn olive (Rolfe and Bazzaz, 1975) and spruce (Keller and Zuber, 1970) exhibited reduced rates of photosynthesis with increased soil lead concentrations. Like most of the lead-effects experiments, the effective rooting-media lead concentrations were usually very high as compared with the concentrations of lead encountered by almost all plants growing naturally or in monoculture. Because of the techniques and experimental procedures involved, few correlations have been made between the lead content of the leaves and their rates of photosynthesis. One study making these measurements was done by Bazzaz *et al.* (1974*a*) who reported for sunflower that at leaf lead concentrations of 193 μg Pb/g dry weight the rate of net photosynthesis was reduced by 50 %. Rolfe and Bazzaz (1975) found no effect of Pb on either photosynthesis or transpiration of loblolly pine (*Pinus taeda*) or autumn olive (*Elaeagnus umbellata*) at tissue concentrations below 60 and 72 μg/g dry weight respectively. At these concentrations photosynthesis was reduced in loblolly pine by 11 % and in autumn olive by 17 %. In other studies carried out by Bazzaz and coworkers (Bazzaz *et al.*, 1974*a*; Bazzaz *et al.*, 1974*b*; Carlson *et al.*, 1975; Rolfe and Bazzaz, 1975) there was a strong correlation between lead-effected decreases in photosynthesis and concomitant decreases in rates of transpiration. The suggestion from this is that decreases in the rates of whole-plant photosynthesis may be due to induced closure of stomata rather than a direct effect on the process of photosynthesis residing directly within the chloroplasts. This point may be questioned, however, as reports are available of *in vitro* effects of lead on isolated chloroplasts (Miles *et al.*, 1972; Bazzaz and Govindjee, 1974; Wong and Govindjee, 1976).

The possibility of *in vivo* effects of lead on chloroplasts that may be responsible for cell death has been reported after studies with the hydrophyte *Ceratophyllum demersum* (Rebechini and Hanzely, 1974). In

this study, striking changes in chloroplast fine structure resulted from growth in an aquatic medium containing $Pb(NO_3)_2$ chelated with EDTA. Lead-exposed leaf cells exhibited a reduction in grana stacks, a reduction in the amount of stroma in relation to the lamellar system, and showed an absence of starch grains.

In bean, however, Yordanov and Merakchiiska (1976) found that 10^{-3} M $PbCl_2$ stimulated photosynthesis on a leaf-area basis, but effected a 15–20 % reduction on a mg-chlorophyll basis. These authors also reported no significant effect on chloroplast lamellar protein composition or spectral properties of the pigment system. The membranes of proplastids in the meristematic cells of lettuce root tips did show destruction of the marginal membranes after treatment with tetramethyl lead (Herich and Bobak, 1974).

The respiration of corn root tips decreased by 10–17 % after a 1-h treatment with 20 mM lead, and by 28–40 % after a 3-h treatment (Koeppe, 1977). These respiratory decreases were accompanied by a concomitant decrease of the energy charge of the treated tissue.

Lead added to a soybean rooting media of sand–vermiculite, reduced pod fresh weight by 35 % with daily waterings of 300 μM lead (Huang *et al.*, 1974). This reduction was correlated with decreases in shoot, root, leaf and nodule dry weight; and with nodule ammonia, protein and carbohydrate content. Decreases in the rate of photosynthesis and nodule acetylene concentration were also observed.

Pigment concentration changes in leaves are often a visible sign of elemental toxicity or deficiency. In $PbCl_2$-treated *Acer rubrum* (red maple) the relative anthocyanine content increased as much as 270 % (Davis and Barnes, 1973). In another quantitative study, $Pb(NO_3)_2$ in solution culture for 21 days effected a decrease in chlorophyll content of *Avena sativa* (oat) by approximately 26 % (Fiussello and Molinari, 1973). Other researchers have referred to the chlorotic appearance of leaves following lead treatment, but have usually not quantitated the loss of chlorophyll. In these studies there have been few attempts to correlate a chlorotic plant appearance only with lead accumulation.

In a study by Walker *et al.* (1977) with *Zea mays* L. (corn), $PbCl_2$ was added to a sandy loam soil in concentrations of 125–250 μg/g dry soil weight. After 20–30 days treatment the plant concentrations of boron, manganese, copper and zinc were reduced from 20–39 %. The mode of lead action in this study was not questioned. Kannan and Keppel (1976) did observe in short-term experiments with pea (*Pisum sativum*) seedlings that lead in a Hoagland's nutrient medium was highly inhibitory to the

absorption of Fe^{2+}, Mn^{2+}, and Zn^{2+} ions. They reasoned that the inhibition was of a physical nature, like blocking the entry or binding of the ions to the ion-carrier.

Several researchers have measured the activities of enzymes in generally crude homogenates of lead-treated plants. Lead acetate (6×10^{-2} M) in the liquid media of germinating rice effected about a 50% decrease in the activities of protease and α-amylase in endosperm after four days (Mukherji and Maitra, 1976). The activity of RNase was reduced by 17%, but that of DNase was stimulated by 12%.

A comprehensive study of enzymes, selected nutrients and nitrogenous compounds of soybean leaves following 10 days of $Pb(NO_3)_2$ treatment in solution culture, led Lee *et al.* (1976) to conclude that lead caused an advance in senescence of the seedlings. At lead concentrations of 100 μg/ml, leaf respiration was increased 22% while several enzymes showed the following effects when compared to zero-lead treated controls: acid phosphatase, +23%; peroxidase, +154%; malic dehydrogenase, +20%; α-amylase, +280%; and glutamine synthetase, −89%. Under the same lead treatment phosphorus, calcium and nitrate decreased, but total free amino acids, soluble protein, ammonia and lead increased.

In lead-treated young corn plants Maier (1978) found that the activity of acid phosphatase decreased in the roots but increased in the leaves. In experiments with other enzymes Maier (1977*a*) observed that NAD^+-dependent malate dehydrogenase increased in leaves of *Medicago sativa* and *Zebrina pendula*, but decreased in the *Medicago* roots. Esterase analyzed after *in vivo* lead treatment by Maier (1977*b*) increased, or decreased, as did its number of multiple forms, with the length of lead treatment and the temperature.

Several investigations have found that various forms of lead produce chromosomal lesions or other disturbances. Included in these are tetramethyl lead effects on *Lactuca sativa* (Sekerka and Bobak, 1974), lead acetate on onion root tip cells (Mukherji and Maitra, 1976), and di- and trialkyl lead on onion root tip cells (*Allium cepa*) in which spindle disturbances were noted (Ahlberg *et al.*, 1972).

Yet another interactive effect of lead is suggested in several studies, in which the hypothesis is advanced that the real toxic effect of lead is not direct on any cellular component, but rather indirect by killing cells through deprivation of phosphate. This was recently argued by Schulze and Brand (1978) following experiments with *Chlamydomonas*, and had been earlier argued with less data by Merakchiiska *et al.* (1976) in studies with lead effects on *Phaseolus vulgaris*.

3.5. Lead Effects on *In Vitro* Plant Systems

Numerous reports from the animal literature have shown that lead forms mercaptides with the —SH group of cysteine and less-stable complexes with other amino acid side chains (see Vallee and Ulmer, 1972 for review). Lead has also been shown to bind and alter the activity of many membranous subcellular particles including mitochondria, chloroplasts, nucleotides and nucleic acids and chromosomes (see Vallee and Ulmer, 1972 for review).

Using mitochondria isolated from etiolated corn seedlings Koeppe and Miller (1970) reported that $PbCl_2$ stimulated the oxidation of exogenous NADH, but inhibited the rate of succinate oxidation by more than 80 %. In a reaction medium containing sucrose or KCl as an osmoticant the effect of lead on the oxidation rates was immediate as was the reversal of the lead-induced succinate inhibition with the addition of inorganic phosphate. Inorganic phosphate did not reverse the stimulation of the rate of NADH oxidation by lead, possibly suggesting an irreversible binding of lead or that lead effected an irreversible change in the mitochondrial complex responsible for NADH oxidation. Other data strongly suggested lead-induced decreases in oxidative phosphorylation. In a subsequent study with isolated plant mitochondria (Bittell *et al.*, 1974) the concentration of lead effecting a 50 % inhibition of succinate oxidation was not different from similarly effective concentrations of cadmium or zinc. Lead (0·1 mM) caused an extensive increase in swelling of mitochondria in a reaction medium containing KCl. In binding studies it was observed that the maximum capacity of a mitochondrial suspension to bind lead was nearly 10 times greater than the 58 nM of Cd^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} bound/mg mitochondrial protein. The affinity was also observed to be very high for lead. Studies by Goyer and other workers (Goyer, 1968; Goyer and Kroll, 1969; Goyer *et al.*, 1968 and Scott *et al.*, 1971) detailing the effects of lead on animal mitochondria indicate that lead has very similar effects on both plant and animal mitochondria.

Effects of lead on isolated chloroplasts were first reported by Miles *et al.* (1972). These researchers observed that lead inhibited photosynthetic electron transport associated with photosystem II, but had no effect on electron transport through photosystem I. Fluorescence induction curves indicated that the primary site of lead inhibition was on the oxidizing side of photosystem II, between the primary electron donor of photosystem II and the site of water oxidation. Bazzaz and Govindjee (1974) later observed that $PbCl_2$ either stimulated or inhibited photosystem II activity depending on the pH of the reaction medium. Further studies with isolated maize chloroplasts by Wong and Govendjee (1976) reported the direct inhibition

by lead of the reaction centre P700 of photosystem I. They hypothesized through observations of the dark decay rates of $P700^+$, that lead altered the rate of electron transfer to $P700^+$. Thus, lead was seen to have two simultaneous effects: an inhibition of P700 photooxidation and an alteration of the kinetics of re-reduction of $P700^+$.

The photosynthetic fixation of CO_2 by isolated spinach chloroplasts was observed by Hampp *et al.* (1973*a*) to be non-competitively inhibited by lead. These inhibitions were most pronounced at concentrations up to 20 μM, and likely were correlated with a drastic reduction in ATP formation by irradiated chloroplasts. Interestingly, even at 200 μM concentrations of lead, 15% of the CO_2 fixation capacity was not inhibited.

As predicted from numerous animal studies, lead also affects the activity of numerous isolated plant enzymes. In extracts of roots or leaves of spinach, Hampp *et al.* (1973*b*) observed the inhibition of ribulose-1,5-diphosphate carboxylase and ribulose-5-phosphate kinase activity at 5 μM $Pb(NO_3)_2$. Lead stimulated the activity of pyruvate kinase up to concentrations of 100 μM. Both a stimulation and inhibition, depending on lead concentration, was observed in an NAD^+ or $NADP^+$ glyceraldehyde-3-phosphate dehydrogenase phosphoglycerate kinase system.

In a recent study by Maier (1977*b*) *in vitro* lead inhibitions of esterase activity were observed in enzyme preparations from *Zea mays*, *Medicago sativa* and *Zebrina pendula*.

Lead (2×10^{-6}–2×10^{-3} M) was found in experiments by Zegers *et al.* (1976) to inhibit the IAA-induced elongation of *Avena sativa* coleoptile segments. These authors hypothesized that lead was interfering with a factor(s) directly involved in cell elongation, suggesting the specific involvement of enzymes in the wall and the ATPase associated with the plasmalemma.

It is suspected that many other enzyme systems would be inhibited by lead if the studies were conducted as they have been with *in vitro* animal systems.

4. SUMMARY

As a result of localized pollution, highly elevated levels of lead are becoming associated with plant roots and foliage. Movement of lead into higher plants has been convincingly demonstrated through roots, but not from lead particles deposited on leaf surfaces. Association of lead with foliage is as a topical coating, with more lead deposited on pubescent leaves than

leaves with smooth surfaces. Moderate winds effecting a fluttering leaf motion cause little lead removal. Rainfall does remove large quantities of lead, with slow mist-type treatments being the most effective. Lead deposits on leaves have little effect on stomatal aperture and gas exchange, but are of considerable importance as potential toxins to the microflora associated with leaf surfaces and to grazing herbivores ranging from insects to horses. Quite possibly the most important effect of lead associated with plant foliage is in food chains where plants in essence act as passive lead carriers.

Under certain soil conditions (including low pH, low cation-exchange capacity, low organic matter levels and low phosphorus levels), large amounts of lead can be taken up by higher plant roots. Lead taken up by roots generally has no toxic effect on plants, except at extremely high root-media concentrations that have little relevance to natural conditions. This general lack of lead effect from high lead concentrations in roots has led to the hypothesis that most lead taken up by roots is quickly inactivated through deposition in the roots. Lead phosphate deposits are formed on root surfaces, in peripheral extracellular spaces (including cell walls) and within dictyosome vesicles of root cells outside the endodermis.

Environmental factors, plant age and plant speciation are important variables in lead uptake by plant roots. Generally, an alteration of any soil variable which makes lead more available in soil solution increases root lead uptake. Most studies with lead in hydroponic solution show a direct correlation with time of lead treatment, uptake into and translocation within the plant, and toxic effect.

Lead additions to both *in vitro* subfractionated cellular systems and to roots have produced effects on many different enzymes or other integrated subcellular systems. One is led to suspect that the choice of the enzyme, organelle or whole-plant process under study has been in many instances a result of the expertise and interest of the investigators rather than searches for truly critical lead-effected rate-limiting reactions. Effects of high lead concentration have been reported on photosynthesis, respiration, energy relations and an array of selected enzymes as well as yield (either fresh or dry weight, or grain). Lead has been shown to cause chromosomal aberrations and to become intimately associated with many different cellular and subcellular membranes. This association often results in the manifestation of toxic lead effects such as reductions in the activity of phosphatase and enzyme-bound ATPase.

Lead accumulations in localized areas near pollution sources probably have little direct effect on plants grown in these areas. This lack of effect most often is due to nearly irreversible binding of lead to soil exchange

surfaces and to extracellular root surfaces. Lead aerosol particulates do not enter plants and have no demonstrable physical effect on the foliar surface of plants. At this time, lead can be hypothesized to pose little, if any, problem to plants themselves, with the possible exception of areas where soil fertility, organic matter and colloid content are very low. Here only modest lead binding will occur in the plant's environment, more lead will be directly available for uptake, and some toxic effects of lead might be predicted.

The more significant role of plants with lead in the ecosystem resides in food chain situations where a topical lead coating may be ingested by herbivores grazing on these plants. The potential relevance of this problem is supported by documented deaths of horses (Rabinowitz and Wetherill, 1972) grazing in lead-contaminated roadside areas.

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CHAPTER 3

Cadmium

A. L. PAGE, F. T. BINGHAM and A. C. CHANG
Department of Soil and Environmental Sciences, University of California, USA

1. INTRODUCTION

The absorption of cadmium (Cd) by plants has not been extensively studied until recently. Essentially, all the data regarding Cd concentrations of plant tissue were accumulated during the past decade. The current widespread interest in the Cd content of plants arises from investigations revealing the harmful health effects of high dietary intake of Cd. Although acute Cd toxicity caused by food consumption is rare, chronic exposure to high Cd levels in food could significantly increase the accumulation of Cd in certain body organs. Since the deterimental effect of low-level Cd exposure takes a long time to develop, threshold levels defining maximum safe dietary intake of Cd are difficult to establish. Furthermore, the harmful effects associated with a particular Cd intake level may also be magnified or lessened by concentrations of other interacting elements present in the diet. Zinc (Zn), copper (Cu), iron (Fe) and selenium (Se), are known to influence the safe dietary levels for Cd (Underwood, 1977). The World Health Organization (WHO) (1972) has proposed a maximum tolerable intake not to exceed 400–500 μg Cd/week. Since background levels of Cd in foods contribute approximately 50 % (200–250 μg Cd/week) of the intake limit proposed by the WHO, there is a genuine concern over any increase in the concentration of Cd in foods which reach the market place.

The highly publicized episode of Cd poisoning of humans was first reported in Japan in the mid 1950s (Tsuchiya, 1978). In this case, the source of excessive Cd in the diet came from rice (*Oryza sativa*) grown on paddies which had been contaminated from Zn mining operations. The Cd

concentrations of the rice, sediments from the river and the soil in which the rice was grown were considerably greater than that found in uncontaminated regions nearby.

Even for populations not exposed to Cd contamination, the main source of Cd body burden is also from food. Friberg *et al.* (1974) estimate that the daily ingestion of Cd for most of the world's population ranges from 25 to 75 μg/day. Based upon results of market surveys over a seven-year period, the Food and Drug Administration (Mahaffey *et al.*, 1975) showed an average of 39 μg/day Cd intake for 15 to 20-year-old males in the US. Drinking water and ambient air contribute relatively little to the daily intake. Concentrations of Cd in domestic water supplies rarely exceed a few μg/liter, and at a consumption of 1–2 liters of water/day the daily intake would not exceed a few micrograms (Friberg *et al.*, 1974; Tsuchiya, 1978; Pahren *et al.*, 1979). Ambient air Cd concentrations rarely exceed 0·01 $\mu g/m^3$, and at an intake of 20 m^3 of air/day, the daily Cd intake would not exceed 0·2 μg. Cigarette smoking, however, adds considerably to Cd input via inhalation. Friberg *et al.* (1974) estimate a daily intake of from 2 to 4 μg Cd from smoking one package of cigarettes/day.

The above-cited information illustrates the contribution of Cd in the food chain to the accumulation of Cd in the human body. The concentration of Cd in foods is in turn controlled by its concentration in the plant growing-substrate and by the physical and chemical properties of the substrate. In the following sections the concentration of Cd in foods produced from natural and Cd-contaminated soils as well as the soil and plant factors influencing the accumulation are reviewed.

2. NATURAL OCCURRENCE

Cadmium is a metal seldom found in a pure state in the natural environment. It was first discovered by a German chemist, F. Strohmeyer, in 1817 as a constituent in smithsonite ($ZnCO_3$) obtained from a zinc ore. Geochemically, Cd is closely related to Zn and is often found in association with Zn, Pb–Zn and Pb–Cu–Zn ores. The Cd concentration in these ores is usually relative to their Zn content and increases as the content of Zn increases. However, Cd in the principal Zn ore, zincblend (ZnS), varies from a low of about 0·1 % to a high of 5 % (Chizhikov, 1966). After refining, the Zn concentrates typically contain 0·2–0·4 % Cd, and rarely does the Cd content in Zn concentrates exceed 1 % (Sneed and Brasted, 1955).

The average concentration of Cd in the earth's crust is estimated as

0·15 μg/g (Weast, 1969). In terms of relative abundance in the earth's crust, there are only 14 elements—silver, indium, selenium, argon, palladium, platinum, gold, helium, tellurium, rhodium, rhenium, iridium, osmium, and ruthenium—occurring at lower average concentrations. The data compiled by Waketa and Schmitt (1970) indicate the Cd contents of igneous rocks to be 0·001–1·8 μg/g; metamorphic rocks 0·04–1·0 μg/g; and sedimentary rocks <0·3–11 μg/g (Page and Bingham, 1973). Certain shales are unusually high in Cd (Wedgepohl, 1968). Along the Pacific Coast in the US, the Monterey shale formation in some locations contained as much as 90 μg Cd/g (authors' unpublished data). Marine sediments may also contain high concentrations of Cd. In the western US, marine phosphorite deposits are reported to contain from 60 to 340 μg Cd/g (Auer, 1977). Concentrations of about 100 μg Cd/g are reported for certain marine zinc-bearing phosphorites from the Oceanic Islands (Goldschmidt, 1958).

The concentrations of Cd in cultivated and non-cultivated soils not subjected to contamination, are governed by the quantities of Cd found in the parent materials. Based upon Cd concentrations reported for common rocks, one would expect that, on the average, soils derived from igneous rocks would be lowest in total Cd, soils derived from metamorphic rocks would be intermediate, and soils derived from sedimentary rocks would contain the largest quantities of Cd. Although there are considerable data in the literature on concentrations of Cd in uncontaminated soils, there is only very limited information to relate Cd concentrations in soils to their morphological properties. Results from a national survey in Japan showed mean Cd concentrations for non-polluted rice paddy lands (2746 samples), farmland (722 samples) and orchard soils (268 samples) of 0·4, 0·3 and 0·3 μg/g, respectively (Tsuchiya, 1978). Maximum concentrations of Cd for the non-polluted rice paddies, farmlands and orchard soils were, respectively, 7·1, 1·0 and 1·2 μg/g. Less-extensive studies from other nations show Cd concentrations of non-polluted soils falling in the general range of those reported for Japan. Klein (1972) presents data which show mean Cd concentrations for soils from residential (70 samples), industrial (86 samples) and agricultural (91 samples) areas to be 0·4, 0·7 and 0·4 μg/g, respectively. Reports from Norway (Allen and Steinnes, 1979), Australia (Morley, 1979) and Canada (John *et al.*, 1972) also show soil Cd within the above-mentioned concentration range. Thus it appears that typical soils will contain from 0·05 to 1·0 μg Cd/g with a median concentration of a few tenths of one μg Cd/g. However, as much as 30 μg Cd/g have been observed in non-polluted soils derived from Monterey Shale in the coastal ranges in southern California.

Soils in the vicinity of metal (Zn, Pb and Cu) mining, smelting and finishing operations are potential sites of high Cd levels. Concentrations of Cd in soils near metal mining, processing or refining operations ranging from a few to 40 μg Cd/g have been reported (Thornton *et al.*, 1979; Munshower, 1977; Cartright *et al.*, 1976; Ragaini *et al.*, 1977; Gough and Severson, 1976; Fulkerson and Goeller, 1973, and others). In a somewhat unusual situation, soils derived from old abandoned mine tailings are found to contain as much as 800 μg Cd/g (Thornton *et al.*, 1979).

In regions where solid wastes containing Cd are repeatedly disposed of or recycled on soils, the concentration of Cd in the surface soil may be elevated, as Cd is quite immobile in soils. Following repeated surface applications of the waste, Cd tends to accumulate in soil to the depth to which it is incorporated. Because of the possible pathway for disposed Cd to enter into human food chains via crops grown on contaminated soils, considerable research has been focused on studying soils which have received waste in either recycling or disposal operations. A number of monographs and review articles summarizing heavy metal impacts on land application of waste have been published recently (Loehr, 1977; Elliott and Stevenson, 1977; McKim, 1978; Leeper, 1978; Page, 1974; CAST, 1976; CEP Consultants Ltd, 1979). Investigations on the land disposal of wastes have demonstrated that the Cd concentrations of plants depend not only upon the concentration of Cd in the substrate, but also upon a wide variety of plant and soil factors.

3. SOIL FACTORS INFLUENCING CADMIUM ABSORPTION BY PLANTS

Chemically, Cd, like any other chemical element of the soil, may be dissolved in the soil solution, adsorbed onto organic or inorganic colloidal surfaces, occluded into soil minerals, precipitated with other compounds in soils, and incorporated into biological materials. The distribution of Cd among the various components of the soil matrix is fundamental to its plant availability. A shift from solid-phase forms to that of the soil solution is essential to increase plant-available chemical constituents in the soil. The factors governing the equilibrium between the solid and liquid phases of Cd in soils are complicated and not fully understood. However, observations show that the equilibrium in the multicomponent, multiphase soil system is influenced by soil pH, temperature, organic matter content, oxidation–reduction potential, mineralogical composition and the type and

concentration of other dissolved constituents. Undoubtedly, the availability of Cd to plants is also related to those factors which influence its equilibrium concentration in soil. Because these factors interact chemically, it is difficult to determine the resultant effects applicable to a broad range of soils of varying properties.

Field and greenhouse experiments have demonstrated that Cd concentration and pH of the soil are two important factors influencing the uptake of Cd by food crops. Although oxidation–reduction potential exhibits a dramatic effect on Cd absorption of plants, it is a relatively unimportant factor in food production as most food crops, except for rice (*Oryza sativa*), will not grow under reducing soil conditions. Bingham *et al.* (1976), reported greater concentrations of Cd in rice grown under oxidizing (non-flooded) than under reducing (flooded) conditions. Similar observations have been made by Takijima *et al.* (1973).

3.1. Soil pH Effects

If other soil conditions remain unchanged, the plant tissue Cd concentration would decrease as the pH of the soil increases. Bingham *et al.* (1980) show a progressive decrease of Cd in rice grain as the pH of the soil on which the plants were grown was increased stepwise from pH 5·5 to

TABLE 3.1
CONCENTRATION OF CADMIUM IN SWISS CHARD (*Beta vulgaris* var. *cicla*) LEAVES AS INFLUENCED BY CADMIUM ADDED TO SOIL AND SOIL pH

Cd added to soil[a] (μg/g)	Soil pH		
	4·5	5·2	7·4
	Leaf concn. (μg/g)[b]		
0	1·6	1·8	0·8
0·5	8·4	5·2	3·0
1·0	14	10	3·7
1·5	18	3·9	4·6
2·0	16	7·2	5·3

[a] Cadmium was added to soil in the form of municipal sewage sludge.
[b] Dry weight basis.

TABLE 3.2
CONCENTRATIONS OF CADMIUM IN VARIOUS CROPS AS INFLUENCED BY SOIL pH[a]

Soil pH	Plant species				
	Swiss chard (*Beta vulgaris* var. *cicla*)	Pea (*Pisum sativum*)	Wheat (*Triticum* spp.)	Barley (*Hordeum vulgare*)	Corn (*Zea mays*)
			Leaf concn. (μg/g)[b]		
4·6	14	1·42	0·4	1·6	0·43
7·3	3·7	0·47	0·15	0·5	0·34
			Seed concn. (μg/g)[b]		
4·6	—	0·51	0·20	0·34	0·03
7·3	—	0·17	0·09	0·11	0·04

[a] 1·2 kg/ha cadmium added to soil in the form of municipal sewage sludge.
[b] Dry weight basis.

pH 7·5. Chaney *et al.* (1975) also observed decreasing Cd concentrations in soybean (*Glycine soja*) leaves as the pH of the soil substrate was increased from 5·3 to 7·0. Similar observations have been extrapolated to rape (*Brassica napus*) fodder (Andersson and Nilsson, 1974), wheat (*Triticum* spp.) (Linnman *et al.*, 1973; Hyde *et al.*, 1979), corn (*Zea mays*) (Hyde *et al.*, 1979; CAST, 1976), lettuce (*Lactuca sativa*) (Mahler *et al.*, 1978; Giordano *et al.*, 1979; John *et al.*, 1972), Swiss chard (*Beta vulgaris* var. *cicla*) (Mahler *et al.*, 1978; Chaney *et al.*, 1976), oat (*Avena sativa*) (Chaney *et al.*, 1976), barley (*Hordeum vulgare*) (Chang *et al.*, 1979; Singh and Steinnes, 1976) and sorghum (*Sorghum vulgare*) (Chang *et al.*, 1979). Data presented in Tables 3.1 and 3.2, selected from results of experiments conducted in our laboratory, illustrate the extent to which Cd is accumulated by plants in relation to soil pH. One of the most effective means of minimizing the absorption of Cd by plants grown on acid soils is to increase their pH by liming. Chaney *et al.* (1975), demonstrated that cadmium concentrations of soybean leaves was reduced from 33 to 5 μg Cd/g (dry weight basis) when the pH of the soil on which the plants were grown was increased by liming from pH 5·3 to pH 7·0.

3.2. Effect of Substrate Cd Concentration

During the past decade numerous research reports indicate that under similar chemical, physical, biological and mineralogical conditions in the soil, amounts of Cd absorbed by plants tend to increase as the concentration of Cd in the soil increases. However, the increased Cd

concentration in plants may or may not be related linearly to the increased concentration in the soil. The magnitude of the Cd increase is not always consistent, and depends upon a wide variety of environmental factors.

A large percentage of the studies on Cd accumulation by plants in relation to the Cd concentrations in soil deal with soil that has been amended with municipal sewage sludges. Sewage sludges almost without exception contain more Cd than soils. Consequently, in situations where sludges are applied to soils, the soils tend to become enriched in Cd (Page, 1974; Sommers, 1977). It is beyond the scope of this chapter to review in detail Cd absorption by plants grown in sludge-amended soils. It has been the subject of several recent reviews and symposium proceedings (Baker and Chesnin, 1976; Chaney and Hornick, 1977; Dowdy *et al.*, 1976; Kirkham, 1976; Chaney and Giordano, 1977; Page, 1974; Information Transfer Inc., 1978; CEP Consultants Ltd, 1979).

Both field and greenhouse experiments have been conducted to evaluate the effect of Cd level in soil on its concentration in plants. Studies conducted by Page and Chang (1978) and by De Vries and Tiller (1978) demonstrate that plants grown on Cd-enriched soils in containers in the greenhouse absorb more Cd than the same plants grown on the same soil amended with identical amounts of Cd in the field (Fig. 3.1). A probable reason for the differential Cd absorption characteristics between container-grown and field-grown plants is in the root development. The roots of the container-grown plants are subjected to contaminated soil exclusively, whereas in the field the roots may extend to depths below the Cd-contaminated layer.

In order to illustrate the relationship between the amounts of Cd added to soils and the corresponding Cd concentration in a variety of vegetables, we choose results accumulated from field studies in our laboratory (Chang *et al.*, 1979) and those of Giordano *et al.* (1979). Data presented in Table 3.3 show progressive increases in concentration of Cd in foliage and the edible part of carrot (*Daucus carota sativa*) and radish (*Raphanus sativus*), and in the foliage of Swiss chard (*Beta vulgaris* var. *cicla*) and lettuce (*Lactuca sativa*) as the amounts of Cd added to the soil in the form of municipal sewage sludge is increased from 0·8 to 6·4 kg/ha. Results derived from field experiments conducted by Giordano *et al.* (1979), except for potato (*Solanum tuberosum*), also show increased Cd concentrations in a wide variety of vegetable crops upon addition of Cd to soil at a rate equivalent to 11·2 kg/ha (Table 3.4).

Not only the amount of Cd added, but also its chemical form will influence the amount absorbed by plants. This effect, although not entirely understood at this time, is no doubt caused by chemical reactions which

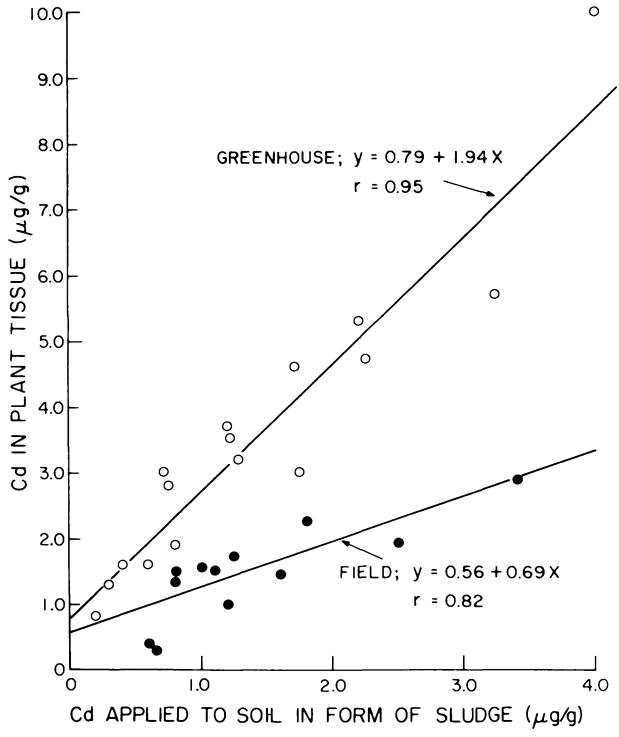

FIG. 3.1 Relationship between concentration of Cd in Swiss chard plants grown in the greenhouse and in the field. (After Page and Chang, 1978.)

occur in the soil in relation to changes in the form of added Cd. Cunningham *et al.* (1975) studied the uptake of Cd by corn (*Zea mays*) and rye (*Secale cereale*) grown in soils amended with different types of sewage sludges and observed differences in Cd accumulation by plants where the concentration of Cd in soil was approximately the same but the sludge source of Cd differed. Mahler *et al.* (1978) added $CdSO_4$-spiked sludges to soil to produce concentrations of Cd in soil equal to 10 and 20 kg Cd/ha. They reported concentrations of Cd in Swiss chard (*Beta vulgaris* var. *cicla*) plants of 15 and 26 μg Cd/g whereas Chang *et al.* (1979) reported, respectively, 4·8 and 6·6 μg Cd/g tissue where plants were grown under similar conditions, but with Cd added to the soil entirely in the form of a composted sewage sludge. Similarly, Korcak and Fanning (1978) added Cd to soil (2 mg Cd/kg) in the form of inorganic salts ($CdSO_4$) and an equivalent amount in the form of municipal sewage sludge and determined

TABLE 3.3
CONCENTRATION OF CADMIUM IN VARIOUS VEGETABLES IN RELATION TO CADMIUM ADDED TO SOIL[a]

Crop	Tissue	Application rate (kg Cd/ha)				
		0	0·8	1·6	3·2	6·4
		Cd concn. (μg/g)[b]				
Carrot	foliage	0·5	1·64	2·52	3·89	3·91
(*Daucus carota sativa*)	edible part	0·44	0·91	1·64	2·44	2·61
Radish	foliage	0·48	0·70	1·86	2·81	5·01
(*Raphanus sativus*)	edible part	0·19	0·36	0·63	0·88	1·88
Swiss chard	foliage	0·2	0·5	0·7	2·1	2·6
(*Beta vulgaris* var. *cicla*)						
Lettuce	foliage	0·5	2·2	3·1	4·4	—
(*Lactuca sativa*)						

[a] Cadmium was added in the form of municipal sewage sludge. Crops were grown under field conditions in a calcareous soil.
[b] Dry weight basis.

the amount of Cd absorbed by corn (*Zea mays*) foliage. Even though the pH of the soils amended with sewage sludge were slightly lower (pH ~6·0) than that of the same soil amended with $CdSO_4$ (pH ~6·5), the amount of Cd accumulated by the corn foliage from the soil amended with $CdSO_4$ was from 5 to 18 times greater than the amount accumulated by the foliage from the soil amended with the sewage sludge.

A number of studies suggest that the length of time soils treated with Cd are incubated also influences the availability of Cd to crops. Bates *et al.* (1975) followed the Cd concentrations from successive planting of crops grown on soils which were treated with the same amount of Cd prior to each planting. He reported that the concentrations of Cd in the first crop of ryegrass (*Lolium* spp.) were approximately the same as their concentrations in the following three crops. Hinesly *et al.* (1976) and Dowdy *et al.* (1978) reported similar observations with corn (*Zea mays*) and snap beans (*Phaseolus* spp.), respectively. Hinesly *et al.* (1979) also reported the availability of Cd to corn plants for a five-year period after the last application of Cd (in the form of sewage sludge). Results show a consistent decline in the Cd concentration of corn leaves and grain with time following cessation of Cd applications. At the fifth year, the concentration of Cd in corn grain decreased to background levels. Although the Cd concentration of the corn foliage progressively decreased, it did not drop down to background levels after the five-year period. Observations made by Street *et*

TABLE 3.4
INFLUENCE OF CADMIUM APPLICATIONS TO SOILS ON THE CADMIUM CONCENTRATIONS OF VARIOUS VEGETABLE CROPS[a]

Crop	Tissue	Application rate (kg/ha)[b] 0	11·2
		Concn. (μg Cd/g)[c]	
Lettuce (*Lactuca sativa*)	edible part	0·86	3·56
Broccoli (*Brassica oleracea botrytis*)	edible part	0·27	0·89
Eggplant	foliage	0·81	2·02
(*Solanum melongena*)	edible part	0·54	1·64
Tomato	foliage	1·11	3·61
(*Lycopersicon esculentum*)	edible part	0·52	1·04
Potato	foliage	0·80	0·69
(*Solanum tuberosum*)	edible part	0·11	0·10
Corn	foliage	0·29	16·3
(*Zea mays*)	edible part	0·10	1·83
Squash	foliage	0·15	1·40
(*Cucurbita* spp.)	edible part	0·15	0·27
Pepper	foliage	0·90	7·51
(*Capsicum* spp.)	edible part	0·25	1·30
Bean	foliage	0·16	0·72
(*Phaseolus* spp.)	shelled beans	0·07	0·21
Cabbage (*Brassica oleracea capitata*)	edible part	0·16	0·19
Carrot (*Daucus carota sativa*)	edible part	0·71	1·25
Cantaloupe (*Cucumis melo*)	edible part	0·21	0·44

[a] Derived from data published by Giordano *et al.* (1979).
[b] Cadmium was applied in the form of municipal sewage sludge.
[c] Dry weight basis.

al. (1978) and Chaney and Hornick (1977) are somewhat contrary to those of Hinesly *et al.* (1976, 1979), Bates *et al.* (1975) and Dowdy *et al.* (1978). After a 16-week incubation period, Street *et al.* (1978) observed that the availability of Cd to corn seedlings decreased when Cd was added to soils in the form of inorganic salts ($CdSO_4$), but the uptake increased when it was added through sludge applications. He suggested that increased availability of Cd to plants grown in soils treated with sewage sludge as incubation time increased was due to the release of organically adsorbed Cd through microbial decomposition of organic matter. Chaney and Hornick (1977)

reported elevated concentrations of Cd in plants grown on soils long after the soil was treated with sludge.

3.3. Other Soil Factors

Although other soil factors have been reported to influence the absorption of Cd by terrestrial plants, their effects are not nearly as consistent and straightforward as those of soil pH and Cd content of the soil. Haghiri (1974) reported a decrease in the amount of Cd absorbed by oat shoots (*Avena sativa*) as the cation-exchange capacity (CEC) of the soil was increased by adding organic matter. However, information derived from data published by Mahler *et al.* (1978), and presented in Table 3.5, show no consistent pattern in Cd absorption by either lettuce (*Lactuca sativa*) or Swiss chard (*Beta vulgaris* var. *cicla*) in relation to soil CEC. Data presented by Stenstrom and Lonsjo (1974) also show no change in the trend of Cd absorption by wheat grain (*Triticum* spp.) as the CEC of the soils increased. It is quite possible that adjusting the CEC of a particular soil by introducing a foreign substance to the soil may cause a decrease in Cd absorption by plants because the introduced substance exhibited strong reactions with Cd. But over a broad range of soils with varying CEC, effects of other soil chemical properties tend to overshadow any consistent pattern in the absorption of Cd in relation to increased CEC.

TABLE 3.5

ACCUMULATION OF Cd BY LETTUCE (*Lactuca sativa*) AND SWISS CHARD (*Beta vulgaris* var. *cicla*) IN RELATION TO CEC OF SOIL[a]

Soil series	pH	CEC meq/100 g	Cadmium added (μg/g)							
			0	5	10	20	0	5	10	20
			Cd concn. for lettuce shoots[b]				Cd concn. for chard leaves[b]			
Redding	5·4	7·6	0·9	21	40	77	2·1	33	65	133
Altamont	4·7	9·8	0·6	21	42	64	1·3	55	98	225
Hanford	5·0	18	0·8	19	32	48	2·1	33	57	110
San Miguel	5·7	38	0·8	29	50	71	2·2	41	66	115
Domino	7·5	6·5	0·9	51	88	111	1·5	29	51	81
Simona	7·7	7·4	0·5	31	52	71	1·2	25	42	58
Arizo	7·4	16	1·3	29	42	71	1·4	15	26	45
Holtville	7·7	27	3·0	49	62	99	2·7	24	40	59

[a] From: R. J. Mahler, Ph.D. Thesis, University of California, Riverside, California.
[b] Dry weight basis.

Several investigators suggested that oxides of iron and manganese exhibit highly specific adsorption affinity for trace metals, including Cd (Jenne, 1968; Forbes *et al.*, 1976). There is also limited information to support the thesis that the quantity of Cd absorbed by plants can be influenced by the content of iron and manganese oxides in soil (White and Chaney, 1976).

The synergistic and antagonistic effects of other trace metals in the soil substrate on the absorption of Cd by plants has been examined by a number of investigators. The interactive effects of Cd, Zn, Cu and Ni were studied by Mitchell *et al.* (1981). Increasing concentrations of both Cu and Ni in soil consistently reduced concentrations of Cd in the leaves of lettuce (*Lactuca sativa*) plants. However, the effect of Zn depended upon the Cd concentration in the soil. At low Cd levels ($\simeq 0{\cdot}1$ μg/g), increasing levels of Zn reduced the concentration of Cd in lettuce leaves, but at higher Cd levels the added Zn either showed no effect or increased the Cd concentration. In solution culture experiments, Lagerwerff and Biersdorf (1972) observed a similar Cd adsorption pattern for radish (*Raphanus sativus*). However, Haghiri (1974) presents data which show an increased uptake of Cd by soybean (*Glycine soja*) plants with Zn additions to the soil up to approximately 100 μg Zn/g; followed by a progressive decrease in Cd uptake at concentrations of Zn added to the soil greater than 100 μg Zn/g. Observations similar to those presented by Haghiri (1974) are reported by Chaney *et al.* (1976). Other investigators (Iwai *et al.*, 1975; John, 1976; Cunningham *et al.*, 1975; Maclean, 1976) have observed that Zn added to substrates has little or no effect on the concentration of Cd in plants. An examination of data reported on interactive effects of Zn and Cd suggest that other soil chemical properties may be operative and in certain situations tend to offset any possible synergistic or antagonistic effect Zn may have on the absorption of Cd by plants.

In addition to Zn, Cu and Ni, other elements in soils which have been reported to reduce Cd uptake by plants include Se (Francis and Rush, 1974), Mn (Chaney and Hornick, 1977) and P (Williams and David, 1977; Street *et al.*, 1978). Phosphorus additions to non-calcareous soils may reduce the solubility of Cd in the soil solution and thereby decrease plant-available Cd (Santillan-Medrano and Jurinak, 1975).

Limited information indicates that the absorption of Cd by plants may also be influenced by the temperature of the substrate in which the plants are grown. Giordano *et al.* (1979) reported significant increases in the concentration of Cd in broccoli (*Brassica oleracea botrytis*) and potato (*Solanum tuberosum*) with increased temperature of the soil substrate.

Haghiri (1974) made similar observations for soybean (*Glycine soja*) seedlings. Although increased temperatures of the soil substrate tend to increase the absorption of Cd by some plants, the effect has not been consistently observed for all plants (Giordano *et al.*, 1979; Sheaffer *et al.*, 1979).

4. PLANT FACTORS INFLUENCING CADMIUM ABSORPTION BY PLANTS

The concentrations of Cd in plants vary among species and cultivars. Different plant parts (leaves, stems, fruit, roots) accumulate different amounts of Cd. The concentration of Cd in a particular plant part is also influenced by its physiological state of development.

4.1. Natural Levels of Cd in Plants

Cadmium is a naturally occurring element present in all soils in at least trace quantities. For this reason, all plants contain detectable concentrations of Cd. Data compiled in Tables 3.6, 3.7 and 3.8 show natural Cd levels in a variety of fruits, leafy and legume vegetables, and grains. The concentrations of Cd presented in the tables pertain to situations where the soils on which the various crops were grown were not subjected to any known outside source of Cd contamination. They should be representative of natural background levels of Cd present in the crops listed.

The natural variation in concentration of Cd observed for crops varies substantially (Tables 3.6, 3.7 and 3.8). In the case of fruits (Table 3.6), concentrations generally vary by a factor of 4–6. However, there are some notable exceptions. The data of Tiller *et al.* (1976) show concentrations for tomatoes (*Lycopersicon esculentum*) which vary by a factor of 24 (0·002–0·048 μg Cd/g fresh weight basis), and those of Fuchs *et al.* (1976) show a similar variation for strawberry (*Fragaria* spp.) ($<$0·001–0·029 μg Cd/g fresh weight basis). Mean concentrations for all fruits reported range from a low of $<$0·001 to a high of 0·07 μg Cd/g, but, based upon the information presented, typical concentrations for fruits are of the order of a few hundredths of one μg/g (fresh weight basis).

Variations in natural levels of Cd in leafy, legume and root vegetables (Table 3.7), and grains (Table 3.8), are somewhat comparable to those observed for fruits. No doubt the concentration of Cd in foods grown on soils in various regions throughout the world are related to the properties of the soil on which they are grown, to the background Cd concentrations in

TABLE 3.6
CONCENTRATIONS OF CADMIUM IN VARIOUS FRUITS GROWN ON NON-POLLUTED SOILS

Type	No. of samples	Concn. (mg/kg)[a]		Reference
		Mean	Range	
Apple	14	0·01	0·005–0·027	Pfeilsticker and Markard, 1975
(*Malus* spp.)	17	<0·000 2	—	Fuchs *et al.*, 1976
	12	0·07	—	Anke *et al.*, 1976
Cherry	4	0·04	0·002–0·076	Pfeilsticker and Markard, 1975
(*Prunus cerasus*)	14	<0·000 2	<0·000 2–0·004	Fuchs *et al.*, 1976
Plum	7	0·036	0·014–0·067	Pfeilsticker and Markard, 1975
(*Prunus domestica*)	15	0·000 1	<0·000 2–0·005	Fuchs *et al.*, 1976
Strawberry	5	0·03	0·02–0·07	Tanaka *et al.*, 1977
(*Fragaria* spp.)	25	0·006	<0·000 2–0·029	Fuchs *et al.*, 1976
Peach	10	0·002	<0·000 2–0·006	Fuchs *et al.*, 1976
(*Prunus perisca*)				
Okra	4	0·08	0·04–0·11	Tanaka *et al.*, 1977
(*Hibiscus esculentus*)				
Tomato	3	0·03	0·02–0·04	Tanaka *et al.*, 1977
(*Lycopersicon esculentum*)	12	0·03	—	Anke *et al.*, 1976
	10	0·02	0·01–0·08	Thomas *et al.*, 1972
	30	0·01	<0·000 2–0·03	Fuchs *et al.*, 1976

	10	0·012	0·002–0·048	Tiller *et al.*, 1976
	12	0·1 (DW)[b]	—	Chang *et al.*, 1979
	3	0·08 (DW)	—	Dowdy and Larson, 1975
Eggplant (*Solanum melongena*)	3	0·03	0·02–0·04	Tanaka *et al.*, 1977
Pear	6	0·03	0·01–0·09	Thomas *et al.*, 1972
(*Pyrus communis*)	3	0·011	0·010–0·013	Pfeilsticker and Markard, 1975
Orange	15	0·002	<0·001–0·007	Fuchs *et al.*, 1976
(*Citrus sinensis; C. aurantium*)				
Grapefruit	3	<0·01	<0·01–0·01	Tanaka *et al.*, 1977
(*Citrus paradisi*)				
Lemon	5	0·01	0·01–0·04	Tanaka *et al.*, 1977
(*Citrus limon*)				
Rhubarb	9	0·025	0·01–0·057	Pfeilsticker and Markard, 1975
(*Rheum rhaponticum*)	10	0·010	0·005–0·016	Tiller *et al.*, 1976
Pepper, sweet	5	0·029	0·015–0·043	Fuchs *et al.*, 1976
(*Capsicum* spp.)	3	0·04	0·03–0·05	Tanaka *et al.*, 1977
Cucumber	3	0·01	—	Thomas *et al.*, 1972
(*Cucumis sativus*)	29	0·003	<0·0002–0·014	Fuchs *et al.*, 1976

[a] Except where indicated by (DW), all results are expressed on a fresh weight basis.
[b] (DW) = dry weight basis.

TABLE 3.7

CONCENTRATIONS OF CADMIUM IN VARIOUS LEAFY, LEGUME, AND ROOT VEGETABLES GROWN ON NON-POLLUTED SOILS

Type	No. of samples	Concn. (mg/kg)[a]		Reference
		Mean	Range	
Spinach, leaf	4	0·11	0·03–0·31	Tanaka *et al.*, 1977
(*Spinacia oleracea*)	9	0·045	0·019–0·070	Fuchs *et al.*, 1976
Lettuce, leaf	5	0·093	0·037–0·198	Pfeilsticker and Markard, 1975
(*Lactuca sativa*)	9	0·054	0·031–0·147	Pfeilsticker and Markard, 1975
	3	0·03	0·02–0·05	Tanaka *et al.*, 1977
	31	0·029	<0·000 2–0·06	Fuchs *et al.*, 1976
	11	0·012	0·006–0·02	Tiller *et al.*, 1976
	6	0·56 (DW)[b]	0·2–0·71 (DW)	Chang *et al.*, 1979
	16	1·23 (DW)	0·30–3·76 (DW)	Giordano *et al.*, 1979
	18	0·4 (DW)	0·4–0·5 (DW)	John and Van Laerhoven, 1976
Parsley	6	0·081	0·043–0·170	Pfeilsticker and Markard, 1975
(*Petroselinum crispum*)				
Kohl rabi	12	0·08	—	Anke *et al.*, 1976
(*Brassica oleracea gongylodes*)	14	0·026	0·01–0·071	Pfeilsticker and Markard, 1975
Celery	5	0·06	0·01–0·22	Tanaka *et al.*, 1977
(*Apium graveolens dulce*)	3	0·04	<0·01–0·05	Thomas *et al.*, 1972
Cabbage	23	0·04	0·01–0·15	Thomas *et al.*, 1972
(*Brassica oleracea capitata*)	9	0·031	0·022–0·094	Pfeilsticker and Markard, 1975
	11	0·006	0·002–0·010	Tiller *et al.*, 1976
Brussels sprout	16	0·03	0·01–0·11	Thomas *et al.*, 1972
(*Brassica oleracea gemmifera*)	4	0·027	0·017–0·036	Fuchs *et al.*, 1976
Asparagus	3	0·02	0·01–0·04	Tanaka *et al.*, 1977
(*Asparagus officinalis*)				
Cauliflower	12	0·01	0·003–0·021	Fuchs *et al.*, 1976
(*Brassica oleracea botrytis*)	3	0·01	—	Tanaka *et al.*, 1977
Broccoli	5	0·01	—	Tanaka *et al.*, 1977
(*Brassica oleracea botrytis*)				

Swiss chard	6	0·22 (DW)	0·20–0·27 (DW)	Chang *et al.*, 1979
(*Beta vulgaris* var. *cicla*)	6	1·22 (DW)	0·49–3·6 (DW)	CAST, 1976
Pea	5	0·004	0·003–0·005	Fuchs *et al.*, 1976
(*Pisum sativum*)	3	<0·03 (DW)	—	Dowdy and Larson, 1975
Bean, kidney	8	0·04	0·02–0·08	Tanaka *et al.*, 1977
(*Phaseolus* spp.)				
Bean	12	0·042	0·019–0·075	Pfeilsticker and Markard, 1975
(*Phaseolus* spp.)				
Carrot	8	0·13	0·09–0·22	Thomas *et al.*, 1972
(*Daucus carota sativa*)	3	0·07	0·03–0·10	Tanaka *et al.*, 1977
	47	0·041	0·003–0·160	Fuchs *et al.*, 1976
	14	0·038	0·016–0·088	Pfeilsticker and Markard, 1975
	11	0·005	0·001–0·010	Tiller *et al.*, 1976
Potato	19	0·08	0·01–0·17	Thomas *et al.*, 1972
(*Solanum tuberosum*)	12	0·04	—	Anke *et al.*, 1976
	63	0·016	0·005–0·055	Fuchs *et al.*, 1976
	8	<0·05	—	Baerug and Martinsen, 1977
	10	0·064	0·016–0·18	Tiller *et al.*, 1976
Onion	12	0·08	—	Anke *et al.*, 1976
(*Allium cepa*)	11	0·04	0·01–0·09	Thomas *et al.*, 1972
	21	0·011	<0·0002–0·036	Fuchs *et al.*, 1976
	4	0·006	0·002–0·02	Tiller *et al.*, 1976
Parsnip	5	0·057	0·016–0·111	Fuchs *et al.*, 1976
(*Pastinaca sativa*)			—	
Rutabaga	15	0·016	0·010–0·026	Fuchs *et al.*, 1976
(*Brassica napobrassica*)				
Beet, red	13	0·041	0·012–0·069	Fuchs *et al.*, 1976
(*Beta vulgaris*)	15	0·036	0·01–0·09	Tiller *et al.*, 1976
Turnip	5	0·03	0·01–0·08	Tanaka *et al.*, 1977
(*Brassica rapa*)	3	0·12 (DW)	—	Chang *et al.*, 1979
Radish	6	0·016	0·011–0·027	Pfeilsticker and Markard, 1975
(*Raphanus sativus*)	6	0·15 (DW)	0·08–0·19 (DW)	Chang *et al.*, 1979

[a] Except where indicated by (DW), all results are expressed on a fresh weight basis. [b] (DW) = dry weight basis.

TABLE 3.8
CONCENTRATIONS OF CADMIUM IN VARIOUS GRAINS GROWN ON NON-POLLUTED SOILS

Type	No. of samples	Concn. (mg/kg)[a]		Reference
		Mean	Range	
Rice, unpolished	34	0·13	—	Kjellstrom *et al.*, 1977
(*Oryza sativa*)	35	0·11	<0·001–0·31	Tsuchiya, 1978
Sorghum	36	0·033	0·01–0·1	Chang *et al.*, 1979
(*Sorghum vulgare*)				
Wheat	9	0·055	0·02–0·08	Hyde *et al.*, 1979
(*Triticum* spp.)	110	—	<0·005–0·045	Tiller *et al.*, 1976
spring	31	0·068	0·018–0·136	Kjellstrom *et al.*, 1975
autumn	48	0·035	0·005–0·077	Kjellstrom *et al.*, 1975
common, hard	5	0·10 (DW)	—	Zook *et al.*, 1970
common, soft	4	0·07 (DW)	—	Zook *et al.*, 1970
	43	0·10 (DW)	0·01–0·23 (DW)[b]	Crossman and Egels, 1975
Rye	19	<0·01	—	Crossman and Egels, 1975
(*Secale cereale*)				
Buckwheat	3	0·10	0·06–0·14	Tanaka *et al.*, 1977
(*Fagopyrum* spp.)				

Barley	37	0·05 (DW)	0·0104–0·11 (DW)	Vlamis and Williams, 1980
(*Hordeum vulgare*)	29	0·027 (DW)	0·01–0·07 (DW)	Chang *et al.*, 1979
	7	0·37 (DW)	0·08–0·65 (DW)	Munshower, 1977
	60	0·05 (DW)	0·01–0·75 (DW)	Crossman and Egels, 1975
Oat	12	0·04	—	Anke *et al.*, 1976
(*Avena sativa*)	8	0·014	—	Kjellstrom *et al.*, 1974
	8	0·011	—	Kjellstrom *et al.*, 1974
	67	0·05 (DW)	0·01–0·28 (DW)	Crossman and Egels, 1975
	6	0·09 (DW)	0·04–0·22 (DW)	Chaney and Hornick, 1977
Corn	11	0·065 (DW)	0·035–0·148[c]	Garcia *et al.*, 1974
(*Zea mays*)	24	0·05 (DW)	0·01–0·08 (DW)	Crossman and Egels, 1975
	9	0·02 (DW)	0·01–0·04 (DW)	Hyde *et al.*, 1979
	16	0·08 (DW)	<0·04–0·22 (DW)	Pietz *et al.*, 1978
	7	0·15 (DW)	0·10–0·18 (DW)	Hinesly *et al.*, 1979
	4	1·5 (DW)	1·1–1·7 (DW)	Sheaffer *et al.*, 1979
	3	<0·02 (DW)	—	Dowdy and Larson, 1975
	5	0·12 (DW)	0·02–0·3 (DW)	CAST, 1976
Soybean	5	0·17	0·05–0·48	Tanaka *et al.*, 1977
(*Glycine soja*)	6	0·21 (DW)	0·13–0·36 (DW)	Chaney and Hornick, 1977

[a] Except where indicated by (DW), all results are expressed on a fresh weight basis.
[b] (DW) = dry weight basis.
[c] Range of six samples collected from six different regions in the US.

the soil, to different absorption characteristics of species and cultivars, and possibly other environmental factors.

4.2. Concentrations in Various Plant Parts

The concentrations of Cd tend to vary among the various plant parts analyzed. When Cd concentrations in plants are expressed on a dry weight basis, the concentration of cadmium in leaves is greater than that of fruits, which is in turn greater than that of seeds. The concentration of cadmium in the leaves and seeds of pea (*Pisum sativum*), wheat (*Triticum* spp.) and corn

TABLE 3.9

CADMIUM CONCENTRATIONS OF VEGETATIVE AND REPRODUCTIVE TISSUES FROM PLANTS GROWN ON SEWAGE SLUDGE-AMENDED SOILS IN RELATION TO SPECIES, APPLICATION RATE AND SOIL pH

Cd added from sludge (kg/ha)	Soil pH	Cadmium concentrations (μg Cd/g)[a]					
		Pea (*Pisum sativum*)		Wheat (*Triticum* spp.)		Corn (*Zea mays*)	
		Leaves	Seeds	Leaves	Grain	Leaves	Grain
0	4·4	0·94	0·20	0·19	0·06	0·11	0·03
2·2	4·6	4·40	0·64	0·82	0·25	1·44	0·06
4·5	5·0	3·80	1·11	1·10	0·32	1·83	0·08
9·0	5·2	6·05	1·45	0·89	0·58	3·37	0·09
0	7·8	0·43	0·14	0·14	0·04	0·07	0·01
2·2	7·6	0·43	0·17	0·18	0·12	0·14	0·02
4·5	7·4	0·42	0·18	0·25	0·23	0·41	0·03
9·0	7·3	0·48	0·30	0·33	0·34	0·92	0·03

[a] Dry weight basis.

(*Zea mays*) (Table 3.9) are typical of results reported in the literature. From data presented earlier (Table 3.4), it is shown that the concentration of Cd in the foliage (leaves) is greater than that of the fruiting part of the plant.

4.3. Plant Species and Cultivars

The Cd contents of the most recently matured leaf at early bloom stage, for 22 crops grown to maturity on a calcareous soil treated with Cd at rates of 0·1 and 10 μg Cd/g, are presented in Table 3.10. The data show concentrations of Cd in leaves from plants which at the 10 μg Cd/g soil treatment rate vary from $<0{\cdot}1$ (rice, *Oryza sativa*) to 160 μg Cd/g (turnip, *Brassica rapa* L.) on a dry weight basis, and serve to demonstrate the extent

TABLE 3.10

CONCENTRATIONS OF CADMIUM (μg/g)[a] OF THE MOST RECENTLY EXPANDED LEAF AT EARLY BLOOM FOR VARIOUS CROP SPECIES GROWN ON A CALCAREOUS SOIL

Plant species	Cd added to soil (μg/g)	
	0·1	10
Paddy rice (*Oryza sativa* L.)	<0·1	<0·1
Wheat (*Triticum aestivum* L.)	<0·1	11·6
White clover (*Trifolium repens*)	0·2	6·0
Sudangrass (*Sorghum halepense* Pers. var. *sudanense* Hitche)	0·2	5·7
Alfalfa (*Medicago sativa* L.)	0·3	8·2
Bermudagrass (*Cynodon dactylon* Pers.)	0·3	9·4
Soybean (*Glycine max.* Merr.)	0·4	16
Upland rice (*Oryza sativa* L.)	0·4	0·9
Field bean (*Phaseolus vulgaris* L.)	0·6	10
Zucchini squash (*Curcurbita pepo* L. var. *medullosa* Alef.)	0·6	13
Cabbage (*Brassica oleracea* L. var. *capitata* L.)	0·7	39
Romaine lettuce (*Lactuca sativa* L.)	0·8	62
Table beet (*Beta vulgaris* L.)	0·8	47
Tall fescue (*Festuca elatior* L.)	1·4	17
Carrot (*Daucus carota* L. var. *sativa* D.C.)	1·4	38
Swiss chard (*Beta vulgaris* L. var. *cicla* L.)	1·4	42
Turnip (*Brassica rapa* L.)	1·8	160
Curlycress (*Lepidium sativum* L.)	2·4	89
Tomato (*Lycospersicon esculentum* Mill.)	2·6	71
Spinach (*Spinacia oleacea* L.)	3·6	160
Sweet corn (*Zea mays* L.)	3·9	27
Radish (*Raphanus sativus* L.)	4·2	40

[a] Dry weight basis.

to which Cd contents of plants may vary when grown on contaminated soils. Other investigations have observed substantial differences in the Cd concentrations of plant species when grown on soils contaminated with Cd (see Kirkham, 1977; Chaney and Giordano, 1977 and Haghiri, 1973).

Varieties within plant species also show substantial differences in their Cd absorption characteristics. John and Van Laerhoven (1976) grew nine varieties of lettuce (*Lactuca sativa*) in solution cultures under identical conditions. At a substrate solution concentration of 0·5 mg Cd/liter, the concentrations of Cd in lettuce tops among the varieties varied from 30 to 100 μg Cd/g. Hinesly *et al.* (1978) grew 20 corn (*Zea mays*) inbreds on a sewage sludge-amended soil and observed Cd concentrations in leaves

which varied from 3 to 63 μg/g. Petersson (1977) similarly observed that cultivars of wheat (*Triticum* spp.) and barley (*Hordeum vulgare*) differ in their Cd absorption characteristics.

5. Cd PHYTOTOXICITY AND DIAGNOSTIC CRITERIA

Soil and plant tissue analyses are commonly used to assess the nutritional status of crops or the possibility of toxic concentrations. However, the results of chemical analysis of soil and plant tissue can only be interpreted if the chemical tests have been thoroughly and successfully calibrated with crops under actual field conditions. At this point, diagnostic criteria for Cd are based upon experiments carried out under greenhouse conditions with a limited number of soils and crop species—and hence are highly tentative.

An example of calibrating plant tissue analysis under greenhouse conditions are the Cd tolerance studies carried out in our laboratory. Tolerance to Cd was established for a wide variety of food crops. All crops were grown to maturity in pots filled with a slightly alkaline soil (Xerollic Calciorthid) treated with different amounts of Cd. The soil was amended with a municipal sewage sludge enriched with $CdSO_4$ (10 g sludge/kg soil) in amounts to produce soil Cd addition rates of 0, 2·5, 5, 10, 20, 40, 80, 160, 320 and 640 μg Cd/g soil. Approximately 12 kg of Cd-treated soil were placed in plastic pots and cropped with various food crops to observe the Cd uptake and accumulation characteristics of various crop species as well as their yield relationships. The yield of each crop was plotted as a function of the addition rate of Cd, and the Cd-rate producing a 25 % reduction in yield (critical rate) was determined graphically from the yield–Cd-rate curve. Critical leaf values were established in a similar manner, i.e. leaf samples were collected at the early blossom stage from each of the food crops under test for tolerance to soil Cd. These leaf samples were analyzed for Cd; plots of yield versus leaf Cd value were referred to for estimation of the leaf Cd value associated with a 25 % decrement in yield. Additional details are given in the publication by Bingham *et al.* (1975). Table 3.11 contains critical leaf and soil Cd concentrations for 15 food crops. Leaf Cd values ranged from 2·0 μg Cd/g tissue for rice to 160 μg Cd/g tissue for cabbage (*Brassica oleracea* var. *capitata*). The soil Cd rates (for a pH 7·5 soil) reducing yields by 25 %, likewise were found to be highly dependent upon the crop species under test. The data in Table 3.11 show spinach (*Spinacia oleracea*), soybean (*Glycine max.* Merr.), curlycress (*Lepidium sativum*) and lettuce (*Lactuca sativa*) to be relatively sensitive to soil Cd;

TABLE 3.11

Cd CONTENT OF DIAGNOSTIC TISSUE (LEAF, SHOOT, ETC.) OF FOOD CROPS ASSOCIATED WITH A 25% YIELD DECREMENT

Crop species	Diagnostic tissue	Plant tissue Cd at 25% yield decrement (μg/g)	Soil Cd addition at 25% yield decrement (μg/g)
Rice			
Oryza sativa L.			
Flood managed (Paddy)	Top two leaves at early spike	2·0	>640
Non-flood managed (Upland)	emergence stage	2·0	20
Soybean	Most recently matured leaf at early	7·0	5
Glycine max. Merr.	bloom stage		
Field bean	Most recently matured leaf at early	15·0	40
Phaseolus vulgaris L.	bloom stage		
Carrot	Shoot at normal harvest stage	32·0	20
Daucus carota L.			
Wheat	Top two leaves at early spike	33·0	50
Triticum aestivum L.	emergence stage		
Corn	Third and fourth leaf from top at early	35·0	18
Zea mays L.	tassel stage		
Romaine lettuce	Wrapper leaf at early heading stage	48·0	13
Lactuca sativa L. var. *longifolia*			

TABLE 3.11—*contd.*

Crop species	Diagnostic tissue	Plant tissue Cd at 25% yield decrement (μg/g)	Soil Cd addition at 25% yield decrement (μg/g)
Zucchini squash *Curcurbita pepo* L. var. *medullosa* Alef.	Most recently matured leaf at early bloom stage	68·0	160
Radish *Raphanus sativus* L.	Shoot at normal harvest stage	75·0	96
Spinach *Spinacia oleracea* L.	Shoot at normal harvest stage	75·0	4
Curlycress *Lepidium sativum* L.	Shoot at normal harvest stage	80·0	8
Turnip *Brassica rapa* L.	Most recently matured leaf at normal harvest stage	120	28
Tomato *Lycopersicon esculentum* Mil.	Most recently matured leaf at early bloom stage	125	160
Swiss chard *Beta vulgaris* var. *cicla*	Most recently matured leaf at normal harvest stage	150	250
Cabbage *Brassica oleracea* L. var. *capitata* L.	Most recently matured leaf at heading stage	160	170

No yield depression at maximum addition rate of Cd (640 μg Cd/g).

yields were reduced by Cd additions of 4–13 μg Cd/g. In contrast to the Cd-sensitive crops, tomato (*Lycospersicon esculentum* Mil.), squash (*Curcurbita pepo* L. var. *medullosa* Alef.) and cabbage (*Brassica oleracea* L.), were found to be tolerant to addition rates of 160–170 μg Cd/g. Rice (*Oryza sativa*) under flood was tolerant to Cd at all rates of Cd tested ($\leq$640 μg Cd/g). Upland managed rice (*Oryza sativa*), however, was much less tolerant to Cd (Bingham *et al.*, 1976). A DTPA–TEA (diethylenetriaminepentaacetic acid–triethanol amine) extraction of this soil removed approximately 60% of the added Cd; thus, critical soil Cd values for the DTPA–TEA test (Lindsay, 1972) would be approximately 60% of the addition rate that caused a 25% reduction in yield. The correlations between DPTA–TEA soil-extractable Cd and crop yield on tissue Cd concentration are highly crop specific.

More recently, Mahler *et al.* (1978) reported leaf and soil Cd values associated with a yield reduction of Romaine lettuce (*Lactuca sativa* var. *longifolia*) and Swiss chard (*Beta vulgaris* var. *cicla*) grown under greenhouse conditions on four acid and four calcareous soils. Their data clearly showed diagnostic levels for leaf Cd to be not only dependent upon the crop species but also upon the soils. They found leaf Cd to be dependent upon Cd addition rate or a related property and soil pH. For example, leaf Cd content (y) of Swiss chard (*Beta vulgaris* var. *cicla*) grown on four acid and four calcareous soils could be predicted with the following equation:

$$y = 629 + 31{\cdot}8\ Cd_{SE} - 93{\cdot}5\ pH \qquad (R^2 = 0{\cdot}89)$$

where Cd_{SE} represents the Cd concentration in saturation extracts (μg Cd/ml) and pH, the pH of the saturated soil paste. A similar regression equation was developed for Cd addition rate, soil pH and clay content. Results of studies by Bingham *et al.* (1979), Chaney and Hornick (1977) and others show that Cd becomes phytotoxic at lesser concentrations in acid than in neutral or calcareous soils. Because of the dependency of available Cd upon a number of soil properties in addition to soil pH, the prospects of developing a satisfactory soil test for Cd in soils seems remote unless the test compensates or integrates the net effect of parameters influencing Cd solubility and availability.

Symeonides and McRae (1977) reviewed a number of methods which have been proposed to predict Cd concentrations of plants on the basis of the amount of Cd extracted from the soil by various extracting solutions. Seven of the extractants (Table 3.12) were tested in greenhouse studies to determine how well the extractable Cd correlated with the Cd concentration in the plant. Twenty-five surface soils representing a wide range of soil

TABLE 3.12
CORRELATION OF VARIOUS CHEMICAL EXTRACTION PROCEDURES WITH LEAF Cd OF RADISHES (25 SOILS TREATED AT 50 AND 100 μg Cd/g)

Extractant	Cd extracted from soil (μg/g) range	Correlation coefficient with leaf Cd
N Ammonium acetate–acetic acid (pH 4·8)	8·0–77·5	0·50***
5% Acetic acid	9·2–90·8	0·36**
N Ammonium nitrate	0·6–65·9	0·97***
0·05 N EDTA	30·3–101·6	0·29
N Hydrochloric acid	46·9–102·3	0·19
Conc. hydrofluoric acid	50·1–102·5	0·04
Hot conc. nitric acid	39·2–102·5	0·17

** Significant at the 0·01 % level.
*** Significant at the 0·001 % level.

properties were treated at two rates of $CdSO_4$ application (50 and 100 μg Cd/g soil) and then cropped with radish (*Raphanus sativus*) plants. Leaf Cd was used as a reference value to correlate the results of various soil tests for Cd. The results of their evaluation of chemical soil tests, summarized in Table 3.12, showed measurements of total Cd (N HCl, HF, or hot concentrated HNO_3 extractions) did not correlate with leaf Cd concentration of the radish (*Raphanus sativus*) plants grown on the 25 soils. However, they found the N NH_4NO_3 solution to extract Cd in amounts which correlated to a high degree with plant Cd ($r = 0{\cdot}97$).

Korcak and Fanning (1978) correlated Cd extracted with DTPA and double acid (0·05 N HCl–0·025 N H_2SO_4) with Cd concentration of corn (*Zea mays*) shoots of plants grown for 30 days on three acid soils ± lime and Cd applications. Hinesly *et al.* (1977) used 0·1 N HCl to extract available Cd in soil. Cadmium extracted with 0·1 N HCl correlated with the Cd content of corn (*Zea mays*) grain.

Thus, at this junction, there is no commonly accepted diagnostic testing procedure for available Cd. Regardless of the method used, the test undoubtedly will be dependent upon the crop species as well as upon various soil properties.

6. SUMMARY

Geochemically, Cd is a rare element. It is not found in pure state in the natural environment and its concentration in mineralogical material is

usually low. Although there are soils derived from high Cd parent material or soils which have been contaminated by man's activities, the indigenous Cd content for much of the world's agricultural land seldom exceeds a few parts per million. Since the low soil Cd level in its natural state exhibits little phytotoxicity and it is not considered an essential chemical element for plant growth, the effect of Cd on plants until recently has not drawn widespread attention. The intensive studies on the Cd content of plant tissue in recent years result from reports demonstrating potential health hazards of Cd. In an urban community, dietary intake constitutes the major source of long-term low-level body accumulation of Cd. The detrimental impact of Cd becomes apparent only after decades of continuous exposure. Monitoring the Cd content of plant tissue becomes essential to prevent excessive Cd build-up along the human food chain.

It is obvious that chemistry of the soil would play an important rôle in the plant availability of soil Cd. Growing under natural conditions, the amounts of Cd absorbed by plants are usually low. Fruits, vegetables and grains grown on non-polluted soils typically accumulate a few hundredths to a few tenths of a part per million of Cd (fresh weight basis). Recent studies have also shown that plants respond to input of Cd to soils and are susceptible to the Cd injury. Even before the soil Cd reaches a toxicity-producing level, the plant's uptake of Cd may be accelerated. For reasons not yet clear, soil Cd derived from weathering of parent material does not seem to be as readily plant-available as that added to soils in the form of inorganic chemicals or municipal sewage sludges.

The substrate Cd concentration and pH are two important soil factors that influence the plant accumulation of Cd. Under similar conditions, the higher soil Cd content invariably would result in higher plant tissue Cd. A shifting of soil pH toward the acidic range not only results in greater plant uptake of Cd but also results in more acute phytotoxic symptoms. Field observations indicate that the availability of Cd to plants diminishes with time following Cd applications to soils. There are also data suggesting possible synergistic and antagonistic effects of plant tissue accumulation of Cd due to the presence of other heavy metal elements (i.e. Cu, Ni and Zn). Plants also exhibit differential Cd accumulation characteristics. Besides the expected difference between plant species, various parts of a plant are not identical in Cd content. Where Cd concentrations of plants are expressed on a dry weight basis, the leaf of the plant usually is higher in Cd than fruits and roots. Among all physiological segments of the plant, seeds are usually the lowest in Cd.

Diagnostic procedures through leaf tissue and soil analyses are useful

techniques in assessing the detrimental effects of Cd to plant growth. However, it has not been proven effective in predicting the Cd accumulation in plant tissue.

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CHAPTER 4

Copper

NICHOLAS W. LEPP
Department of Biology, Liverpool Polytechnic, UK

1. INTRODUCTION

Copper is one of the transition elements, a group characterized by the possession of a partly filled set of *d*-orbitals. It is one of the few metallic elements to occur in a native (i.e. pure metal) state in the earth's crust; in consequence, it has been exploited by man since 5000 BC, and has, together with iron, been termed one of the cornerstones of civilization (Smith, 1965). It was formerly employed as bronze (a copper–tin alloy) for the production of utensils, weapons and ornaments, but successive changes in metallurgical techniques have resulted in a decline in this use of copper. Today, the main use of copper is in the form of its various alloys, many of which are of great importance in the electrical industry. Estimates of production showed that 7·4 million short tons of copper were used in the Western World in 1964, 4·4 million of which were mined. Usable world copper reserves amount to some 200 million tons, but continued exploration has resulted in the discovery of further substantial deposits (Massey, 1973).

Copper constitutes 70 ppm of the earth's crust, occurring as a constituent of several different ores (Massey, 1973). Of these, chalcopyrite ($CuFeS_2$) is thought to account for about 50 % of total world copper deposits. Other metals associated with copper in ore deposits include arsenic (enargite, tennantite) and antimony (tetrahedrite). Sulphides (chalcolite, covellite), oxides (cuprite, tenerite), carbonates (malachite, azurite), sulphates (antlerite, brochantite) and native copper, constitute the bulk of other copper sources. The extraction of copper from these sources often leads to pollution problems around sites of copper extraction, where the presence of acidic gases and metallic impurities, derived from the copper ores, can exacerbate the biotic consequences (Hutchinson and Whitby, 1974).

The role of copper in biological systems must be viewed in the light of its known chemical properties. Of these, the ready formation of organo–copper complexes, is of great significance. A wide variety of organic ligands are known to complex with copper (Sillén and Martell, 1964); the usual number of monodentate ligands per copper atom is four, although two, five and six are also known to occur. This ease of complex formation, coupled with the high stability of many of the resultant complexes, gives copper the potential to interact strongly with organic compounds in living tissue, or in the immediate abiotic environment. Some authorities believe copper to be at the head of stability series relating to stability of organo–metallic complexes (Irving and Williams, 1953), but departures from such generalizations have been observed (Stevenson, 1976).

This affinity for organic materials is reflected in the essentiality of copper for all forms of life. The main biological role of copper is as a constituent, normally in the prosthetic group, of oxidizing enzymes (e.g. ascorbic acid oxidase, cytochrome oxidase, laccase and tyrosinase). These enzymes are important in oxidation–reduction processes, and copper undergoes changes between Cu(I) and Cu(II) states as the enzymes function. Normally, copper is tightly bound to the enzyme and requires drastic (acid or cyanide) treatment to effect its release (Phipps, 1976).

In view of this, copper deficiency, as well as copper excess, will be of consequence for the integrity of biochemical function in living organisms; in this respect, zinc, copper and manganese stand alone in the category of trace metals, as all produce problems related to deficiency and excess.

When considering interactions between an essential element, such as copper, and plants, one cannot simply look at problems of deficiency or excess in isolation. The sources and availability of the element must be considered, together with uptake mechanisms, factors affecting re-distribution within the plant, and the overall pattern of elemental circulation between the plant and its abiotic environment. Superimpose upon this the question of chemical speciation of the element in various compartments of the soil/plant/atmosphere continuum, and the potential for interaction with other constituents of this system, and a remarkably complex picture emerges. A comprehensive treatment of plant/copper interactions must include these considerations, and their understanding is an essential prerequisite for evaluating the available information.

2. SOURCES AND AVAILABILITY OF COPPER TO PLANTS

A brief description of the primary geochemistry of copper has been given above. These details provide few clues to the complexity of the interactions

between copper and the other soil constituents, in relation to copper availability to plants. One approach to this problem is to fractionate the total soil copper pool. McLaren and Crawford (1973*a*,*b*), using 24 British soil types, established five main soil copper fractions. These were: (a) Copper in the soil solution and freely exchangeable copper; (b) copper weakly bound to inorganic sites; (c) organically bound copper; (d) copper occluded by free oxides; (e) residual copper, held mainly in clay lattice structures. These authors suggested that the bulk of available soil copper reserves were held in (c). These findings have been substantiated by Elsokkary and Låg (1978).

Interactions between copper and organic compounds are widely recognized. Copper can be complexed with humic acids (Bloomfield *et al.*, 1976). Lewis and Broadbent (1961) demonstrated complex formation with carboxyl and phenyl groups. Both Dawson and Nair (1950) and Ennis (1962) showed complex formation between copper and sulphydryl groups in peat.

It is widely believed that over 90 % of the 'available' soil copper pool is in the form of organo–copper complexes (Geering and Hodgson, 1969; Hodgson *et al.*, 1966). Bloomfield and Saunders (1977) showed that the preponderance of such complexes were with small organic compounds. Any organo copper complexes isolated from soils show a high degree of stability (Stevenson, 1976). The importance of the presence of soluble organic compounds on copper extractability from a peaty, copper-deficient soil was convincingly demonstrated by Nielsen (1976). Pots of copper-deficient peaty soil were either planted with cereals or left unplanted. After 44 days, planted pots showed a significant increase in extractable copper levels, in comparison with the unplanted pots; the author attributed this to the production of root exudates and release of organic products of root decay, which complexed with the non-organic copper fraction in the test soil.

The relative availability of copper in a soil will determine plant response. Problems of copper deficiency are associated with particular soil types, and the type of crops in cultivation. In the UK, Caldwell (1971) lists areas of copper-deficient soils in relation to cereal cultivation. In general, these soils are either shallow peats or sandy loams; derived from a variety of parent materials. Reuther and Labanauskas (1966) list a variety of soils in which copper deficiency can be expected. These include peats, sandy soils, leached sandy soils, calcareous sands, leached acid soils and soils which receive heavy N fertilization. Increased rates of nitrogenous fertilizer application have been shown to increase copper deficiency in cereals (Fleming and Delaney, 1961; Davies *et al.*, 1971), and phosphate fertilizers may have a

similar effect (Bingham *et al.*, 1958). Liming can also reduce copper availability to cereals (Caldwell, 1971; Tähtinen, 1976). However, studies by Elsokkary and Låg (1978) on industrially polluted and non-polluted soils in Norway, indicate that Fe and Mn contents of soils may influence the distribution of copper in different soil fractions. Those authors concluded that other soil cations had little effect on soil copper fractionation.

Soil copper excess is usually confined to specialized situations. Soils derived from copper-bearing ore bodies may contain very high copper levels, and occur in many parts of the world. These soils may often support a specialized flora; in the Zambian copper belt, the occurrence of *Becium homblei* is characteristic of high copper soils (Jacobson, 1967; Drew and Reilly, 1972) and has been used for geobotanical prospecting. Various species of 'copper mosses' (*Merceya*, *Mielichhoferia*) are exclusively confined to acidic, copper-rich substrates (Persson, 1956). Not all such sites possess a specialized flora. Dykeman and De Sousa (1966) investigated a natural copper swamp forest in New Brunswick, Canada. Soil copper levels were excessive, but the peaty soil overlying the copper ore body prevented excessive 'available' soil copper levels, presumably due to the formation of organic complexes or copper binding.

Copper excess can also arise as a result of prolonged application of copper-based fungicides (Bordeaux mixture) in old orchards or vineyards (Reuther and Labanauskas, 1966; Alloway *et al.*, 1979). In such soils, copper is primarily associated with the organic matter fraction (Alloway *et al.*, 1979). Soils contaminated by industrial activity or past mining activities can also possess high copper levels (Gadgil, 1969; Hutchinson and Whitby, 1974; Wu and Bradshaw, 1972). In this case, the copper sources and form of the emitted copper will dictate, to some extent, its subsequent distribution and availability, although acidity, often found in mine spoils, can also be an important factor in determining copper availability in such soils (Massey, 1972).

3. UPTAKE AND DISTRIBUTION OF COPPER WITH THE PLANT

3.1. Root Uptake

In natural conditions, free copper ions are only rarely available for uptake from the soil water solution. The preceding section has described the chemical form of copper in soils, and the overwhelming evidence is in

favour of organically complexed copper being the predominant species in the soil water. Even laboratory studies using, for example, $CuSO_4$ as a copper source are investigating the uptake of a relatively unstable copper complex. In solution, $CuSO_4$ forms the hexaquo copper complex $[Cu(OH_2)_6]^{2+}$, and if used at pH values higher than 7, this forms a $Cu(OH)_2$ suspension (Marquenie-van der Werff and Ernst, 1979). This aspect of copper chemistry should be carefully considered in studies on copper uptake from solutions.

The mechanism of copper uptake by roots is unclear. Most available evidence points to a passive uptake, the bulk of this coming from short-term excised-root or whole-plant studies. Bowen (1969), using sugar cane leaf slices, suggests that uptake may be an active process, but root studies (Veltrup, 1976, 1977; Goren and Wanner, 1971; Cathala and Salsac, 1975) firmly suggest a passive uptake process. This discrepancy may be due to differences in the experimental material. Further evidence against active copper uptake comes from studies with boiled v. fresh roots (Smith, 1953), where little difference in copper uptake between the two types was evident; studies on the Q_{10} of copper uptake (Goren and Wanner, 1971) reveal a value of 1·1–1·2, indicative of a non-metabolic process. Cathala and Salsac (1975) showed that uptake was not affected by low temperatures, DNP or nitrogen atmospheres. Marquenie-van der Werff and Ernst (1979), working with the submerged aquatic plant *Elodea*, showed a biphasic pattern of copper uptake into the plants; this was concluded to be evidence for active uptake. However, Harrison *et al.* (1981) have demonstrated that boiled roots can also show a biphasic uptake of copper; an artefact resulting from errors in data handling. Evidence for similar biphasic patterns of zinc adsorption by clays (Shuman, 1976) exists, in a system which possesses no active component. Great care should be taken in drawing mechanistic conclusions from such data.

In view of the overwhelming predominance of organically complexed copper in soils, it is surprising that so little attention has been paid to the uptake of organo–copper complexes. Copper EDTA, EDDHA and, in some cases, DTPA and HEEDTA are used commercially as copper fertilizers. The uptake characteristics of these important chemical forms of copper have scarcely been examined. In the actual root-uptake process, the charge of the applied copper is of considerable importance. Coombes *et al.* (1977) looked at uptake of a variety of copper complexes, differing in overall charge and structure, by excised *Hordeum* roots. Cu EDTA (−ve) was taken up very poorly over an extended (24-h) time period. Cu–glycine (0) was taken up more readily and two (2+) complexes were taken up very

readily. Whilst charge is an important consideration in uptake, the chemical form of the applied copper is important in subsequent binding in the root. Coombes *et al.* (1978) demonstrated this using three copper complexes, two (2+) and one (0) overall charge. A considerable portion of root-absorbed copper derived from a $CuSO_4$ solution was freely exchangeable with Ca^{2+} ions, whereas for both the (2+) complex bisethylenediamine copper and the (0) complex, copper glycine, a much smaller portion of the total root copper pool was exchanged with Ca^{2+} ions. Complex charge governs uptake, but complex structure and, perhaps, stability, governs subsequent binding.

Several workers have interpreted copper uptake patterns in terms of a single multiphasic isotherm (Bowen, 1969; Veltrup, 1976, 1977; Harrison *et al.*, 1978). Accurate resolution of experimental data, a prerequisite for repeatable and valid results, is difficult for copper. Normal radiotracer techniques cannot be readily used, due to the short half-life of all the natural radioactive copper isotopes (12 h or less). The lack of a suitable radioisotope places great reliance on precision, and on post-uptake treatment of roots prior to analysis. It is difficult to devise satisfactory desorption treatments for use in copper uptake studies, due to the high affinity of copper for organic binding sites. This is one important reason for poor resolution in determining uptake phases. A recent refinement of experimental technique (Harrison *et al.*, 1979) has allowed much greater precision in such studies. Using a 60-min low-temperature (5 °C) desorption, with $Pb(NO_3)_2$ as the desorbing agent, it has proved possible to remove the great proportion of free space copper, resulting in a more accurate resolution of the uptake isotherm. Even so, the subsequent data handling process to produce the isotherm requires great caution. Harrison *et al.* (1981) have compared several widely used methods for curve-fitting such data and have shown that considerable variation can occur. Injudicious method selection can result in anomalous conclusions; uptake of copper by boiled roots can, using certain data analysis procedures, be erroneously demonstrated as biphasic, a characteristic others have interpreted as indicative of active uptake (Marquenie-van der Werff and Ernst, 1979). Whilst a multiphasic model requires the existence of a carrier, transport does not necessarily have to be active.

Excised-root and short-term whole-plant studies are also useful in indicating both the occurrence and magnitude of interactions that may occur between copper and other ions during uptake. It has been widely held that copper and zinc can produce interferences during simultaneous uptake (Bowen, 1969; Schmid *et al.*, 1965). Using intact barley roots, Veltrup

(1977) demonstrated that both absolute uptake, and phasic uptake patterns of copper were unaffected by zinc over both short (2 h) and long (24 h) term uptake periods. Copper and lead interact in uptake processes. Goren and Wanner (1971) demonstrated interferences of copper uptake by lead, and vice versa, in intact *Hordeum* roots. This appeared to be a non-physiological competition, as the Q_{10} for uptake of both ions was in the range 1·1–1·2.

However, these conclusions should be treated with a degree of caution. In many cases, the rate of copper application is high, and effects noted at elevated levels may have little relevance to the real world. Concentrations of copper and other micronutrients in the soil solution are low; active processes may occur at such levels, but may be masked at higher application rates. Wheat plants can receive an adequate copper supply from 0·01 μM copper (in solution) (Loneragan, 1975). Experiments using high application rates (Larsen, 1966; Jarvis, 1978) will possess little relevance to a field situation. Using low (5 μM) levels of copper and zinc, Hawf and Schmid (1967) noted severe inhibition of zinc uptake by copper in intact *Phaseolus* plants, a finding substantiated by Chaudhry and Loneragan (1972) for wheat seedlings. When these observations are coupled with antagonisms observed in field (Dunne, 1956) and glasshouse (Chaudhry and Loneragan, 1970) experiments, the question of copper/zinc interactions in root uptake is still awaiting resolution.

Copper uptake by whole plants has been widely studied, from the viewpoint of both deficiency and excess. The main feature of this process is the concentration of copper in the root system. Pettersson (1976), using cucumbers, showed that, from a range of metals tested, copper had the second lowest shoot/root metal ratio after 12 days growth in metal-containing nutrient solution. Similar observations have been made for cotton (Sowell *et al.*, 1957), corn and sunflower (Cathala and Salsac, 1975) and tomato (Whitby and Hutchinson, 1974). In the roots, staining techniques have indicated that copper may be accumulated in epidermal cells, and also in the endodermis and pericycle (Brams and Fiskell, 1971). Subcellular fractionation of roots of the grass *Agrostis tenuis*, grown at normal (0·064 ppm) nutrient copper levels, showed that most copper was associated with the residues obtained after ultracentrifugation (Turner, 1970). Copper-tolerant plants of the same species showed accumulation of copper in the cell walls. This type of investigation tells us little about the situation in the intact root, as the disturbances of the cells and cell contents prior to centrifugation could result in a redistribution of copper, which, with its high affinity for organic binding sites, may concentrate in tissue

fractions offering the highest proportion of such sites (i.e. cell walls) and competitively displace other ions which may have previously occupied these sites.

One important factor regulating copper uptake in whole plants is the nitrogen status of the soil. Applications of N fertilizers to low-copper or potentially copper-deficient soils can lead to an exacerbation of the problem. This occurs as a result of a reduction in copper levels in the plants (Lucas, 1945; Chaudhry and Loneragan, 1970; Cheshire *et al.*, 1967). This may be due to a growth stimulation of tops, relative to root growth (Chaudhry and Loneragan, 1970), which, due to the low mobility of copper, may result in sub-optimal foliar concentrations. Other evidence in this area is, at best, confused. Relationships may exist between copper and N for both cereals and legumes (Rasheed and Seeley, 1966) or for cereals but not legumes (Gladstones *et al.*, 1975). Also, there appears to be a relationship between cereal grain protein content and copper deficiency (Nambiar, 1976*a*,*b*). In legumes, more complex copper–N interactions may occur. Legumes generally contain more copper in tops than non-legumes (Gladstones *et al.*, 1975), perhaps due to their ability to fix N. Further aspects of this relationship are discussed in Section 4.1 below.

Interactions between copper and other trace metals may also occur. The presence of copper can reduce uptake of both cadmium and nickel in intact soybean seedlings (Cataldo and Wildung, 1978), this apparently occurring competitively. The presence of high chromium levels reduces copper uptake (also zinc and nickel) in corn and rye (Cunningham *et al.*, 1975). However, in experiments on metal uptake by sycamore (*Acer pseudoplatanus*) seedlings, Lepp and Eardley (1978) could find no effect of the presence of high chromium, zinc or nickel levels on copper accumulation in roots and tops from sewage sludge-amended soil. This may be due to the copper–N interactions discussed above, but similar considerations would also apply to the experiments of Cunningham *et al.* (1975), where chromium had a significant competitive effect on copper uptake. Veltrup (1979), using intact *Hordeum* plants has demonstrated effects of cadmium, cobalt and nickel on copper accumulation and uptake in the roots. In the short term (2 h) the other elements increased copper uptake, but over longer periods (24 h), cobalt reduced uptake.

Plant species differ in their ability to take up copper, and much variation can be encountered. Wide differences in copper content have been noted in trees (Denaeyer De-Smet, 1973; Stone, 1968; Young and Guinn, 1966) and in herbaceous forms (Gladstones *et al.*, 1975) growing on the same soil type. This is an important consideration in low copper soils, where some crops

may be more susceptible to deficiency than others (Graham, 1978). Likewise, in conditions of copper excess, certain species can accumulate high levels without any apparent deleterious effects (Drew and Reilly, 1972; Wu and Bradshaw, 1972; Antonovics *et al.*, 1971) and the expression of tolerance within a population may be very rapid (Wu and Bradshaw, 1972).

3.2. Transport of Copper Within the Plant

Copper can be transported in both xylem and phloem transport systems. The involvement of each system depends upon the source of copper and the type of transport (primary or secondary redistribution).

In the xylem, the main source of copper will be from root uptake. The chemical form of copper during its passage from soil water to xylem element lumen is not known. Graham (1979) measured copper concentrations in roots, root cell sap and xylem exudate of sunflower. He showed, using a copper-sensitive electrode sensitive only to free copper ions, that $< 1\,\%$ of the total copper in this system was ionic. From this, it can be inferred that there is a potential for copper movement from soil water to xylem in a complexed form, but the identity of the organic agents involved is unclear. Equally so, chelation may occur following uptake, but there is a lack of evidence at the present time to support the veracity of one or other of these processes under natural conditions. All available evidence (Tiffin, 1972, 1977; Graham, 1979) points to chelation of copper prior to ascent of the xylem conduits. Copper, applied as ^{64}Cu, can be detected in a $-$ve charged form of xylem exudate, and evidence points to the occurrence of different, concentration-dependent organic agents (Tiffin, 1972). The identity of the organic agents remains unresolved, but amino acids have been suggested as possible carriers. Support for this comes from the high affinity of Cu^{2+} ions for N and S (Corwin, 1950), and from known associations of copper with proteins and amino acids in animals (Harris and Sass-Kortsak, 1967; Gregoriadis and Sourkes, 1968). Claims have also been made for the association of copper with soluble proteins in plants (Ozolinya and Lapinya, 1976).

In N-sufficient plants, the ratio of amino acids to copper is at least 500:1 (Pate *et al.*, 1975), so little problem relating to the availability of potential organic partners can be envisaged, unless associations are highly specific. Changes in copper supply, N supply and levels of other cations could, however, alter the picture. The stability of the organo–copper complexes, although unknown, can be assumed to be high. Large changes in elemental composition at the sites of complex formation could result in imbalances of stability developing, and changes in the composition of the xylem sap

during ascent will undoubtedly lead to changes in chemical equilibria between the various organo–metallic complexes ascending the conduits. Interactions with the fabric of the conduits are also a distinct possibility, which may be enhanced or diminished by the presence of other constituents of the xylem sap. Some evidence for the above has been obtained. Dollard (1979), working with xylem tissue of sycamore (*Acer pseudoplatanus* L.) has shown that both free copper ions and copper supplied as a copper–glycine complex will displace calcium ions from *Acer* xylem. Calcium ascends the xylem by a process of passive ion exchange (Shear and Faust, 1970; Ferguson and Bollard, 1976); thus, it is the major occupant of the −ve charged binding sites in the xylem tissue (Dollard and Lepp, 1978). In a comparison with lead, nickel and zinc (free and complexed with glycine), copper proved to be the most efficient calcium-displacing agent, regardless of its chemical form. Further studies (Dollard, 1979) revealed that the presence of copper interfered with binding of lead and zinc to *Acer* xylem tissue. There is clearly the potential for interactions between metals during ascent of the xylem, and copper, due to its affinity for −ve charged binding sites, may be an important factor in such processes, particularly if present at elevated levels.

The study of phloem transport of copper presents further problems. The pH of phloem sap is approximately two units higher than xylem sap. Evidence from both soils (Hodgson *et al.*, 1966) and model chemical systems (Osterberg and Sjoberg, 1968) indicates that increasing alkalinity increases the formation of stable copper complexes. Thus the potential for copper chelation is greater in the phloem sap than in xylem sap, and copper:N ratios are correspondingly high (Tammes and van Die, 1966; Pate *et al.*, 1975). Pate *et al.* (1975) also examined concentration ratios of elements between phloem and xylem sap. Their results showed a nine-fold concentration of copper in phloem sap, greater than the ratio for poorly exchanged calcium, but less than the ratio for rapidly exchanged potassium.

Copper is classed as an element of intermediate phloem mobility (Williams and Moore, 1952; Bukovac and Wittwer, 1957). Evidence for phloem mobility following foliar applications to copper-deficient plants must be interpreted cautiously (Kester *et al.*, 1961). Copper deficiency may interfere with processes associated with sieve element loading, or may induce changes in the anatomy of the transport tissue. There is also no guarantee that copper is translocated in the complexed forms associated with an adequate copper supply. In addition, the assessment of phloem mobility

is open to doubt. Many of the techniques which are judged to measure this may also measure xylem movement. Further work is clearly required in this area.

Movement of copper between various plant organs, on a seasonal basis, has also received investigation. In wheat, it has been demonstrated that copper does not move from leaves to developing grains as readily as N (Linser *et al.*, 1975). Hocking and Pate (1977) showed that leaves, pods and seed coats of three legume species lost 20–50 % of their maximum copper content at a time between maximum content and full senescence. In this respect copper resembled other micronutrients, such as iron and zinc. Other species show marked seasonal variations in copper content. Guha and Mitchell (1966) followed changes in foliar copper levels in three tree species throughout the growing season. They found a steady decline in foliar copper levels from leaf emergence to senescence, which could be interpreted as due to re-export to other plant parts, or loss via leaching. Dollard (1979) has shown distinct patterns of change in copper contents of various tissues of sycamore (*Acer pseudoplatanus* L.) during the course of the growing season. Major events are a general rise in bark and wood copper content at the time of leaf emergence, and an increase in bark copper content at leaf senescence. There is also evidence for interchange of copper between bark and wood during the dormant period. Tyler (1971) has documented seasonal changes in copper content of two salt marsh herbs, the rush *Juncus gerardii* and the grass *Agrostis tenuis*. Both species showed a high shoot copper content at the start of the growing season, followed by a sharp decline and an eventual levelling off. The absence of a rise in shoot copper content at the end of the growing season indicates some degree of redistribution from senescing leaves; other non-essential elements studied in the same species showed rises at senescence, indicating a degree of immobility.

Finally, some comments on the influence of plant copper status on copper transport should be made. Loneragan *et al.* (1976) showed that redistribution of copper from old leaves of copper-deficient wheat plants was slower than in copper-sufficient plants. Similar conclusions could be drawn for the effect of plant copper status on N translocation. There is also evidence that copper deficiency depresses the proportion of total plant copper which moves into cereal grains (Scharrer and Schaumlöffel, 1960). It may be appropriate to describe copper as a nutrient which exhibits variable mobility in plants, the rate and pattern of mobility being governed by internal and external factors.

3.3. Copper Loss from Plants

Plants can lose copper via several distinct pathways. These can be listed as root exudation, guttation, leaching and abscission. We have very little knowledge of either the magnitude or factors affecting the first two processes. Harrison (personal communication) has detected copper in guttation fluid from *Hordeum* seedlings, grown in solution containing 5 ppm copper, but the significance of this finding awaits further evaluation.

In the preceding section, many workers observed seasonal changes in foliar copper contents. These could be due to retranslocation, or to loss via leaching. As leaching of cations is thought to proceed via ion-exchange processes (Mecklenberg *et al.*, 1966), copper loss from leaves could theoretically proceed via this mechanism. Coombes (1979) details the only experimental evidence on copper leaching to date. At increasing rates of copper supply to roots of beans (*Phaseolus vulgaris*) the total amount of copper leached from leaves of such plants showed a slow but steady increase. Using ion-exchange columns, it was found that both +ve and −ve charged forms of copper could be detected in the leachates, and at low copper application rates nearly 50 % of the total leached copper was present in an anionic form. This fraction remained constant in overall copper content as total leached copper levels increased, so at higher copper application rates, the proportion of −ve charged forms of copper in the leachate decreased. The significance of this −ve charged fraction is difficult to assess, especially in the absence of a characterization of the other agents associated with copper in the leachates. The −ve charged fraction may be more readily absorbed by leaves underlying those being leached by natural means, but on return to the soil or litter surface, the −ve charged forms may be broken down due to microbial activity. This area requires further investigation.

Copper can also be lost from the plant via abscission of the various organs borne on the stem. Rates of loss via this route will depend upon the type of plant, the nature of the abscised organs and time of abscission. In the tree *Fagus sylvatica*, Denaeyer De-Smet (1973) has demonstrated that over 50 % of the total absorbed copper is lost in the course of a single growing season, the majority of this loss occurring via leaf abscission. This author also examined copper circulation in the conifer *Picea abies*, from the same site. Here, total copper loss was less on a seasonal basis due, in part, to the persistent nature of *Picea* foliage. Similar data for other species is not forthcoming, so further comparisons cannot be drawn, but herbaceous species must obviously return all shoot copper, save that present in seeds, to the total soil and litter copper pool at the end of the growing season. A full

account of copper circulation in plants and other major components of woodland ecosystems is given by Lepp (1979). Further general principles of metal circulation are given by Hughes *et al.* (1980), Hughes (Vol. II, Chapter 3) and Martin and Coughtrey (Vol. II, Chapter 4).

3.4. Role of Copper in Plant Metabolism

Copper was first demonstrated to be an essential element for plant growth in 1931 (Sommer, 1931; Lipman and MacKinney, 1931), since which time it has been shown to act as an important factor in several biochemical processes. Many enzymes have been shown to require copper as a co-factor (Phipps, 1976; Nason and McElroy, 1963; Evans and Sorger, 1966) and copper also has a strong affinity for other non-catalytic proteins. In plants, enzyme systems known to require copper are chiefly oxidases. In this group are ascorbic acid oxidase (James and Cragg, 1943; Hallaway *et al.*, 1970), tyrosinase (polyphenol oxidase) (Mulder, 1949; Judel, 1972), diamine oxidase (Hill, 1973) and phenol oxidase (Judel, 1972; Graves *et al.*, 1979).

In photosynthesis, copper is an essential constituent of plastocyanin, a copper protein isolated from higher plants and green algae (Bishop, 1964) (pp. 140–1). This molecule accounts for 50 % of the total chloroplast copper content, and the two copper atoms are an integral part of the molecule's structure, being unaffected by added chelating agents. Plastocyanin is an important intermediary in the electron transfer process linking photosystems II and I (Bishop, 1966). It has been stated by early authors (Neish, 1939; Gilbert, 1951) that the bulk of the foliar copper content of copper-sufficient plants is concentrated in the chloroplasts, but this has not been substantiated. Certainly, the chlorosis associated with copper deficiency reduces photosynthesis (Loustalot *et al.*, 1945), but this cannot be held to be a definitive result of lack of chloroplast copper, as other factors may also be involved in this response.

Other evidence for the essential biological rôle of copper in plants is somewhat fragmentary. A body of information links copper to processes associated with floral induction. Copper supply has been shown to interfere with floral initiation in various *Lemna* species (Hillman, 1961, 1964) and *Chrysanthemum morifolium* (Graves and Suttcliffe, 1974; Graves *et al.*, 1979). In the latter species, low copper levels are associated with low IAA-oxidase levels (Davies, 1973). There is some relationship between copper, IAA-oxidase activity, high IAA levels and LD for floral initiation in *C. morifolium* (Davies, 1973; Tompsett and Schwabe, 1974). (See Section 4.1 below.) There is also an observation on copper inhibition of auxin transport in roots (Mitchell and Davies, 1975).

Some evidence also exists for a role of copper in ethylene production. Copper can catalyze ethylene production from linolenic acid, a process sensitive to the action of diethyldithiocarbamate (a known inhibitor of copper enzyme systems) (Lieberman and Mapson, 1964). Copper may also stimulate ethylene production from methionine (Hansen, 1966). Ethylene generation in response to copper has been further reviewed by Abeles (1973) (pp. 98–100).

It is clear that copper performs an essential function in the metabolic activities of higher plants; however, the extent of this role requires further investigation. The fragmentary evidence for a copper requirement for processes not associated with either photosynthesis or oxidase enzyme activity requires more careful scrutiny, to establish if less tenuous links can be forged between these observations and the overall biological role of copper in higher plants.

4. COPPER DEFICIENCY

4.1. General Aspects of Copper Deficiency in Plants

Copper deficiency in pastures, crops and animals has been responsible for widespread losses in many parts of the world (Reuther and Labanauskas, 1966). In plants, marginal copper deficiency can reduce both yield and quality of fruit and grain, whereas severe deficiency may also reduce vegetative yield. In addition, low copper content of pasture herbage can reduce the level of copper ingested by grazing animals to an extent where healthy growth is impaired. Also, the botanical composition of pasture herbage may alter. In Australia, valuable pasture species such as subterranean clover (*Trifolium subterraneum*) show reduced persistence and seed yield in mixed swards established on copper-deficient soils (Riceman, 1948; Carter and Day, 1977).

Diagnosis of a copper-deficient soil is difficult. Testing soil copper levels is very dependent on the nature of the extractant employed (Cox and Kamprath, 1972). Analysis of vegetation is also subject to error; the copper content of the tops of copper-deficient plants may exceed or equal that found in copper-sufficient plants (Piper, 1942; Steenbjerg, 1951). In wheat, Loneragan *et al.* (1976) showed that foliar copper content in wheat, in relation to soil copper level, was a function of leaf age. Reduced soil copper levels slowed down the rate of copper redistribution from older leaves. Thus, the primary leaves of plants grown in copper-deficient soil often

contained more copper than corresponding leaves on copper-sufficient plants. Techniques used to diagnose copper deficiency will be discussed later.

The most apparent morphological effects of copper deficiency in plants are related to lignification. When severe copper deficiency restricts vegetative growth, the deficient plants are weaker, and show reduced lignification and thickening of cell walls of xylem elements, fibres and epidermal cells (Schütte and Mathews, 1968). Where deficiency is more pronounced phloem and well as xylem differentiation can be impaired (Rahimi and Bussler, 1973). Bussler (1981) has suggested that reduced or non-existent lignification is a specific indicator of copper deficiency. Pissarek (1974) is even more specific; this author suggests that reduced lignification of stem sclerenchyma is a more sensitive indicator of deficiency than reduced lignification of xylem elements. It could be argued that observed changes in anatomy result indirectly from a reduced copper supply, but the widespread observations of this response in copper-deficient plants points to a specific involvement of copper in the process. The presence of copper as a co-factor in phenoloxidase enzymes (Judel, 1972) may be the key to this response.

As well as impairing vascular development, copper deficiency also interferes with the growth and expression of microstems. In oats, severe copper deficiency markedly decreases the rate of tiller production in the early stages of growth (Piper, 1942) but at the final harvest the same plants tiller profusely. However, the deficient plants failed to flower; the observed increase in tillering in the later stages of growth may have been due to death of earlier tillers and subsequent redistribution of nutrients (Piper, 1942). In most cases where copper deficiency results in depressed stem extension or increased tillering there is a marked delay in the transition from vegetative to reproductive growth (Hill, 1978). Such extreme symptoms are only evident in cases of severe copper deficiency. In less extreme cases, plants appear most sensitive to copper deficiency during reproductive growth; low copper levels can depress grain yield whilst vegetative yield remains near normal (Piper, 1942; Lipman and Mackinney, 1931). Other plants show similar responses; both Riceman (1948) and Rossiter (1951) showed reduced seed production in *T. subterraneum* in response to copper deficiency. This was due to a reduced rate of seed pod production, as a consequence of reduced flowering, rather than a reduction in the numbers of seeds per pod (Riceman, 1948), but Rossiter (1951) also invoked floral abortion and reduced seed set as causal agents for the overall response. Graham (1975) showed reduced anther growth and diminished production

of viable pollen in copper-deficient wheat. Similar observations have been made for corn (Bailey and McHargue, 1943).

In *Chrysanthemum*, Graves *et al.* (1979) have shown that copper is essential for flower production, and that varietal differences modified the response. A large experiment using 21 different cultivars highlighted this aspect. These ranged from complete suppression of flowering under inductive conditions, to a delay in flowering or reduction in bloom number in the less-sensitive cultivars. Hillman (1964) reports that a low concentration of copper may interfere with floral induction in two species of duckweed (*Lemna minor* and *L. gibba*); promoting the flowering of the former in long days, and inhibiting the flowering of the latter under similar conditions. Adams *et al.* (1975) have also demonstrated an interaction between low copper levels and boron deficiency in suppression of flowering in *C. morifolium*. Both conditions independently depress floral induction, but together their effect is greater. This interaction could be linked to effects of boron deficiency on activity of copper-requiring enzymes (Brown, 1979), as accumulation of phenolic compounds was suggested as one cause of low copper inhibition of flowering in *C. morifolium* (Adams *et al.*, 1975). Brown (1979) showed an enhancement of ascorbate oxidase activity in boron-deficient tomato plants; suggesting that boron deficiency could result in copper-deficient plants remaining vegetative.

Symptoms of copper deficiency can often be enhanced by the application of N to marginal or copper-deficient soils (Fleming and Delaney, 1961; Hooper and Davies, 1968; Chaudhry and Loneragan, 1970; Cheshire *et al.*, 1967). In wheat, excess N lowered total plant copper levels by increasing total growth, and by increasing top growth in relation to root growth. Attempts to correlate copper and N levels in tops of whole plants supplied with adequate copper have produced conflicting results (Rasheed and Seeley, 1966; Gladstones *et al.*, 1975). These can, in part, be explained by differences between the experiments, and by use of different species. Nambiar (1976*a*,*b*) suggests another general relationship between copper and N in cereals. This author found a correlation between high N content in copper-sufficient grain and susceptibility to copper deficiency at marginal rates of copper supply. This suggested (Nambiar, 1976*b*) that cereals with a relatively high grain protein content at adequate copper supply are potentially more susceptible to copper deficiency than those with a relatively lower grain protein content. Further information is required to substantiate this interesting hypothesis.

There is also evidence that legumes may have a separate requirement for copper in N fixation, as has been suggested for both Ca and Mo. Evidence

for this comes from the work of Greenwood and Hallsworth (1960) and Hallsworth *et al.* (1964). In *T. subterraneum*, acetylene reduction/g root nodule decreases with decreased copper supply (Snowball *et al.*—cited by Hill (1978)). In this species, there may be a requirement for copper in the N fixation process, but the relationship between this copper requirement and the copper requirement for overall growth of *T. subterraneum* is still uncertain.

4.2. Diagnosis and Correction of Copper Deficiency

Diagnostic criteria for copper deficiency in crops are difficult to establish. Soil analyses may prove of initial value in some cases (Cox and Kamprath, 1972), but due to the influence of soil type on availability of copper, and the yield response of crops on a particular soil (King and Alston, 1975), it is impossible to produce a critical value for soil copper levels applicable in all conditions. Organic soils present the greatest problems (Lucas and Knezek, 1972) due to the strong affinity of copper for humic acids (Stevenson and Ardakani, 1972; McLaren and Crawford, 1973*a*,*b*).

Diagnosis of deficiency from plant symptoms is also difficult, even though in severe cases these may be very characteristic. Early stages of deficiency are characterized by general restriction of root and shoot growth (Piper, 1942) with no attendant specific symptoms. Such changes could also be attributed to factors other than copper deficiency. By the time characteristic symptoms (i.e. wither tip in cereal) become evident, growth may be severely reduced, and even with immediate corrective measures yields may fail to reach their full potential. Further complications can arise in cereals. Copper levels may be sufficient for normal vegetative growth, but insufficient for floral development and subsequent grain production (Piper, 1942; Lipman and Mackinney, 1931; Riceman *et al.*, 1940). Remedial measures are ineffective in such circumstances.

Deficiency symptoms for a wide variety of plants have been given by Hewitt (1963) and Reuther and Labanauskas (1966). In herbaceous crops, these are usually chlorosis of young leaves and gradual death of older leaves (a symptom associated with a nutrient of low phloem mobility), whilst woody plants show progressive die-back of terminal shoots, and increased production of gummy excrescences both within and on the surface of fruits and twigs.

The use of plant copper levels as indicators of deficiency conditions can only provide a superficial indication of the potential deficiency problem. In annual species, the stage of growth at which the tops are sampled is critical; copper content of copper-sufficient plants declines with age (Piper, 1942;

Gladstones *et al.*, 1975), as does the copper content of tree foliage (Guha and Mitchell, 1966). Indeed, midway through the growing season, the copper content of copper-sufficient tops may be very similar to that of deficient plants (Loneragan *et al.*, 1976). In addition, copper is an element for which Piper–Steenbjerg yield curves can occur (Piper, 1942; Steenbjerg, 1951). These curves have been reported for several nutrients and tissues (Bates, 1971). In a Piper–Steenbjerg curve for copper in cereals, copper content in the tops of deficient plants sometimes exceeds that of marginally copper-sufficient plants. No one explanation can be advanced for the various instances of this phenomenon. For copper, Piper (1942) suggested that the high copper levels in the tops of copper-deficient oats resulted from the delayed senescence of these plants. As the copper content of tops decreases with age in copper-sufficient plants, copper deficiency could halt this decline by delaying senescence. Copper deficiency also inhibits the growth of new tissues, and, in deficient plants, a greater proportion of the shoot biomass will be composed of older leaves. In consequence, levels of copper in the older leaves will be a greater factor in regulating the total shoot copper levels in deficient plants. In wheat, the delayed senescence of these old leaves is associated with a delay in the redistribution of copper and N from these organs (Loneragan *et al.*, 1976). Clearly, the reduced rate of copper export and the delay in senescence experienced as a result of copper deficiency, will maintain the levels of copper in the tops of deficient plants, whilst levels in similar aged copper-sufficient plants are falling. One further complication this produces is the uncertainty involved in determining plant copper status by analysis of old leaves (Loneragan *et al.*, 1976). Use of the younger leaves results in clear differentiation of deficient, marginal and sufficient plants until late in the growing season (Loneragan *et al.*, 1976). This tends to avoid Piper–Steenbjerg effects for copper, although these have been reported for other elements in young tissues, e.g. zinc/sugar beet (Rosell and Ulrich (1964)).

The correction of copper deficiency is usually achieved by application of the element at recommended rates, which will vary for both soil type and crop. Both soil applications and foliar feeding have proved beneficial for a variety of crops. Copper may be applied as sulphate, oxide, as part of a slag mixture containing other trace elements, or in a soluble chelated form (Reuther and Labanauskas, 1966). Chelates with EDTA and EDDHA are often used, and, more recently, chelates or complexes with natural products, such as lignosulphonates (Korkman and Virta, 1979). Synthetic chelates, such as copper–EDTA may be lost from upper soil horizons by leaching, but copper–lignosulphonate or copper sulphate can remain fixed

in the upper soil layers (Korkman and Virta, 1979). In some cases, rapid alleviation of deficiency can be achieved by spraying with copper-based fungicides, such as Bordeaux mixture (Reuther and Labanauskas, 1966), but a more permanent solution is often soil application. Problems of copper excess may also arise following many years usage of copper fungicides (Anne and Dupuis, 1953).

One alternative to the use of copper fertilizers as corrective measures is to breed crops that show efficiency in copper nutrition. In cereals, there are differences in copper efficiency between wheat and rye; the former being less efficient at utilizing available soil copper. Rye has been shown to evade copper deficiency in the poor sandy soils of Western Australia (Nambiar, 1976*a*) in contrast to wheat, oats and barley. The hybrid cereal *Triticale* (a wheat/rye hybrid) has been produced in hexaploid (6*n* *Secale cereal* × *Triticum durum*) and octoploid (8*n* *Secale cereal* × *Triticum aestivum*) forms. Graham (1978) has demonstrated the significant enhancement of copper efficiency in 6*n* *Triticale* on low-copper soils, in contrast to its parents and 8*n* *Triticale*. This could, in part, be attributed to adequate copper nutrition in *Triticale* preventing male sterility and maintaining pollen viability. It is thought that the genes for copper efficiency are carried on a single chromosome in rye (Graham, 1978) and as such could be incorporated into other cereals by selective breeding. A similar single-gene control of copper efficiency has been reported in oats (Pugsley—cited by Graham, 1978). Recently, Graham *et al.* (1981) have reinforced these conclusions with respect to rye, wheat and *Triticale*. Studies using ^{64}Cu have provided some evidence that the gene for copper efficiency in rye may function by increasing the surface area of the root system as well as affecting the rates of absorption/unit area of root. There may be important future developments in breeding for trace element efficiency in valuable agricultural crops; the above results indicate what can be achieved, and provide promising prospects for further improvements.

5. COPPER EXCESS

5.1. General Aspects of Copper Excess in Plants

Problems of copper excess may arise from a variety of causes. These may be due to natural soil enrichment, due to the presence of natural copper mineralization, a phenomenon known from many parts of the world. In such areas, specialized copper-tolerant floras have arisen, and use of

specific indicator species has frequently been employed in biogeochemical prospecting.

The remaining cases of copper excess in soils arise as a consequence of industrial or agricultural activities. Copper smelting and refining industries, manufacturers of copper products and sewage disposal processes all contribute to the man-made input of copper to the biosphere. Use of copper-based agrochemicals over extended periods can lead to excessive accumulations of soil copper in localized areas. Use of copper as a supplement to animal feeds can, in intensive farming, lead to the appearance of lagoons of contaminated animal wastes; this is especially true for pig husbandry (Batey *et al.*, 1972). Finally, use of contaminated sewage sludge as a soil amendment can be responsible for introduction of excess copper to farm land; in the UK, a series of guidelines have been developed to regulate the extent of this practice (Chumbley, 1971).

5.2. Symptoms of Copper Excess

Copper toxicity in plants is generally manifested as a general chlorosis and stunting of growth (Foy *et al.*, 1978). The chlorotic condition may result from iron deficiency, as foliar application of inorganic $FeSO_4$ (Hewitt, 1953) or soil application of iron chelates (Leonard and Stewart, 1952) can alleviate this condition. Analysis of foliage from such chlorotic plants reveals a low iron content (Smith *et al.*, 1950; Roth *et al.*, 1971). The extent of copper-induced iron chlorosis may be effected by climatic and other soil conditions not connected with copper toxicity (Lucas and Knezek, 1972), phosphorus status of both soil and plants (Spencer, 1966) and iron status of the soil (Reuther and Smith, 1954). This latter point is emphasized by the fact that copper toxicity can arise on low-iron sandy soils (Reuther *et al.*, 1953), but not on high-iron sandy soils (Walsh *et al.*, 1972).

Crop stunting due to copper excess can arise from a combination of factors. These include specific effects of copper on the plant, antagonism with other nutrients, or reduced root growth and penetration into the soil (Foy *et al.*, 1978). In the case of copper, toxicity is experienced initially in the root tips (Brams and Fiskell, 1971; Daniels *et al.*, 1972), with subsequent inhibition of the development of lateral roots. Such a restriction in root length could lead to macronutrient depletion in the restricted rooting zone, and a consequent growth inhibition.

Recently, Savage *et al.* (1981) have used scanning electron microscopy to observe specific effects of excess copper on the root morphology of developing lettuce seedlings. These included reduction in root length, reduction in numbers of root hairs, reduction in numbers of secondary root

initials and abnormal development of the root cap. At toxic concentrations, root extension was negligible.

No satisfactory explanation can be put forward to account for the inhibitory effect of copper on root extension. Changes in IAA-oxidase activity may be important (Mukherji and Das Gupta, 1972; Coombes *et al.*, 1976), but other mechanisms have also been implicated. Hunter and Welkie (1977) have suggested that the initial phases of copper toxicity are reversible, as treatment with EDTA will reverse copper effects if applied soon after commencement of the treatment (1 h). This reversibility declines with time; 6–12 h after onset of copper treatment, EDTA is ineffective.

These symptoms can be masked or enhanced by other factors. Crop cultivars which show differing iron efficiencies respond differently to excess copper (Brown and Ambler, 1973; Brown and Jones, 1977*a*,*b*). Early growth may be normal, but later growth chlorotic (Reilly and Reilly, 1973). Interactions between soil iron, soil P and copper have been observed in copper-induced chlorosis of citrus (Spencer, 1966). At low P levels, severe stunting with little chlorosis was observed. When soil P levels were increased, growth was near normal, but severe chlorosis was evident.

5.3. Effects of Excess Copper on Metabolic Processes

Whilst the general expression of copper toxicity in whole plants is based on the interaction of several factors, the basic deleterious effect of copper on growth is related to the root system (Daniels and Struckmeyer, 1973; Mukherji and Das Gupta, 1972; Coombes *et al.*, 1976; Daniels *et al.*, 1972, 1973; Hallsworth *et al.*, 1965; Sowell *et al.*, 1957). Copper can affect mitosis (Kostal, 1971), but more specific effects of copper have been postulated in relation to the sensitivity of various enzyme systems to excess copper. Mukherji and Das Gupta (1972) invoked an enhancement of the activity of catalase, IAA-oxidase and peroxidase activity in lettuce seedlings, due to elevated copper levels, as a mechanism for copper toxicity. Das Gupta and Mukherji (1977), in a more extensive investigation of copper effects on rice seedlings, demonstrated numerous effects which could be attributed to excess copper. These included reduced nucleic acid content, especially in the embryo, reduced activity of both α-amylase and RNase and reduced protease activity in the endosperm. The most pronounced effects occurred at 5 mM $CuSO_4$ treatment. Some of these effects were reversible; treatment with GA reversed the inhibition of α-amylase activity, and both GA and cAMP reversed inhibition of RNase activity. However, none of these effects were correlated to actual tissue copper levels. The importance of this was demonstrated by Coombes *et al.* (1976). In a study of the effects of excess

copper on IAA-oxidase activity in barley roots, these authors found different responses in enzyme sensitivity to copper in seedlings and older plants. IAA-oxidase activity was stimulated at all copper levels in seedlings, but three-week-old plants showed a progressive loss of IAA-oxidase activity after 1–4 days exposure to elevated copper levels. Tissue analysis revealed that, in seedlings, over 90 % of the total plant copper was located in the seed. Roots contained much lower levels. In older plants, root copper contents rose rapidly, and when a certain critical concentration was reached, IAA-oxidase activity rapidly declined. Root copper levels found in the seedlings were in the range where IAA-oxidase activity was promoted in the older plants. As rice behaves in a very similar manner to barley, in respect to seedling growth, results obtained from rice seedlings not accompanied by tissue copper analyses should be viewed with caution. Wu *et al.* (1975) have shown a reduction in L-malate dehydrogenase activity in roots of the grass *Agrostis stolonifera* at up to 50 μM copper, which was attributed to reduced protein synthesis; this effect was reduced in the case of copper-tolerant plants of the same species.

In addition to effects on plant roots, excess copper may also reduce metabolic activity in the soil. Ruhling and Tyler (1973) showed that spruce needle litter, contaminated with copper and zinc, possessed reduced dehydrogenase activity when compared to uncontaminated material. Tyler (1974) showed that soils contaminated with these two elements had reduced urease and acid phosphatase activity and a depressed respiration rate. Later work by Tyler (1976) largely attributed reduction in soil acid phosphatase activity directly to elevated soil copper levels. The interaction between elevated soil copper levels and inhibition of plant growth is clearly a complex process.

Other plant processes are also sensitive to copper. Whilst the general chlorosis induced by copper toxicity will depress photosynthesis, specific effects of copper on the photosynthetic reaction have been reported. Most of the observations relate to algae, and, as such, fall outside the scope of this review, but Cedeno-Maldonado *et al.* (1972) have reported that copper (25 μM) inhibits photosynthetic electron transport in isolated chloroplasts. This may be due to an alteration in the structure of chlorophyll. Gross *et al.* (1970) observed that photosynthesis in the green alga *Chlorella* was inhibited by copper, and coincident with this was a change in the absorption spectrum of the algal chlorophyll. Reckendorfer (1954) suggested that leaf scorch, arising as a result of excessive application of metal-based pesticides (including copper) could be due to metal replacement of magnesium in the chlorophyll molecule.

It is clearly difficult to separate specific effects of copper on plant growth from the general copper-induced changes in metabolism that result from excessive soil copper levels. Work with isolated plant parts, or with specific metabolic pathways is clearly required to clarify this question, but the relevance of specific copper-induced changes in plant metabolism must be counterbalanced by the strong evidence for factors other than the presence of this element, at sites of metabolic activity, being responsible for the general growth inhibitions that can be observed in whole plants.

5.4. Counteraction of Copper Excess

In some soils, excessive levels of copper may be present without adversely affecting plant growth (Dykeman and De Souza, 1966). The lack of toxic response in such cases is due to the interaction of copper with other soil factors; in the cited example, a very high soil organic matter content served to immobilize the excessive copper levels due to strong binding. In agriculture, liming to pH 7·5 or 8 is considered an effective remedy for most soils.

The value of fertilizer treatments, applied to copper-tolerant *Agrostis tenuis* growing on copper mine spoil, has been reported by McNeilly and Johnson (1978). These authors observed significant increases in growth with either calcium or N additions of 50 or 100 kg/ha, but no additional response to added P above 50 kg/ha. Additional growth increases were noted when 50 kg/ha P was added in combination with 50 or 100 kg/ha N. Both P and calcium treatments reduced shoot metal content, but N was less effective. For further details of the general principles of reclaiming metalliferous wastes, see Williamson and Johnson (Vol. II, Chapter 6).

6. CONCLUDING REMARKS

The interaction between copper and plants is complex. The chemical properties of this element, which confer the potential for considerable interaction with the organic constituents of both the abiotic environment and the plant tissues, pose considerable problems in interpreting various aspects of this interaction. The resolution of factors regulating copper availability in soils, copper uptake by plants and circulation of copper in the biosphere, are of primary importance. The impact of copper deficiency on agriculture depresses potential food production in many parts of the world; equally so, the continued use of copper in industrial processes generates increasing quantities of contaminated by-products, whose disposal

becomes more problematical. The solution to such problems can only be achieved by a clearer understanding of the relationship between copper and plants, which in the future may enable more efficient action to correct copper imbalances in the biosphere.

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CHAPTER 5

Zinc

J. C. COLLINS
Department of Botany, University of Liverpool, UK

1. INTRODUCTION

In many parts of the world Zn deficiency is a common phenomenon, often occurring in economically important crops. At the other end of the scale many of man's activities are increasing Zn levels in the environment, often to toxic levels. Whether the problem is deficiency or toxicity there is a need for knowledge to describe how Zn exists in the soil, enters and is transported within the plant, and the role it plays in the biochemistry and physiology of the plant.

In this chapter, aspects of Zn–soil relations of relevance to plant uptake will be reviewed, followed by a review of Zn–plant relationships. Where possible recent work will be cited; earlier work in this field has been reviewed by Lindsay (1972*a*,*b*), and reviews of many aspects of this topic can be found in Mortvedt *et al.* (1972).

2. ZINC IN THE SOIL

The soil chemistry of Zn has been covered extensively in several recent reviews (Hodgson, 1963; Lindsay, 1972*a*; Krauskopf, 1972), accordingly the following brief account is confined to aspects directly relevant to plant uptake of Zn.

Zinc is not one of the more abundant metals in nature with an average

concentration in the lithosphere of about 80 ppm.† Soil levels usually fall in the range 10–300 ppm, though the soil level is usually lower than that of the parent material. Zn occurs mainly as the sulphide mineral, sphalerite or zinc blende, though it does substitute for Mg in silicate minerals. Zn exhibits 4-coordination, and very rarely 6-coordination with oxygen.

Salts of Zn with the common soil anions are all relatively soluble and hence do not persist in the soil solution under neutral and acidic conditions. Zn is held in the soil mainly on inorganic and organic surfaces and substituted into common soil minerals. It is thought that the principal matrix which holds many of the less abundant heavy metals is due to occlusion and co-precipitation with the hydrous oxides of Fe and Mn.

The solubility of Zn in the soil has been expressed by the following empirical relationship (Lindsay and Norvell, 1969):

$$Zn^{2+} + \text{soil} \rightleftharpoons Zn\text{—soil} + 2H^{+}$$

Log K for this reaction was determined as 6·0, thus:

$$(Zn^{2+}) = 10^{6}(H^{+})^{2}$$

The nature of the principal solid phase involved in this equilibrium was not identified. This approach is rather simplistic; a much better fit can be obtained by the use of the competitive Langmuir adsorption isotherm, relating Zn adsorption and Zn in soil solution to pH and Zn concentration (Bar-Yosef, 1979). The empirical relationship holds up to pH 5, but deviates from observed values markedly at higher pHs. It seems certain that the predominant Zn species below pH 7·7 will be Zn^{2+}, above this $Zn(OH)_2$ will predominate. The zincate ion, $Zn(OH)_4^{2-}$, is not likely to play an important role in the soil (Lindsay, 1972*a*).

Zinc is found dispersed through the mineral fractions of the soil; and throughout the soil profile total Zn usually remains fairly constant. Extractable Zn, on the other hand, is usually found to drop off sharply with depth in the profile. This decrease is due to the concomitant decrease in organic matter. Most analyses show a strong correlation between available

† *Units and abbreviations:* When possible S.I. units have been used, the major exception being the retention of ppm and ppb. The use of the correct S.I. unit, mole/unit weight, is preferable especially for physiological considerations. However, the use of the units ppm and ppb is extremely widespread and has been retained here to avoid confusion. The use of ppm implies parts per million dry matter unless stated otherwise.

The following abbreviations have been used in the text: EDTA, Ethylenediaminetetraacetate; DTPA, Diethylenetriaminepentaacetate; EDDHA, Ethylenediaminedi(*o*-hydroxyphenylacetate); DNP, Dinitrophenol.

Zn (Zn that can be extracted chemically, usually by chelation) and soluble organic matter. Thus it is common for plants growing in an area where topsoil has been removed to show Zn deficiency; this can often be rectified by the addition of manure or organic wastes.

Zinc is held by cation exchange reactions, involving electrostatic or coulombic bonds, but also it enters into specific adsorption processes involving covalent bonds. Adsorption of Zn involves the release of protons by a reaction which can be described as a surface promoted hydrolysis; this will occur even at pH 4–5. Desorption will involve proton uptake (Quirk and Posner, 1975). This is important in the diffusion of Zn in the soil and in the root–soil interface where root and rhizosphere flora activity will locally influence the pH.

Soluble and insoluble organic fractions of the soil will associate with Zn. The former fraction is assumed to comprise relatively simple molecules, organic and amino acids, and the more complex water-soluble fulvic acids. Insoluble complexes are likely to be associated with the humic fraction, especially the humic acids. The organic fraction is involved in several essential soil processes: transport to the root surface; weathering; downward transport (leaching); and pollution. Ellis and Knezek (1972) and Stevenson and Ardakani (1972) have given extensive reviews of this subject.

Concentrations of simple soluble molecules in soils can be high, for instance at a 20% moisture content the level of acetic acid has been estimated at 3·7–5 mM (Stevenson and Ardakani, 1972). Leaching and podsolisation could occur from the downward movement of complexes of Zn with such compounds. Hodgson *et al.* (1966) showed that on average 60% of the Zn in displaced soil solutions was in a complexed form. Later work showed the complexes to be mainly low molecular weight dialysable compounds (Geering and Hodgson, 1969).

The high molecular weight humic substances (2000 to over 300 000) form an important part of the soil organic fraction. Their ability to form stable complexes with metal ions is due to their oxygen-containing functional groups: —COOH, alcoholic, phenolic, enolic OH, and C=O. Nitrogen- and sulphur-containing groups are also important, with respect to the binding of Zn. Randhawa and Broadbent (1965) found at least three sites responsible for the binding of Zn in humic acids, the major part was the least strongly bound and involved phenolic OH and —COOH groups. A more stable fraction was linked to highly acidic —COOH groups; less than 1% of the Zn was strongly bound, but the importance of this fraction is that it represents a preferential binding of Zn. A significant recent development in this difficult area of study is the synthesis of humic-like substances, thus

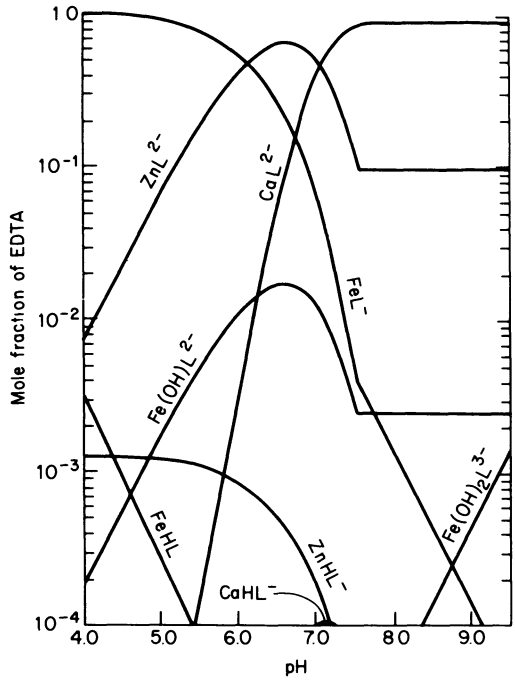

FIG. 5.1 Mole fraction diagram for EDTA in soils when Zn^{2+}, Fe^{3+}, Ca^{2+} and H^{+} are the competing metal ions at 0·003 atm CO_2 (after Lindsay and Norvell, 1969).

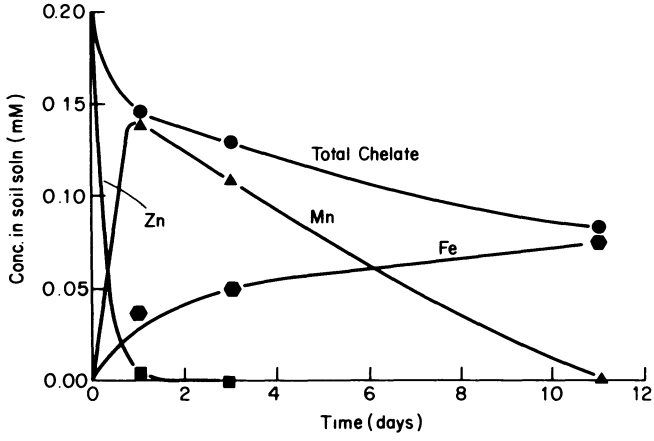

FIG. 5.2 Fate of 0·2 mM Zn EDDHA added to Platner loam (pH 7·0) over an 11-day period (after Lindsay, 1974).

allowing the study of specific functional groups which can be incorporated into the molecule (Zunino *et al.*, 1979).

3. ZINC CHELATES IN THE SOIL

Zinc levels in the soil solution are usually low; in calcareous soils the concentration is often less than 2 ppb and Zn deficiency symptoms frequently occur in plants growing on these soils. In the face of these inherently low Zn levels in the soil solution, chelation processes in the soil play a vital role in making Zn available to the plant. Lindsay (1974), in reviewing aspects of chelation, said: 'were it not for this phenomenon, most soils would be devoid of plant growth because Fe and, in some cases, Zn, Cu, and Mn are too insoluble to maintain adequate levels of soluble nutrients'.

Chelates raise the level of soluble Zn in the soil solution by releasing adsorbed Zn from soil particles. This increase in 'soluble Zn' concentration can appreciably increase the effective diffusion coefficient (D_e) of Zn in the soil, thus increasing the Zn flux to the root surface. However, the interactions of chelates with the soil and soil solution are complex, though the recent introduction of soil chelate stability–pH diagrams (Lindsay and Norvell, 1969; Lindsay, 1974) have allowed the prediction of the predominant chelate forms in the soil–chelate equilibrium at different pH values. Figure 5.1 shows the levels of various metal chelate complexes of EDTA as a function of pH, the competing cations being Ca, Fe and Zn. At pH 6–7 $ZnEDTA^{2-}$ predominates, below this $FeEDTA^{-}$ and above $CaEDTA^{2-}$. A further complexity is that chelate levels in the soil solution vary with time: Lindsay and Norvell (1969) added ^{14}C-ZnEDDHA to soil and followed the fate of the added chelate over an 11-day period (Fig. 5.2). Mn more or less totally displaced Zn from the complex within the first day, followed by a gradual replacement of Mn by Fe. This shift in the nature of the metal complex must have a profound effect on metal availability to the plant. Initially Zn would be readily available, but eventually Zn deficiency would occur in a soil low in Zn.

Elgawhary *et al.* (1970*a*) showed the dramatic influence that added chelate can have on the movement of Zn in soils. Addition of $1{\cdot}5 \times 10^{-8}$ mole ZnEDTA/g soil increased D_e for Zn from $0{\cdot}36 \times 10^{-9}$ to $9{\cdot}0 \times 10^{-9}$ $cm^2\ s^{-1}$, addition of $1{\cdot}5 \times 10^{-8}$ mole EDTA/g soil gave a 10-fold increase in D_e. They attributed the increase in D_e to a release of Zn from the solid phase. In an attempt to simulate the soil–root situation Elgawhary *et al.* (1970*b*) used a porous ceramic tube embedded in a ^{65}Zn-labelled soil, then

various chelate solutions were slowly passed through the tube. Solute diffused from the 'tube' and ^{65}Zn diffused into the tube. It was found that EDTA caused a marked increase in ^{65}Zn movement into the tube, with 10^{-3} M EDTA giving a 17-fold increase in the rate of ^{65}Zn transport compared to water alone. Unfortunately most of the data on this subject concern applied chelates, such as EDTA. However, it is interesting to note that in the above experiments 10^{-3} M citrate also significantly increased the rate of ^{65}Zn transport.

4. MOVEMENT OF Zn TO THE ROOT SURFACE

Ions move to the root surface by two main processes, convection and diffusion, though the continued growth of the root system into non-depleted areas of soil is also an important factor.

Though gravitational movement can be important, the major source of convection or mass flow in the soil is due to the removal of water from the soil by plant transpiration. Movements due to other factors, osmotic or temperature gradients for instance, are relatively unimportant under normal conditions. The mass flow of solution carries ions at their concentration in the soil solution to the root surface, thus a knowledge of the amount of water transpired and the ionic concentration in the soil solution gives a value for the convective flux of an ion. Calculation of ionic concentrations in the soil solution is hazardous, and average values are usually the best estimates to be obtained (Barber *et al.*, 1963; Wilkinson, 1972).

Rate of supply to the root surface might exceed root demand for an ion, so a build up of concentration at the root surface with consequent diffusion back into the bulk solution will occur. This is an unlikely situation for Zn as the soil solution levels are usually very low. In fact demand is more likely to exceed convective supply, leading to a depletion zone at the root surface. For maize, Elgawhary *et al.* (1970*a*) found a transpirational water flow of 250–350 g water/g dry matter and a tissue requirement for Zn of about 15 ppm. They calculated that growing in a calcareous soil where the soil solution concentration of Zn could well be below 2 ppb, less than 5 % of this requirement was met by convective flow to the root surface.

Diffusion in the soil can be described by Fick's first law

$$F = -D\frac{dc}{dx}$$

Where F is the amount moving in unit time across unit area, D is the

diffusion coefficient of the diffusing species and dc/dx is the concentration gradient. Diffusion coefficients of ions in the soil are considerably reduced from their free solution values, and it is the effective diffusion coefficient in the soil, D_e, which is of real interest. The various influences that determine D_e are given in the following expression:

$$D_e = D_1 \theta f_1 \gamma (\Delta C_1 / \Delta C)$$

Where D_1 is the diffusion coefficient in free solution, θ is the volumetric moisture content, f_1 is the tortuosity factor, γ accounts for viscosity and negative adsorption factors and $\Delta C_1/\Delta C$ is the buffering capacity. These parameters are not independent, and interaction occurs. Thus, increasing θ reduces f_1 and increases the cross-sectional area for diffusion.

Warncke and Barber (1972) found an increase in D_e as θ increased in four soils. D_e was in the range 10^{-11}–10^{-8} at 13 % water content and increased to 10^{-9}–10^{-8} $cm^2\ s^{-1}$ at 45 % water content (near saturation). However, in two soils there was no increase in D_e, as these soils contained high Zn levels, increasing θ probably reduced $\Delta C_1/\Delta C$ with the net result being that D_e remained unaltered. It is interesting to note that Nambiar (1975) has demonstrated roots to absorb adequate Zn from relatively dry topsoils (4 % moisture content), provided they have an adequate water supply from the subsoil. Mucilage secreted by the root surface may play an important part in this process, also it is important that more information is gained on the microscopic pathways open to ion movement in the vicinity of the root.

The concentration of Zn in displaced soil solutions varies little over the range 0·3–10 bar (Wilkinson *et al.*, 1968), but the buffering capacity $\Delta C_1/\Delta C$, has a marked effect on D_e. Factors which act on $\Delta C_1/\Delta C$ will be a major influence on the diffusion of Zn. Both pH and Ca level will alter the ratio of adsorbed to solution Zn, thus altering D_e.

The presence of chelates in the soil influences D_e. There is a reduction, primarily due to the molecular size, but this is compensated for by the increase in soluble Zn level due to chelation, thus increasing $\Delta C_1/\Delta C$. Usually the net result is an increase in D_e. Thus Elgawhary *et al.* (1970*a*) found a 10-fold increase in D_e for Zn on addition of EDTA to soil.

The relative roles of diffusion and mass flow in Zn supply to the root surface have been demonstrated by allowing roots to absorb ions from soil uniformly labelled with ^{65}Zn. An autoradiograph of the soil–root system shows the ionic distribution after a period of uptake. Wilkinson *et al.* (1968) using this technique found a narrow depletion zone around the root. This pattern was independent of transpiration rate, showing that mass flow makes little contribution to the movement of Zn to the root.

The evidence is clear that transfer of Zn to the root surface is mainly diffusive in nature and is likely to occur in the 1–2 mm zone adjacent to the root. This zone will be influenced by the root activity determining pH and producing chelates, and also by changes in moisture content and microbiological activity. In particular endo- and ectotrophic mycorrhizae have been shown to increase the uptake of Zn by plants, presumably hyphae extend beyond the depletion zone and transfer Zn to the root.

5. UPTAKE OF ZINC

Active or passive? In the past this has been the controversy. However, most would now agree that Zn entry into the plant is metabolically mediated. Controversy arose mainly because in many of the early reports, the rapid initial absorption into and adsorption onto the cells walls (the so-called free space) masked the active component. Schmid *et al.* (1965) showed the importance of the first, physical, phase of uptake. When this was allowed for, Zn uptake into excised barley roots was sustained at a steady rate for several hours. This rate was reduced at low temperature, and by anaerobiosis, DNP and azide. However, the uptake rate, 4 μmole g^{-1} fresh weight day^{-1}, was extremely high. Such a rate, if maintained, would lead to the development of toxicity. Uptake of Zn by intact plants, growing in recirculated culture solutions, has been examined by Carroll and Loneragan (1969). At low external concentrations they found the rate to be almost directly related to the concentration supplied. For eight plant species the uptake rate varied from 2 to 4 nmole g^{-1} fresh weight roots day^{-1} at 0·01 μM Zn, but this rate was inadequate at very high rates of growth and deficiency symptoms were observed. Maximum yields were obtained between 0·1 and 2·5 μM Zn where the uptake rate was in the range 10–30 nmole g^{-1} fresh weight roots day^{-1}. No deficiency symptoms were observed.

The uptake rates from the two sets of data above are very different, the excised barley root values being one to two orders of magnitude higher than the flowing culture values. The reasons for this large discrepancy were examined by Chaudhry and Loneragan (1972*a*). They studied Zn uptake in intact wheat plants, and when care was taken to avoid depletion of the medium, found an uptake rate of 332 nmole g^{-1} fresh weight roots day^{-1} at 20 °C from an external concentration of 1 μM Zn. The Q_{10} for the process was high; 6·1 in the range 2–10 °C, dropping to about 1·8 over the range 10–29 °C. It seems that the major contributions to the discrepancy come from comparing data gained at different temperatures and using media with

different macronutrient contents, but some differences might well be expected when comparing data from intact plants and excised roots. It is worth noting that Chaudhry and Loneragan (1972*a*) did give evidence that short-term uptake studies are of relevance to long-term studies of Zn nutrition.

The high value of the Q_{10} for Zn uptake at 2–10 °C found by Chaudhry and Loneragan (1972*a*) in wheat seedlings is noteworthy, for the soil temperature in many parts of the world will fall in this range, at least in the spring, and early root growth will take place at these temperatures. In this range an increase in temperature will cause a dramatic increase in Zn uptake. This may partly explain the frequent observation of Zn deficiency symptoms in a cold spring with subsequent recovery as the temperature rises.

Studies with the macronutrient ions have established their compartmentation on a sub-cellular basis, the most common methods used being compartmental analysis using a convenient radio-isotope and insertion of ion-specific microelectrodes into the cell. No suitable ion-specific microelectrodes exist for Zn, but ^{65}Zn is a suitable isotope for compartmental analysis studies. Studies on the grass *Deschampsia caespitosa* show that the Zn concentration is higher in the vacuole than in the cytoplasm, and that at low external Zn concentrations there is an active flux into the cytoplasm across the plasmalemma and an active extrusion from the cytoplasm to the vacuole (Brookes *et al.*, 1981). The active nature of the Zn fluxes accords with earlier work, and it will be of interest to determine the nature of the Zn in the cytoplasm and vacuole. A suitable ion-selective electrode for Zn would greatly facilitate determining whether Zn is appreciably complexed within the cell.

The uptake of chelated Zn by plant roots has not been examined in any great detail. The importance of chelation in the soil in supplying Zn to the root surface makes this an important omission. There is evidence that Fe is released from the complex prior to absorption (Chaney *et al.*, 1972), but for Zn evidence is lacking. A recent study on the uptake of Cu chelates may be of some relevance. Goodman and Linehan (1979), from a study of the electron paramagnetic resonance spectra of Cu and Cu chelates, showed that wheat roots absorbed Cu from Cu chelates only after prior release of Cu from the complex. A soluble Cu species reappeared in the root tissue, this was thought to be an amino acid complex.

What evidence there is for Zn chelate uptake seems to show that chelation depresses uptake. DeKock and Mitchell (1957) showed uptake of Zn by mustard was depressed by DTPA. Similarly, Halvorson and Lindsay

(1971) found that DTPA caused Zn deficiency in maize at pH 7·5, though growth was normal at pH 5. This was attributed to a depression of free Zn level by DTPA, at pH 7·5 the level was calculated to be $10^{-11\cdot7}$ M. Halvorson and Lindsay (1977) confirmed these findings, showing that added Zn corrected the deficiency. They calculated a critical level for normal growth of about $10^{-10\cdot6}$ M Zn. This is much lower than the value of 10^{-7} M calculated by Carroll and Loneragan (1969) from flowing culture experiments. Halvorson and Lindsay (1977) have suggested that a depletion zone in the free space could have developed in the flowing culture experiments. They proposed that only free Zn is taken up by the root and that the released chelate acts to maintain soluble Zn levels adjacent to the absorbing zones. It is noteworthy that the hypothesised soluble Zn level in calcareous soils is only slightly higher than the calculated critical level.

Mycorrhizal infection has been shown to increase Zn contents of infected plants (Lambert *et al.*, 1979). This is to be expected as the mechanism of vesicular–arbuscular endotrophic mycorrhizal action, whereby hyphae extend from the root surface for several centimetres into the soil, is likely to be most important in the uptake of ions which have narrow diffusion zones, such as Zn. Swaminathan and Verma (1979) found a significant increase of Zn in infected plants of maize, wheat and potato.

6. INTERACTION WITH MACRONUTRIENTS AND HYDROGEN IONS

In their experiments with intact wheat seedlings, Chaudhry and Loneragan (1972*a*,*c*) showed that the anion made little difference to Zn uptake, but that a range of cations depressed uptake. The important soil cations, Ca, Mg, K and Na, all inhibited Zn absorption at a concentration of 750 μM. Hydrogen ions were also inhibitory. Inhibition was Ca sensitive at low Ca concentrations, but at higher Ca concentrations H inhibition was Ca independent (Chaudhry and Loneragan, 1972*b*). In the soil it is probable that the effects of Ca, H, and Cu would be additive, though the role of soil chelates should be taken into account.

7. INTERACTION WITH MICRONUTRIENTS

Schmid *et al.* (1965) found that Zn uptake in excised barley roots was strongly inhibited by Cu, but that Mn had little effect. Similarly Bowen

(1969) stated Zn uptake in sugar cane tissue was competitively inhibited by Cu, whilst Mn was absorbed by a separate mechanism. In a thorough study of Zn absorption by wheat seedlings, Chaudhry and Loneragan (1972*b*) found Cu and Co to be competitive inhibitors, Cu strongly so but Co only weakly. Mn and Fe did not interact with Zn uptake. However, they did point out that the situation in soil and water culture could be very different, due in particular to the presence of chelates and Cu–colloid interaction in the soil.

In a recent examination of Zn uptake by excised barley roots Veltrup (1978) found no inhibition by Cu, rather a slight stimulation, and so concluded that Zn and Cu are taken up by separate mechanisms. He was able to fit Zn uptake to a triphasic pattern over the concentration range 1·5 μM to 1·38 mM Zn.

8. ZINC–PHOSPHORUS INTERACTION

It is frequently observed that an elevated supply of P to plants can cause symptoms similar to, or indistinguishable from, Zn deficiency; this condition is referred to as 'P-induced Zn deficiency'. The mechanism and underlying causes of this interaction have been the subject of many investigations; much of this work has been reviewed by Olsen (1972).

Various possibilities have been suggested to explain the interaction:

(a) Zn–P interaction in the soil.
(b) Growth promotion due to increased P causes dilution of Zn in the plant.
(c) Inhibition of Zn uptake due to cations added with P.
(d) Metabolic disorder due to P–Zn imbalance.
(e) Slower rates of Zn transport at increased P levels.

Many of the early investigations assumed that the formation of insoluble Zn phosphate in the soil would render Zn unavailable to the plant. However, Jurinak and Inouye (1962) showed that even at pH 8 the solubility of Zn phosphate is great enough to give a concentration of 15·7 μM soluble Zn, and that Zn concentrations rise as the pH is decreased. These levels would be more than adequate for the plant's needs. Indeed it has been shown that Zn phosphate acts as an efficient source of both Zn and P when applied as a fertiliser (Boawn *et al.*, 1957). Nevertheless, phosphate can increase the adsorption of Zn to Fe and Al oxides and hydroxides in the soil (Bolland *et al.*, 1977), and this decrease in soluble Zn can, on occasions,

be the cause of reduced Zn uptake (Stanton and Burger, 1967; Loneragan *et al.*, 1979).

Zinc deficiency, due to dilution brought about by P-enhanced growth, has been observed in many investigations, and is certain to be a major factor where the soil supply of P and Zn are barely adequate (Boawn *et al.*, 1954; Loneragan *et al.*, 1979). This phenomenon is not confined to P, and a similar interaction can often be found where N enhances growth (Chaudhry and Loneragan, 1970).

The work of Chaudhry and Loneragan (1972*a*) on Zn uptake in wheat seedlings clearly shows that cations such as Ca, Mg and K at low levels of Ca can drastically reduce the uptake of Zn. Although these experiments were conducted in water culture, inhibition was found at cation levels likely to be encountered in the soil solution, and cation-inhibition of Zn uptake due to cations added with P-fertiliser must be considered a likely cause of P-induced Zn deficiency in some cases.

In some cases P-induced Zn-deficiency is observed where the concentration of Zn in the plant tops is not reduced (Boawn and Brown, 1968), so this is obviously not a dilution effect. Neither is it related to the formation of Zn phosphate, for the solubility is too high. This would seem to be a different phenomenon from Zn deficiency and it may be due to the action of P on a Zn-dependent enzyme. In sand-grown plants of *Trifolium subterraneum*, high levels of P can induce P toxicity, the symptoms of which are leaf bronzing, interveinal chlorosis and necrosis in older leaves. These symptoms are similar to those of P-induced Zn deficiency, though in this case Zn levels are not reduced, rather P levels are increased. Symptoms can be alleviated by Zn application which promotes growth and dilutes the P (Loneragan *et al.*, 1979).

Some workers have concluded that high P levels can depress Zn transport, but the mechanism of this interaction, if it exists, is unclear. As pointed out by Olsen (1972) it may be that the plant part nearest the source of supply (the root) receives its nutritional supply first before significant transport to the tops occurs. In some cases the restriction of transport has been noticed at abnormally high levels of Zn and P where solubility problems may well have occurred.

Safaya (1976) has made a detailed study of Zn–P interaction in maize, demonstrating some interesting features. Increasing P supply decreased tissue Zn and the Zn flux into the root. Zn-deficient plants showed increased tissue P levels. P flux into the plant was mostly reduced by Zn, the P flux being more or less steady in the presence of Zn at 14 $\mu g\, g^{-1}\, day^{-1}$ at all P levels. However, in the absence of Zn, the P flux varied greatly. Additionally

it was shown that both P and Zn reduced Cu absorption, and that Zn depressed Mn and, to some extent, Fe absorption. It was concluded that Zn regulates P uptake, and that excess P inhibits first Zn translocation and then Zn uptake. This is an important piece of work, for it is unusual for the ion fluxes to be measured. This approach shows that not only does the tissue Zn level fall as a result of P-enhanced growth, but the Zn flux falls as well.

Mycorrhizal infection enhances Zn uptake, but increased P levels have been shown to reduce the incidence of mycorrhizal infection (Tinker, 1975), thus there might well be a depression of Zn absorption via this mechanism.

It is obvious that Zn–P interactions are complex, and that there can be several contributory phenomena involved in the interaction. A recent report (Loneragan *et al.*, 1979) shows that for *Trifolium subterraneum* at least three mechanisms were operative in P-induced Zn deficiency in sand-grown plants, depending on the levels of Zn and P applied and the nature of the sand. Added complications arise when results from water culture and soil are compared, as in the former case Zn–P soil interaction is removed together with mycorrhizal interaction.

9. TRANSLOCATION

The level of Zn in the xylem fluid is usually in excess of the level supplied to the root (Table 5.1). The Zn is probably transported as the ionic species, not in chelated form. Tiffin (1967) could find no evidence from electrophoretic studies for a Zn chelate, such as is found for Cu, Ni and Fe^{3+}. However, the nature of the transported Zn species is an important question and needs further examination, perhaps by such chromatographic methods as have been successfully employed in determining the Zn ligands in milk (Evans *et al.*, 1979).

TABLE 5.1

CONCENTRATION OF Zn IN THE XYLEM EXUDATE OF EXCISED SEEDLING ROOTS OF MAIZE. PLANTS GROWN IN ONE-TENTH DILUTION LONG ASHTON SOLUTION. (COLLINS, UNPUBLISHED DATA)

Concentration Zn in medium (μM)	Concentration Zn in xylem exudate (μM)
0·1	84·2
50	250
100	460
200	650
400	690

Van Goor and Wiersma (1976) have reported that Zn is transported in the phloem of *Ricinus* as an anionic complex. They found that all the Zn in phloem exudate was complexed; the complex had a molecular weight of 1000–1500 and probably contained some P.

Zinc is an element of intermediate mobility. When plants are supplied with luxury levels, Zn tends to accumulate in the root; however, if this level is not maintained, there is ultimately transport from root to shoot (Loneragan, 1977). Evidence for retranslocation of Zn comes from the work of Massey and Loeffel (1967) who showed that Zn accumulation in the developing ear of maize, between tasselling and maturity, exceeded total uptake during that period by 50%. The excess Zn came from the surrounding stem and leaf tissue.

The level of Zn supply can determine the extent of translocation. In *Trifolium subterraneum* grown at luxury Zn levels, 25% of the Zn was transported from old leaves and petioles to developing fruits, but when grown at marginal or deficient levels, little or no Zn was retranslocated (Riceman and Jones, 1958).

Little Zn appears to be lost from the aerial parts by leaching. Thus Tukey *et al.* (1958) found less than 1% of total foliar Zn lost from squash during a 24-h water mist treatment. However, Beauford *et al.* (1975) have shown that Zn is one of the elements given off into the atmosphere in particulate form by plants. This is probably in the form of epicuticular wax particles and, although it was shown to be a variable phenomenon, it does seem to represent a significant heavy metal load to the atmosphere from a cover of vegetation.

10. ZINC AND ENZYME FUNCTION

It is only recently that the importance of Zn in enzyme function has begun to be fully understood. The first Zn enzyme discovered was carbonic anhydrase (Keilin and Mann, 1940) and since then more than 80 Zn-dependent enzymes have been recorded. Zn is a component of many dehydrogenases (Hewitt and Smith, 1974). The previous handicaps of poor analytical methods and contamination have been largely overcome, and it is now known that Zn plays an important role in protein synthesis, carbohydrate, nucleic acid and lipid metabolism (Vallee, 1976).

Zinc-dependent enzymes are metalloproteins in which the Zn is closely associated, often with covalent bonding, and give colourless solutions. Frequently, Zn can be replaced by other metals, notably Co, with at least

partial activity being retained. The Zn in the enzyme is thought to be in a chemically modified state, the so-called 'entatic state' (state of tension); this lowers the activation energy for the reaction prior to enzyme–substrate complex formation (Vallee and Riordan, 1969).

Zinc is known to form stable complexes with DNA and RNA and is thought to be involved in DNA and RNA stability. Thus in *Euglena gracilis*, Zn deficiency causes growth to be arrested, and cell volume increases together with an increase in the number of osmophilic granules and a doubling of the DNA content. Both RNA content and protein synthesis decrease, and peptides and unusual proteins accumulate. It is believed that all these phenomena are related to the role of Zn in maintaining the integrity of the ribosome and stabilising DNA and RNA (Price *et al.*, 1972). This action at the level of the ribosome seems to be one of the first and most important manifestations of Zn deficiency.

The importance of Zn in intermediary metabolism is now well described, thus Vallee (1976) has stated: 'it is apparent that Zn is essential to multiple processes, critical to the development, division and differentiation of cells'.

11. ZINC DEFICIENCY

An inadequate supply of Zn results in chlorosis and stunted growth, with a drastic reduction in yield. In maize, the condition known as 'white-bud' is a response to Zn deficiency as is 'rosette' or 'little-leaf' in fruit trees. In a study of Zn deficiency in eight species, Rahimi and Bussler (1978) found a violet–red coloration in the leaf as the first symptom, initially as dots then spreading over the entire leaf; this was most pronounced in young leaves. General symptoms were retarded growth, short internodes, small leaves, chlorosis and poor root formation. Zinc deficient plants show a reduced level of Zn in their tissues, usually in the range 0–15 ppm dry matter, healthy plants contain 10–100 ppm.

Sensitivity to Zn deficiency varies among crops. Most fruit trees are sensitive, as is corn, bean (*Phaseolus vulgaris*), onions and potatoes. Grasses, legumes and small-grained cereals are usually relatively insensitive. Also there is often a range of sensitivities within a species. Shulka and Raj (1976) examined the response to Zn deficiency in eight maize genotypes and found a great variation in the severity of 'white-bud'. Under Zn stress the reduction of shoot dry matter production varied between 74 and 95%, and the Zn level in the tissue varied from 7·4 to 20·5 ppm dry matter. They concluded that the variation was due to the

differing capabilities of the genotypes to exploit soil Zn and/or translocate Zn to the shoot.

Several specific physiological processes are known to be altered in Zn deficiency. Ohki (1976, 1978) found that Zn-deficient cotton had a lower photosynthetic rate, and that carbonic anhydrase activity was depressed. A similar situation existed in soybean, where severe deficiency increased dark respiration. Maximum photosynthetic activity was established at a leaf level of 12 μg/g Zn (12 ppm dry matter) (Fig. 5.3). Thomson and Weier

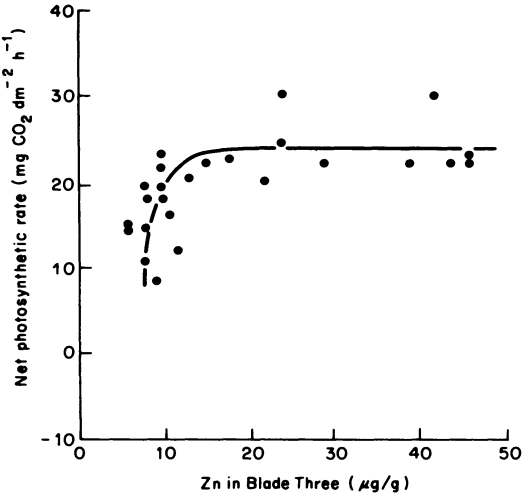

FIG. 5.3 Relationship between Zn concentration in blade three of Soybean and net photosynthesis (after Ohki, 1978).

(1962) showed that the absence of Zn in *Phaseolus vulgaris* interrupted chloroplast development; grana formation was incomplete and large vacuoles were found in the chloroplasts. It has also been shown that starch metabolism is sensitive to Zn deficiency. Jyung *et al.* (1975) noted that starch content, soluble starch synthetase and size and number of starch grains were all decreased in Zn-deficient *P. vulgaris*. These effects were much more marked in a susceptible variety (Sanilac) than in a less susceptible variety (Saginaw). The involvement of Zn in starch metabolism might be direct or indirect.

One of the first processes influenced by Zn deficiency is RNA metabolism. There is a rapid decrease in RNA levels and the number of ribosomes, which leads to a decrease in the level of normal protein (Price *et*

al., 1972). The characteristic internode shortening has been ascribed to an alteration of auxin metabolism. Salami and Kenefick (1970) found that the symptoms of Zn deficiency in maize could be alleviated by additions of Zn or tryptophane. However, Takaki and Kushizaki (1970) found that high levels of tryptophane accumulated in Zn-deficient maize. It is difficult to marry these two reports, though it seems certain that Zn is involved at some stage in auxin metabolism.

Common factors that induce Zn deficiency are: low soil soluble Zn; high rainfall; calcareous soils; low soil organic matter; high soil P levels; liberal N applications; restriction of root zones; low soil temperatures.

Zinc deficiency is of widespread occurrence, particularly on neutral or alkaline soils, and as a loss in yield and delay of maturation occurs, it is important that deficiency be corrected. Fortunately Zn deficiency is easily corrected by foliar spray or soil fertiliser application. Lindsay (1972*a*) has reviewed agricultural practices of Zn deficiency correction. Crop consumption is usually around 0·5 kg ha^{-1} $year^{-1}$, application of fertiliser is at about 0·5–10 kg ha^{-1} for sensitive crops. Boawn (1974, 1976) has shown a rapid conversion of applied Zn to forms not extractable in the first year after application. At equilibrium, 85–90% of fertiliser-Zn was unextractable. He found that an application of 11·2 kg ha^{-1} gave extractable Zn adequate for four years. There is a general consensus that Zn applications give a residual effect for about five years.

12. ZINC TOXICITY

Some soils contain abnormally high levels of Zn. This can be a geochemical phenomenon, for instance where the soil overlays Zn-rich minerals, or it may be a man-made condition. Examples of man-made conditions are spoil heaps at mining sites, sewage sludge often rich in heavy metals applied for agricultural purposes, or excess Zn oxysulphate persisting in the soil after use as a pesticide. Plants growing on such soils often show signs of Zn toxicity, though in some cases a distinct flora develops which is capable of growing and completing its life cycle in such conditions (Antonovics *et al.*, 1971).

The general symptom of Zn toxicity is a retardation of growth, plants being stunted and chlorotic. Takkar and Mann (1978) described Zn toxicity symptoms in wheat and maize: light blue–green tinge develops at the leaf tips and spreads to the base, older leaves are chlorotic, finally the leaf dries out. These symptoms are similar to those of P toxicity. Associated with

these symptoms, the plant contains high Zn levels. Takkar and Mann (1978) found that soil level of 7 ppm Zn (DTPA-extractable) was toxic to wheat and 11 ppm Zn toxic to maize. The tissue levels at these soil levels were 60 and 81 ppm dry matter, respectively. Somewhat higher levels have been recorded by Beckett and Davis (1977) and Davis and Beckett (1978) for barley and ryegrass tissue. These authors observed tissue contents of Zn to vary considerably depending on the growth conditions, but found that the minimum tissue concentration necessary to cause toxic symptoms was relatively independent of growth conditions. Above the upper critical level of Zn the yield was found to decrease in approximate proportion to the logarithm of the tissue concentration.

Several specific actions of elevated Zn levels on physiological processes have been documented, though in many cases the mechanism of action is unclear and often it is not clear whether the action is direct or indirect. Hampp *et al.* (1976) showed Zn to inhibit CO_2 fixation in isolated spinach chloroplasts, inhibition being half maximal at 22·5 μM Zn and non-competitive in nature; non-cyclic electron transport was also affected. De Filippis and Pallaghy (1976) showed Zn inhibition of photosynthesis and respiration in *Chlorella*, inhibition was accompanied by chlorophyll loss, though in this work the Zn levels were very high (1 mM), and the inhibition observed was an initial response followed by later recovery. Zn has been shown to inhibit oxidation of succinate by isolated corn mitochondria (Bittell *et al.*, 1974), a 50% inhibition being obtained at about 0·1 mM Zn. Kleiner (1974) has postulated that Zn binds somewhere between ubiquinone and b cytochromes in the electron transport chain. He found that the binding site was specific for Zn, even Co could not replace Zn. However, the picture is still unclear and more work is needed relating the action of elevated Zn levels to photosynthesis and respiration. In particular, it is difficult to equate isolated-organelle work with *in vivo* studies in the absence of a knowledge of cytoplasmic Zn levels. Also it must be borne in mind that many metabolites (in particular organic acids) bind Zn strongly.

Another important inhibitory effect of Zn is on phloem translocation. Samarkoon and Rauser (1979) found that 0·2 mM Zn severely restricted carbohydrate translocation in *Phaseolus vulgaris*. Within 1–2 days following treatment transport to the sinks was drastically reduced, with a concomitant accumulation of sucrose, reducing sugars and starch in the primary leaves. The mechanism of this inhibition was not determined. Although Zn caused callose deposition on sieve plates, this was not thought responsible (Peterson and Rauser, 1979). Samarkoon and Rauser (1979) concluded that the inhibition of phloem transport is 'an example of an

"essential to life" process which has failed due to metal phytotoxicity' (q.v. Foy *et al.*, 1978).

Many reports show that Zn alters membrane permeability and it is tempting to speculate that this is one of the primary mechanisms of Zn toxicity. In particular it has been shown that high Zn levels cause membranes to become 'leaky' to K. Thus Wyn-Jones and Sutcliff (1972) found that 10 μM Zn enhanced K leakage from maize roots. De Filippis (1979) found that Zn levels above 0·1 mM induced K leakage from *Chlorella* and that the action of Zn was strongly correlated to the strength of the Zn–sulphydryl bond. However, it has not been shown that the action of Zn in this aspect is a direct one, it might be mediated indirectly through an interruption of metabolism linked to the transport processes.

The chlorotic response in Zn toxicity has frequently been attributed to an interference with Fe metabolism, and indeed chlorosis can often be overcome, at least partially, by the addition of Fe. However, the chlorosis is not associated with decreased leaf Fe levels, and Fe appears to retard the absorption and translocation of Zn (Rosen *et al.*, 1977). Ambler *et al.* (1970) produced evidence that high levels of Zn reduce the amount of reductant necessary for Fe translocation by soybean roots; addition of Fe chelate overcame this problem.

A recent discovery has been the existence of metallothioneins, low molecular weight proteins which bind specific heavy metals (Cherian and Goyer, 1978). Until very recently they have been described for animal tissues only. However, Lerch (1980) has isolated a Cu-binding metallothionein from *Neurospora crassa*. The protein, which binds 6 moles of Cu to a 2200 molecular weight fraction, is high in cysteine and contains no aromatic residues. The Cu is thought to be bound in the cuprous state. Production of the protein is proportional to Cu concentration over a restricted range. This protein is remarkably similar to the Zn and Cu metallothioneins described for animal tissues, which are thought to function in detoxification and storage of the elements. This is an exciting discovery and such molecules may well prove to play an important role in many facets of Zn metabolism in plants.

Just as there is a marked difference in susceptibility of species and genotypes of species to Zn deficiency, the same is true for tolerance to Zn toxicity. For the soybean cultivars 'Wye' and 'York', grafting experiments have shown that tolerance to excessive Zn levels resides in the shoot genotype. The root stock genotype controlled Zn absorption and translocation. However the differences in yield due to the susceptibility of the shoot genotype were not related to leaf Zn content. Thus it must be

concluded that, at least in this case, tolerance is associated with a more subtle factor than gross leaf Zn content (White *et al.*, 1979).

13. PROSPECT

A careful perusal of plant nutrition studies over recent years shows the increase in interest in Zn as a plant micronutrient. This is matched by advances in understanding the relations of Zn in the soil and in the plant. The factors controlling Zn availability in the soil, and in particular the importance of chelates, are now fairly well described. The factors controlling Zn transfer to the root surface and the subsequent uptake of Zn are not as well understood. This is a key area, and it is clearly necessary to determine the precise role and nature of soil chelates in this process. There is a great need for reliable data both from physiologists and soil scientists.

The role of Zn in the physiological and biochemical processes of plants is not fully understood. In most cases it has not been determined whether the physiological actions of Zn are direct or indirect responses. A determination of the primary actions of Zn in physiological processes is of major importance. The possibility that Zn exists in the plant cell in a complexed form is worthy of investigation, for there is little or no evidence to show the form of Zn in the cytoplasm or the vacuole.

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CHAPTER 6

Nickel

T. C. HUTCHINSON
Department of Botany, University of Toronto, Canada

1. HISTORY OF REPORTS OF NICKEL IN PLANTS AND NICKEL AS AN ESSENTIAL ELEMENT

Though nickel was identified as an element as early as 1751 by Cronstedt, its occurrence in plants was not recognized for a further hundred years, when Forchhammer (1855) found nickel in oak wood. More routine examination of plant material for the presence of nickel did not occur until Tschugaeff (1905) developed the dimethyl glyoxime method for determining trace quantities. Kraut (1906) found nickel in peat and brown coal ashes, while Cornec (1919) found it in marine algae. Later, McHargue (1925) analysed a wide range of plant, animal and soil samples for nickel, copper, manganese and cobalt and concluded that all were widely present in these materials and may therefore function as essential elements. A brief review of these early studies on nickel is given in Vanselow (1966).

The essentiality of nickel for the proper growth of plants has not been yet proven beyond doubt, though evidence for this continues to accumulate. Recent studies have shown it to be necessary for the functioning of certain enzyme systems (Bartha and Ordal, 1965; Bertrand, 1974; Dixon *et al.*, 1975; Polacco, 1977). There are also frequent reports of slightly beneficial plant growth responses to the addition of trace levels of nickel. Roach and Barclay (1946) found increased yields of potatoes when the plants were sprayed with very dilute solutions of nickel salts, while Dobrolyubskii and Slavvo (1957) reported improved yields of grapes upon similar treatments. Mishra and Kar (1974) report in their review of nickel, that Smith (1943) reported a stimulation of *Pinus radiata* seedlings following nickel additions, while Leyton (1947) suggested nickel has a more general stimulation to tree species.

More recently, a number of studies have shown positive growth responses to nickel additions in nutrient cultures and in soils. Hutchinson and Kuja (1979) reported that metal-tolerant races of the grass *Deschampsia cespitosa* now invading the smelter regions near Sudbury, show a positive response to quite high nickel accumulations in the soil, i.e. stimulation at soil concentrations which are toxic to a number of other test species, and to non-tolerant populations of *D. cespitosa*. Similarly, a positive response has been shown by *Phragmites communis* seedlings raised in nutrient culture at 0·1 and 0·5 ppm Ni. Cox and Hutchinson (1980) reported stimulation of metal-tolerant *D. cespitosa* clones in nutrient culture at 1·0 ppm Ni.

Amongst micro-organisms, Bartha and Ordal (1965) noted the dependence of chemolithotrophic growth of two *Hydrogenomonas* strains of bacteria upon the presence of nickel in the medium. Bertrand (1974) found nickel to be an essential element for the microbiological fixation of N_2 in soil and this could be a key role for nickel in ecosystems. Repaske and Repaske (1976) found nickel to be required for exponential growth of *Alcaligenes eutrophus*.

Although Frieden (1972) did not describe nickel as an essential micronutrient, he did note the strong possibility that it was, while Keeney (1975) went further and described it as essential in trace quantities for crop plant growth. His evidence for this statement may have been based on the work in progress at that time by Dixon *et al.* (1975). They carried out experiments designed to challenge the classical study by Sumner, who in 1926 had isolated the first crystalline enzyme, urease, from jack beans (*Canavalia ensiformis*). He had proposed that enzymes could be proteins devoid of organic co-enzymes and metal ions. Dixon *et al.* (1975) noted 'with some sadness that in this communication we advance evidence which strongly indicates that urease is a nickel metalloenzyme'. They reported $2 \pm 0{\cdot}3$ g atoms of nickel per 105 000 g of purified urease. After denaturing the enzyme with EDTA, they found the re-establishment of activity was a linear function of the nickel content of the denatured enzyme. They also noted that acidification reduced enzyme activity to 36% at pH 3·5 compared with pH 7·0. This reduction coincided with a loss of nickel from the protein during acidification.

Studies by Polacco (1976, 1977) on the nitrogen metabolism of soybean in tissue culture experiments support the key role of nickel in urease activity. He showed that urea utilization and urease synthesis require nickel as Ni^{2+}. When urea is the sole N source, potassium citrate inhibited growth. This toxicity could be overcome by treatments with ammonium

chloride, magnesium chloride at high concentrations or nickel sulphate at low levels. Nickel also stimulated growth in urea-fed cultures in the absence of the potassium citrate treatment. This coincided with a ten-fold stimulus of urease activity. The overcoming of the citrate-induced toxicity of urea-treated plants suggests that trace quantities of nickel are essential specifically for the conversion of urea to ammonia. Support for this hypothesis was provided in further experiments in which nickel stimulated urease levels in arginine-grown cells.

2. NICKEL TOXICITY

The toxic properties of excessive nickel in soils for plant life was first pointed out by Haselhoff (1893), who demonstrated its toxicity to corn and bean plants using solution-culture techniques. Since then, a large number of reports of nickel phytotoxicity have been made. Most describe poor growth accompanied by chlorosis. Vanselow (1966) summarized this literature to that point. Wolff (1913) found 8 ppm Ni in solution rapidly killed barley. Cotton (1930) found that 0·5 ppm nickel produced chlorosis in buckwheat. Brenchley (1938) found 2 ppm Ni to be toxic to bean and barley. Flax (*Linum usatatissimum*) was found to be especially sensitive, showing toxicity at 0·5–5·0 ppm (Millikan, 1949). Oats were also found to be sensitive to elevated nickel levels and have been used by a number of workers to identify nickel toxicity on serpentine soils (Hunter and Vergnano, 1952; Vergnano and Hunter, 1953).

The symptoms of nickel toxicity appear to be a combination of induced iron-deficiency chlorosis and foliar necrosis. Mishra and Kar (1974) describe the main toxic symptoms of nickel as chlorosis or yellowing of the leaves, followed by necrosis. Other toxic symptoms include stunted growth of the roots and shoots, deformation of various plant parts, and unusual spottings on leaves and stems. Vanselow (1966) pointed out that in monocotyledons nickel often showed up as alternate green and light-yellow banding on the leaves, while in dicotyledons the symptoms are of a general chlorotic mottling.

Rauser (1978) studied the early effects of nickel toxicity in bush bean, *Phaseolus vulgaris*, which showed decreased dry matter production, an abnormal vertical orientation of the leaves, an abnormal starch accumulation and an accumulation of apolar soluble phenolics. Anderson *et al.* (1973, 1979) have studied the anatomical development of nickel toxicity symptoms in the leaves of oats, *Avena byzantina*. Nickel excess

induces a predisposing condition in areas of the leaf before the emergence of the coleoptile. These areas give rise to chlorotic bands which develop on the emerged leaf 24 h after emergence. An alternating light and dark regime is necessary for the development of the chlorotic bands. Potential green areas develop under the coleoptile during the day, while the potential chlorotic bands develop at night. Exactly these symptoms were described for oats by Crooke and Inkson (1955) in studies of nickel toxicity on Scottish serpentine soils. Symptoms were worst under nutrient regimes low in calcium or potassium or high in phosphorus.

The toxicity of nickel to micro-organisms is well known. Nickel is used as a fungicide, especially for the control of cereal rusts (Mishra and Kar, 1974). It is also known to be highly toxic to a wide range of algae (Hutchinson, 1973; Stokes *et al.*, 1973; Patrick *et al.*, 1975).

3. NICKEL CONCENTRATIONS IN PLANTS

The nickel concentrations in plants growing on soils other than those with excessive nickel levels, are usually low, i.e. < 10 ppm. Vanselow (1966) provided an extensive tabulation from the literature, which showed that for most crop plants the nickel content of foliage is < 2 ppm of dry weight. He also suggested that tissue concentrations of greater than 50 ppm could be considered excessive. Toxic symptoms of nickel excess showed in young *Citrus* plants containing 55 ppm Ni in leaves. Halstead *et al.* (1969) found a decrease in growth of oats when the nickel content of the grains exceeded 60 ppm, and of the straw 28 ppm Ni. Alfalfa showed a decrease in growth when foliar levels exceeded 44 ppm Ni.

Hutchinson *et al.* (1974) analysed crops and soils from a major horticultural area developed on deep organic muck soils. At the time of harvest, concentrations in lettuce, celery, cauliflower, onion, potato, carrot and parsnip foliage ranged from 2·8 to 6·2 ppm Ni. Keeney and Walsh (1975) obtained values of 0·9–2·5 ppm Ni for rye, sorghum and corn grown on unamended agricultural soils, while Bates *et al.* (1975) found levels of 2·7–3·2 ppm Ni in ryegrass before sewage sludge additions to the soil.

It is apparent that the amount of nickel present in the plant is partially a reflection of that present in the soil. Normally, extraction techniques such as distilled water or ammonium acetate extracts give a better correlation of soil nickel with foliar levels. This is as true for crop plants as it is for native vegetation. The presence of excess nickel in the soil, whether it be from a local geological abnormality, from sewage sludge amendments, or from

TABLE 6.1

NICKEL CONTENT OF HOMOGENIZED LEAF TISSUE COLLECTED AT SITES EITHER (a) 50 km EAST OF THE CONISTON SMELTER IN 1970 OR (b) 60 km SSE OF THE COPPER CLIFF SMELTER IN 1976. DATA ARE EXPRESSED AS ppm DRY WEIGHT

Species	Nickel content	Reference
Vaccinium angustifolium	14	Hutchinson and Whitby, 1974
Acer rubrum	14	Hutchinson and Whitby, 1974
Deschampsia flexuosa	37	Hutchinson and Whitby, 1974
Comptonia peregrina	17	Hutchinson and Whitby, 1977
Betula papyrifera	16	Hutchinson and Whitby, 1977
Populus tremuloides	4	Freedman and Hutchinson, 1980
Acer rubrum	2	Freedman and Hutchinson, 1980
Betula papyrifera	6	Freedman and Hutchinson, 1980
Quercus borealis	6	Freedman and Hutchinson, 1980
Pinus resinosa	11	Freedman and Hutchinson, 1980
Salix humilis	13	Freedman and Hutchinson, 1980
Vaccinium angustifolium	7	Freedman and Hutchinson, 1980
Myrica asplenifolia	30	Freedman and Hutchinson, 1980
Diervilla lonicera	16	Freedman and Hutchinson, 1980
Solidago canadensis	4	Freedman and Hutchinson, 1980
Epilobium angustifolium	7	Freedman and Hutchinson, 1980
Deschampsia flexuosa	6	Freedman and Hutchinson, 1980
Agrostis stolonifera	6	Freedman and Hutchinson, 1980
Equisetum sylvaticum	18	Freedman and Hutchinson, 1980
Polytrichum commune	40	Freedman and Hutchinson, 1980

industrial activity involving atmospheric emissions of nickel such as from smelters and refineries, all add to the amount of nickel taken up by plants and lead to elevated foliar levels. Examples of this are dealt with in a subsequent section.

For terrestrial native species, a greater range of metal concentrations occurs than for crop plants. This is largely because of the wide pH range of natural soils, from highly acidic to alkaline. Especially on acidic soils, nickel uptake is enhanced. In contrast, agricultural crop production is carried out on soils of a narrower pH range, and naturally acidic soils are limed to close to neutrality for agriculture.

Mitchell (1954) recorded nickel levels for a number of native species, quoting ranges in ericaceous dwarf shrubs from acid soils in Britain as *Calluna vulgaris* 0·6–2·6 ppm, *Erica cinerea* 1·5–1·7 ppm and *E. tetralix* as 1·1–1·5 ppm. He also reported foliar levels of 0·7–1·7 ppm Ni in sweet vernal grass (*Anthoxanthum odoratum*), 0·3–3·0 ppm Ni in bullrush

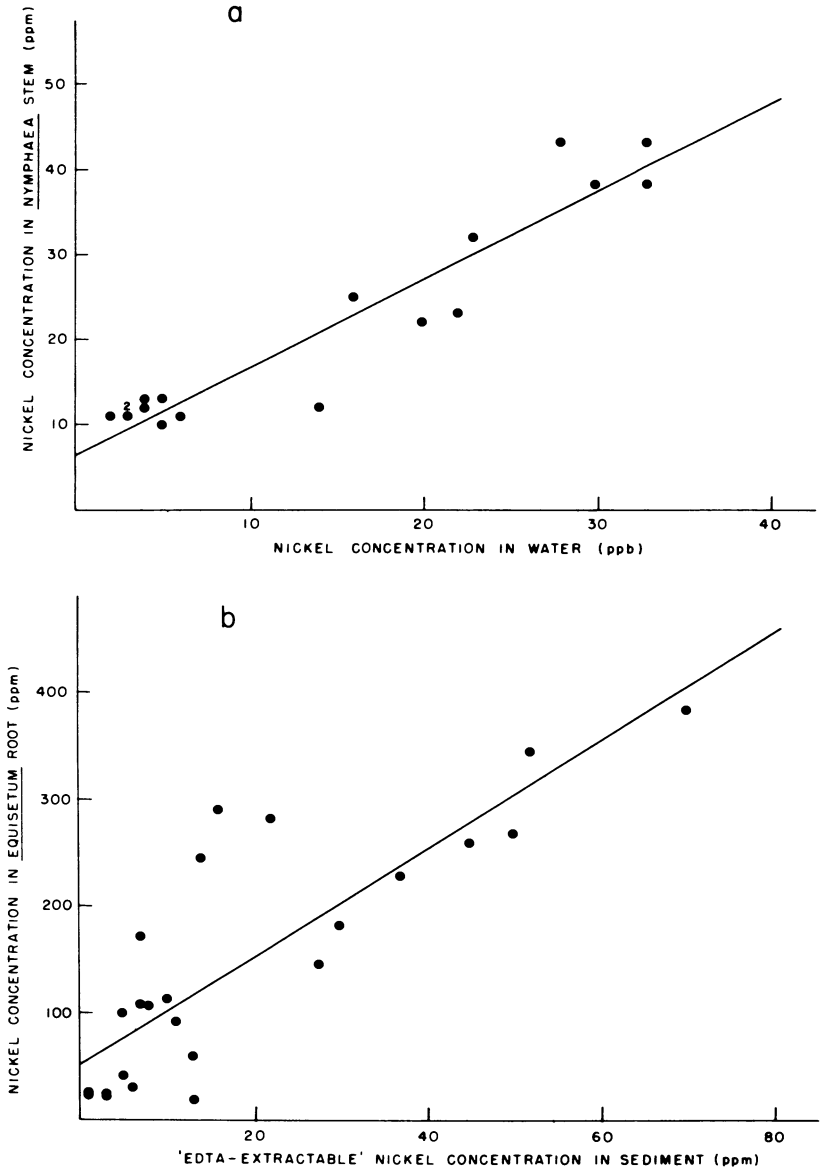

FIG. 6.1 Regression lines of nickel concentrations, in ppm dry weight, of (a) *Nymphaea* stems on the nickel concentration of the water at which the stems were collected, and (b) *Equisetum* roots on the extractable nickel concentration of dried sediments in which they root. Both lines are significant at $p < 0{\cdot}001$. For (a) $r = 0{\cdot}095$, for (b) $r = 0{\cdot}83$.

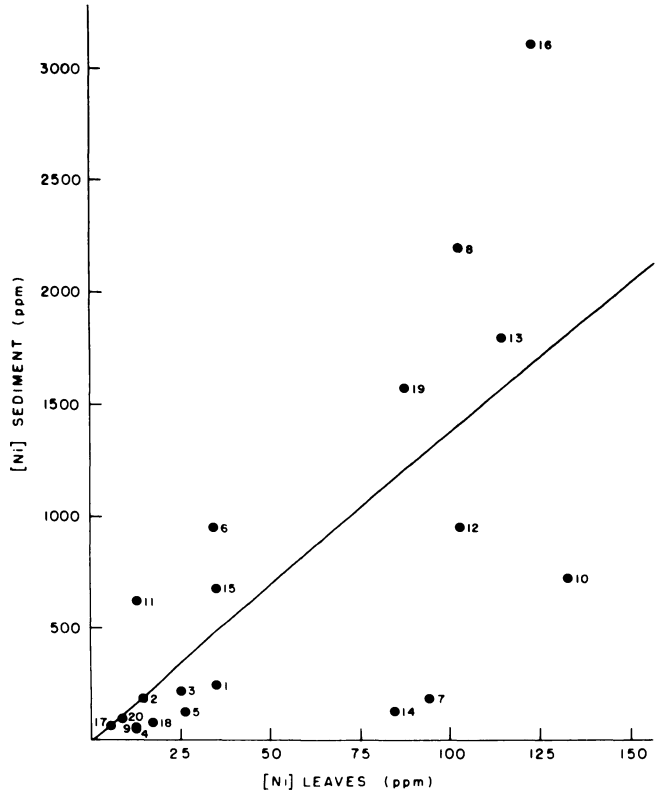

FIG. 6.2 Concentration of nickel in the leaves of *Typha latifolia* in ppm dry weight regressed against the sediment nickel concentration at the 18 sites of collection in the Sudbury area. $p < 0{\cdot}001$, $r = 0{\cdot}72$.

(*Scirpus cespitosus*), 0·46 ppm in timothy (*Phleum pratense*) and 1·9 ppm Ni in red clover (*Trifolium pratense*).

In a series of studies in the Sudbury smelting area of Ontario, nickel concentrations have been determined for vegetation in areas believed to be 'controls', i.e. largely uninfluenced by smelter emissions. At these sites, soil concentrations are also considered background. The data are tabulated in Table 6.1. While levels are generally below 20 ppm Ni, in the Freedman and Hutchinson (1980*a*) data, the high level of nickel in the moss *Polytrichum commune* was notable, and may indicate ion exchange from an aerial source.

Only limited information is available on nickel contents of aquatic

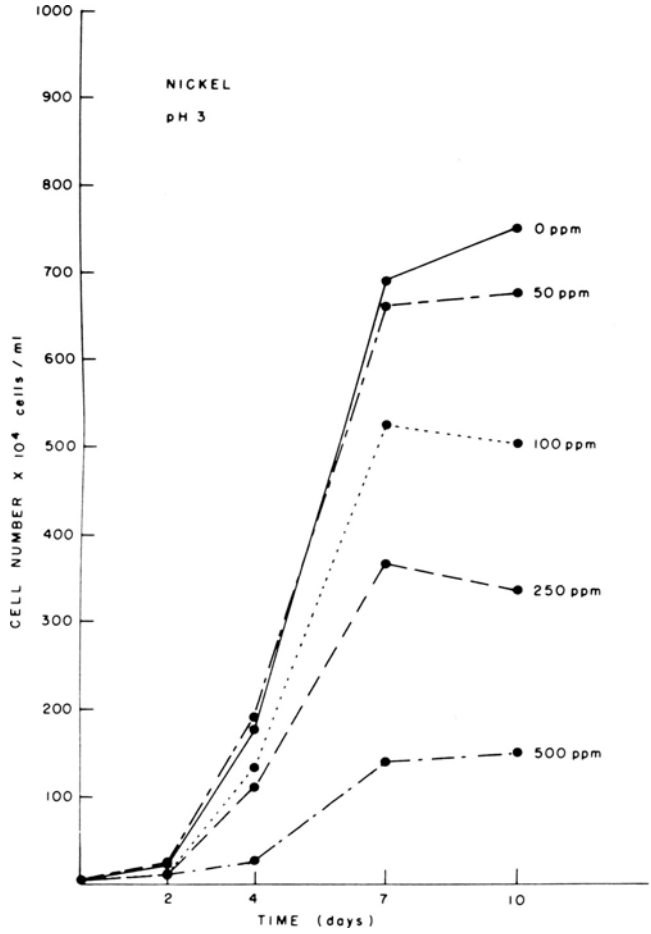

FIG. 6.3 Growth response of *Chlorella saccharophila* collected from a Sudbury area soil to a range of nickel concentrations from 0 to 500 ppm, in a defined algal growth medium (BBM) at pH 3·0. Each point is the mean of three replicate samples from three flasks.

plants. Petkova and Lubyanov (1969) found ash contents of nickel averaging 24·5 ppm for *Elodea canadensis*, *Utricularia* sp., *Ceratophyllum* sp., *Potamogeton* sp. and *Lemna trisulca*, suggesting a dry matter content of 3 ppm. Cowgill (1974) found values of 2·4–4·5 ppm Ni for various parts of the water lilies *Nymphaea odorata*, *Nuphar advena* and *Potederia condata* from Linsley Pond and Cedar Lake, New England, USA.

TABLE 6.2

NICKEL CONCENTRATIONS IN AQUATIC MACROPHYTES AND PERIPHYTON COLLECTED AT 22 SITES, WITH A RANGE OF NICKEL IN WATER FROM 8 TO 42 ppb. DATA ARE GIVEN AS ppm DRY WEIGHT[a]

Species		Range in vegetation (ppm)
Equisetum palustre	roots	24–193
	stems	13–79
Nuphar variegatum	leaves	8–47
	petioles	7–35
	peduncles	3–5
	roots	6–14
Potamogeton sp.	leaves	39–480
	stems	7–255
Anarchis canadensis	leaves	8–510
Periphyton	total plant	43–826

Correlation coefficients of water or sediment nickel with vegetation levels

	r	*p*
Periphyton: nickel in water	0·634	$<0·01$
Eleocharis roots: nickel in sediment	0·54	not significant
Equisetum roots: nickel in sediment	0·54	not significant
Nymphaea roots: nickel in sediment	0·652	$<0·05$
Nuphar roots: nickel in sediment	0·212	not significant

[a] From Hutchinson *et al.* (1975).

In a study of the relationship between water and sediment nickel concentrations and the nickel contents of various aquatic species, it was found that nickel in periphyton was concentrated almost 20 000 times over that of the water, though a significant correlation nevertheless existed between the nickel content of the water at different sites and periphyton contents ($p < 0·01$). Amongst macrophytes, the highest nickel concentration occurred in the leaves of *Potamogeton* spp. with 39 ppm Ni (Hutchinson *et al.*, 1975). Data are summarized in Table 6.2 and Figs. 6.1–6.3.

4. SOIL–PLANT NICKEL RELATIONSHIPS

According to Swaine (1955), soils normally contain from 5 to 500 ppm Ni, with an average of about 100 ppm. Mitchell (1945) divided soils into two groups: (1) those derived from sandstones, limestones or acid igneous

rocks, containing less than 50 ppm Ni, and (2) those soils derived from argillaceous sediments or basic igneous rocks, containing from 5 to more than 500 ppm of nickel. Serpentine soils can also contain up to several thousand ppm of nickel. It is apparent from the literature that the availability of this nickel for plant uptake varies quite markedly between soils and is also substantially plant species specific.

Warren and Delavault (1954) studied the nickel contents of vegetation from a wide range of soils in Canada. They found that generally good correlations existed between soil and plant levels. Halstead *et al.* (1969) examined the effect of soil properties on nickel extractability, using ammonium acetate as an extractant. They also examined the relation between this extraction technique and plant-available nickel, as determined by foliar analyses. They found that both liming and the addition of organic matter reduced nickel availability and extractability from soils, while the addition of phosphate and an increase in pH reduced nickel availability. In a recent study, Haq *et al.* (1980) compared nine different extractants for soil Cu, Ni, Zn and Cd with the concentration of these elements in Swiss chard (*Beta vulgaris*) grown on 46 Ontario soils, these varying in their degree of contamination with metals. The data for nickel and zinc are summarized in Table 6.3. Overall, acetic acid-extractable Ni accounted for 42% of the variability in plant Ni, followed by 38% for a distilled water extract.

TABLE 6.3

LINEAR CORRELATIONS OF PLANT NICKEL AND ZINC, IN FOLIAGE OF *Beta vulgaris*, WITH SOIL NICKEL AND ZINC REMOVED BY VARIOUS SOIL EXTRACTANTS. (MODIFIED FROM HAQ *et al.*, 1980)

Extractant	Correlation coefficient (r^2)[a]	
	Nickel	Zinc
NTA	0·19	0·64
H_2O	0·38	0·35
$HCl + AlCl_3$	0·26	0·53
EDTA	0·17	0·63
DTPA	0·18	0·65
CH_3COOH	0·42	0·60
CH_3COONH_4	—	0·81
$HNO_3 + HCl$ (aqua regia)	0·05	0·31
$(COOH)_2 + (COONH_4)_2$	0·08	0·01

[a] Required r^2 values for significance at 0·05 and 0·01 probabilities are 0·083 and 0·138 for Zn and 0·085 and 0·142 for Ni, respectively.

Approximately 90% of the variability in plant Ni concentration was accounted for if interactions of pH, percentage clay and cation exchangeable capacity with extractable Ni were included in an equation using EDTA as extractant.

Foy *et al.* (1978) emphasized the enhanced nickel availability in acidic soils, as well as the influence of redox, soil adsorption sites and the presence of metal chelating agents on nickel availability. In water culture Proctor and McGowan (1976) found that magnesium additions protected the sensitive oats against high levels of nickel, which would otherwise be toxic. This they related to nickel-toxicity avoidance on serpentine soils. Earlier, Crooke (1956) had shown that in pot experiments, the growth of oats (*Avena sativa*) on serpentine soils was increased by additions of lime, $CaCO_3$ or Na_2CO_3 which increased the soil pH. This enhanced growth correlated with a reduction in ammonium acetate-extractable nickel.

Generally, additions of nickel from sewage sludge, even though much of it is organically bound, leads to an increase in both root and shoot levels of nickel in a wide range of crop plants (e.g. Patterson, 1971; Anderson and Nilsson, 1972; Page, 1974; McLean and Dekker, 1978; Soon *et al.*, 1980). The soils best able to accept nickel-contaminated sewage sludge with a minimal effect on plant uptake of nickel are those of neutral to alkaline pH and with a high organic content.

Even on the same soils, the nickel uptake and concentration in plant foliage can differ markedly between species. Hutchinson and Whitby (1974) report concentrations of 902 ppm Ni in the grass *Deschampsia flexuosa* and 92 ppm Ni in the foliage of *Vaccinium angustifolium* at the same site. Nitric–perchloric acid-extractable nickel in surface soils was 1950 ppm at this site. Petersen (1975) also emphasized species differences in nickel uptake, reporting *Silene maritima* to have 328 ppm Ni in its foliage, compared with 1780 ppm Ni in *Silene acaulis* growing on the same serpentine soil in the Shetlands. In a later paper, Shewry and Petersen (1976) found that levels of nickel in the roots of plants grown on these soils approached or exceeded the soil nickel concentrations.

While both plant foliar and root levels of nickel are generally lower than those in soils, marked exceptions occur with *accumulator* species, of which a substantial number are described. Brooks and Crooks (1980) have described the excellent correlations they found between soil nickel and nickel in the foliage of the nickel-accumulator species *Lychnis alpina* from Europe. On soils high in nickel, the foliage had nickel levels up to twice those of the soil concentrations. Severne and Brooks (1972) and Severne (1974) described the nickel-accumulator *Hybanthus floribundus* from

Western Australia, which had a plant:soil ratio as high as 163. Other genera showed impressive nickel accumulation, with ratios of plant:soil nickel of 54 and 16, respectively, in *H. austrocaledonicus* and *H. caledonicus*.

5. FORMS OF NICKEL IN PLANTS

The forms in which nickel is transported in plants have been the subject of recent studies. Tiffin (1971, 1972) studied the translocation of nickel in the xylem exudate of tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), carrot (*Daucus carota*) and peanut (*Arachis hypogae*). Cut-stem exudate was collected after pre-treatment of plants with from 0·5 to 50 μM Ni, with isotopically labelled ^{63}Ni added. In the nutrient culture all nickel was found as (+)ve charged ion. Low quantities of nickel migrated anodically in tomato exudate, this being presumably bound to an organic carrier. In corn, cucumber and peanut most of the nickel ran anodically and the migration rate of nickel was the same for all these species. The chelate was not identified but was not citrate or malate. This contrasts with iron migration, which is as a citrate or malate complex. The nickel complex was found to be quite stable. The root sap of peanut contained from 14 to 735 μM Ni. Nickel movement as an ionic form seems possible in tomato.

Wiersma and Van Goor (1979) have studied phloem exudate of castor bean (*Ricinus communis*) using both labelled ^{63}Ni and ^{58}Co. The pH of the phloem exudate was 8·2 so that nickel is unlikely to be present as free ions. They found it to be complexed with an organic compound of MW 1000–5000, which is negatively charged. Cobalt gave similar results but not all of it was present as a negatively charged complex. The authors have suggested that polynucleotides or nucleo-proteins may be the complexes involved.

This translocation of nickel via organic complexes has parallels in animal studies. Nomoto *et al.* (1971) examined serum protein from rabbit, which in a survey of mammalian blood had shown the highest nickel concentrations. They found nickel to exist in three forms: (a) as ultrafiltratable nickel (16%), (b) as albumin-bound nickel (40%) and (c) in a nickel–metalloprotein which they named nickeloplasmin (44%). This latter form they showed to migrate as a single protein, and to be a macroglobulin of MW 7×10^5.

In soils, Schnitzer and Skinner (1963, 1967) have studied the stability constants of complexes between fulvic acid and various metals at pH 3·5 and 5·0. Weaker metal–fulvic complexes were formed at the low pH. Nickel formed stronger complexes compared with other divalent cations, except

for copper and iron. The order of stability complexes at pH 3·5 was $Cu^{++} > Fe^{++} > Ni^{++} > Pb^{++} > Co^{++} > Ca^{++} > Zn^{++} > Mn^{++} > Mg^{++}$. In a later study (Schnitzer and Hauser, 1970) it was shown that Al^{3+} also formed a stronger complex with fulvic acid than nickel at low pH.

Wildung *et al.* (1979) have recently published a most interesting account of the occurrence of nickel complexes formed with soil microbial metabolites. Both soil bacteria and fungi, isolated in pure culture from soil on the basis of their metal tolerances and carbon requirements, produced exocellular metabolites which complexed inorganic nickel. A number of different types of complex were identified. These included cationic, anionic and neutral complexes. Out of 239 strains of metal-tolerant bacteria 165 produced nickel-complexing metabolites. These fell into 13 different chemical categories but 136 out of the 169 strains produced complexes of one type. The diversity among the fungi was greater, with the 59 isolates of metal-tolerant fungi all modifying the form of nickel differently. Neutral and anionic nickel complexes with a MW < 1200 from fungal isolates were very mobile in soils, increasing nickel mobility up to 1000 times that of inorganic nickel. A single nickel complex, responsible for the most pronounced increases in nickel mobility, consisted of several ligands capable of complexing nickel independently, and it exhibited both (+)ve and (−)ve charges after separation from the original ligand. These studies of Wildung *et al.* (1979) point to an almost unknown but probably vitally important aspect of heavy metal mobility in soils and the wide range of microbially produced complexing agents.

6. ALGAL RESPONSES TO NICKEL

Algae are known to be rather sensitive to the presence of nickel in water bodies. A review of the literature is given in Spencer (1978). Sparling (1968) studied the effects of copper, zinc, cadmium and nickel on the growth of four genera of blue–green algae: *Anacystis*, *Gleocapsa*, *Merismopedia* and *Nostoc*. He used nickel concentrations of from 0·5 to 10·0 mg/litre and noted decreased growth only at concentrations near 10 mg/litre. This early study, when considered with others over the past 10 years, was early confirmation of the relative tolerance of blue–green algae to elevated nickel levels. It contrasts, for example, with the studies of Hutchinson (1973) on four Chlorophyceae, i.e. *Chlorella vulgaris*, *Haematococcus capensis*, and *Chlamydomonas eugametos* and *Scenedesmus acuminata*. He noted decreased growth between 0·05 and 0·7 mg/litre Ni for all species, except

C. eugametos where a slight stimulation occurred at 0·1 mg/litre, no effect at 0·3 mg/litre and a slight decrease between 0·5 and 0·7 mg/litre. Fezy *et al.* (1979) found the freshwater diatom *Navicula pelliculosa* to be much more sensitive to nickel. In experiments where they added nickel at levels of 0, 100, 300 and 600 μg/litre to cultures at pH 7·2, even the 100 μg/litre concentration ($1{\cdot}7 \times 10^{-6}$ M) reduced the population growth rate by 50%.

The sensitivity of the diatoms (Baccilariophyceae) to nickel has been noted by a number of other workers, including Patrick *et al.* (1975) and Spencer (1978). The relative tolerance of blue–greens (Cyanophyceae) have been noted by Sparling (1968), Spencer (1978), Terlizzi and Karlander (1979) and Patrick *et al.* (1975). The latter group exposed stream communities to nickel concentrations between 0·001 and 1·04 mg/litre and measured the relative abundance of species. They concluded that diatoms were severely affected by increased nickel concentrations, while blue–green algae did not appear to be, in the range of values used. They noted that as the nickel concentration increased, blue–green algae increased in dominance.

Few studies on nickel toxicity to marine algae have been made, though it appears that generally they can tolerate higher levels of nickel than freshwater species. Skaar *et al.* (1974) studied the tolerance to and nickel uptake of the marine diatom (*Phaeodactylum tricornutum*) using radioactive nickel, ^{63}Ni. Growth rate was unaffected when nickel concentrations equalled 500 μg/litre and was only slightly reduced at 1000 μg/litre.

Studies on nickel-tolerant strains and species have been prominent in the past ten years as interest in pollution has increased. In a series of papers (Stokes *et al.* (1973), Hutchinson and Stokes (1975), Stokes (1975*a*,*b*)) the occurrence of nickel-tolerant algal strains in the metal-polluted lakes near to the Sudbury, Ontario smelters have been described. In some of the polluted lakes, nickel concentrations of up to 4·5 mg/litre occur, with in addition, copper up to 0·7 mg/litre. The pH of some of these lakes is acidic, down to pH 4·2. This enhances the heavy metal toxicity, so that a major depletion of the algal flora has occurred. Amongst those few species which have survived are *Scenedesmus acutiformis* var. *alternans*, *Chlorella fusca*, *Euglena mutabilis*. In Stokes *et al.* (1973) comparisons were made between the nickel tolerance of laboratory strains of *S. acuminata* and *Chlorella vulgaris*, with the Sudbury isolates of *S. acutiformis* var. *alternans* and *C. fusca*. Growth of *S. acutiformis* continued in cultures with up to 1·5 mg/litre nickel added. In a later paper Stokes (1975*a*) reported growth at 3 mg/litre nickel. Stokes *et al.* (1973) also noted enhanced tolerances to copper and to silver in the Sudbury isolates. The laboratory strains were inhibited

at 0·1 mg/litre nickel. Recently, Hutchinson and Tam (1981) have isolated another *Chlorella*, i.e. *C. saccharophila* from soils in the Sudbury area, and have found remarkable tolerances to a range of heavy metals, even at low pH. For example, at pH 3·0 and 500 mg/litre nickel, the growth was still 20% of that with no nickel added, Fig. 6.3, and at pH 4·0 and 500 mg/litre nickel, growth was 13% of the control.

In studies of polluted waters in the lower Swansea Valley of Wales, Trollope and Evans (1976) examined metal uptake in the algae growing in local streams. Generally they noted low uptake of nickel even in waters containing up to 2·9 mg/litre. Amongst the species noted in waters containing from 0·1 to 2·9 mg/litre Ni, were *Ulothrix*, *Microspora*, *Oscillatoria* and *Zygnema*, with the former two occurring in waters with highest nickel levels.

The uptake of nickel by algae has also been studied. Skaar *et al.* (1974) noted a nearly linear relationship between uptake per cell and nickel concentration in the external medium for *Phaeodactylum tricornutum*. between 10 and 750 μg/litre Ni. Most nickel uptake occurred within the first 10 h after its initial addition to the medium. They produced evidence that about one-third of the nickel is cell-wall bound. Trollope and Evans (1976) in their Swansea Valley study, found nickel in algae ranging from 0·03 to 1·07 μg/g, with highest concentrations associated with blooms of *Oscillatoria* sp. The high uptake in blue–greens, relative to other green algae, is also reported by Udel'nova *et al.* (1975).

Stokes (1975*a*,*b*) studied nickel uptake in the laboratory and tolerant strains of *Scenedesmus acutiformis*. She found that the ratio of nickel in algae to nickel in supernatant increased as inhibition increased. A concentration factor of approximately 2×10^3 was recorded. The synergistic action of nickel and copper together was emphasized in that the growth effects correlated with enhanced nickel uptake in the presence of copper. In a river system draining the polluted Sudbury area, Hutchinson *et al.* (1975) found the periphyton to show a concentration factor of close to 20×10^3 compared with water levels. Over a range of sites in this river, a significant correlation ($p < 0{\cdot}01$) occurred between periphyton nickel levels and those in the surrounding waters. Trollope and Evans (1976) found a concentration factor of 6×10^3 for *Zygnema*. Both Spencer (1978) and Stokes (1975*a*,*b*) emphasized the initial accumulation of nickel in cell walls, and the subsequent effect of nickel on membrane integrity, affecting the retention of essential metabolites and the ability to assimilate inorganic carbon. Ionic nickel appears to be the toxic form, with chelates and organic matter reducing toxicity.

7. NICKEL IN LICHENS AND MOSSES

Few studies have been made of the response of lichens to nickel. Nieboer *et al.* (1975) pointed out the apparent tolerance of *Stereocaulon paschale* and *Umbilicaria deusta* to the air pollutants in the Sudbury area. These include atmospherically borne nickel and copper. They noted that concentrations of both nickel and copper fell off as a function of distance from the smelters, with the data best fitting reciprocal of distance. At a site 8 km from the stacks, concentrations in *S. paschale* were 300 ppm Ni dry weight. This

TABLE 6.4
BACKGROUND NICKEL CONCENTRATIONS IN BRYOPHYTES (FROM PUCKETT AND BURTON, 1980)

Species	Concentration[a]	Location	Reference
Sphagnum angustifolium	0·3	Canada	Pakarinen and Tolonen, 1976
Sphagnum fuscum	0·2–0·8	Canada	Pakarinen and Tolonen, 1976
Hylocomium splendens	4·6–5·1	Canada	Groet, 1976
Rhacomitrium lanuginosum	3·7	Canada	Rebcz, 1978
Bryophytes (average for 38 spp.)	0·83[b]	USA	Shacklette, 1965
Mosses	0·15–5·0[b]	USA	Shacklette, 1965
Liverworts	0·15–0·7[b]	USA	Shacklette, 1965
Sphagnum fuscum	0·55–2·22	Finland	Pakarinen and Tolonen, 1976
Sphagnum magellanicum	2·5–4·9	Sweden	Ruhling and Tyler, 1970
Pterogonium gracile	6	Denmark	Johnson and Rasmussen, 1977
	On Calcareous Rocks		
Tortella tortuosa	6[c]	Finland	Lounamaa, 1956
Tortella tortuosa	3[c]	Finland	Lounamaa, 1956
Brachythecium glareosum	1[c]	Finland	Lounamaa, 1956
	On Silicic Rocks		
Tortella tortuosa	10[c]	Finland	Lounamaa, 1956
Rhacomitrium lanuginosum	10[c]	Finland	Lounamaa, 1956
Rhacomitrium lanuginosum	3[c]	Finland	Lounamaa, 1956
Hylocomium schreberi	10[c]	Finland	Lounamaa, 1956
Hylocomium splendens	6[c]	Finland	Lounamaa, 1956
Hylocomium triquetrum	3[c]	Finland	Lounamaa, 1956

[a] Data expressed as ppm on a dry weight basis.
[b] Original data expressed as % nickel in ash; ppm/dry weight calculated on the basis of % ash data given for each species.
[c] Original data expressed as ppm in ash: ppm/dry weight calculated on the basis of an assumption that ash weight is 10% of dry weight.

contrasts with mean nickel values obtained for arctic sites by Puckett and Finegan (1980) of 2·5 ± 0·6 ppm for *Cetraria cucullata*, 2·7 ± 0·6 ppm for *C. nivalis* and 2·9 ± 0·9 ppm for *Cladina stellaris*. The ability of lichens to accumulate metals from the air is similar to that reported for mosses, and especially similar to the cation exchange properties of *Sphagnum*. With both lichens and mosses a frequent problem is that of the close adhesion of the plant to the substrate, and the subsequent difficulty of removing substrate particles from the plant before analysis. A summary of background nickel levels recorded for bryophytes is given in Table 6.4.

The property of moss cell walls to accumulate metals has led to the development and use of moss bags for monitoring deposition of metals, especially around man-effected sources (Goodman and Roberts, 1971). *Hylocomium splendens* has a very wide distribution in northern forests so has been used as a monitor of local atmospheric loads (e.g. Ruhling and Tyler, 1970). Nickel is now one of the elements regularly monitored.

One moss which appears to have remarkable tolerance to metal-contaminated habitats is *Pohlia nutans*, e.g. Shacklette (1965), Hutchinson *et al.* (1978). It occurs in highly nickel and copper polluted habitats in the Sudbury region. In 'control' areas the nickel concentration in plants were found to be 0·5–6·0 ppm dry weight. At the polluted sites concentrations as high as 2100 ppm occurred, and the plants were able to grow on soils of low pH, i.e. to pH 3·7 containing up to 6900 ppm nickel.

8. SYNERGISM AND ANTAGONISMS OF NICKEL

While the toxicity of nickel *per se* is well established, it is important to recognize that certain of its interactions with other elements or compounds, both in aquatic and terrestrial systems, can either enhance this toxicity or ameliorate it. The enhancement above a simply additive effect is called synergism and the amelioration to a less than simply additive effect is called antagonism. A number of examples have been described from the literature even though very little research has been done to date on interactions involving nickel. Since in any environment nickel exists along with a wide range of other elements, the probability is strong that many more interactions of a synergistic or antagonistic nature remain to be described.

In aquatic systems, Hutchinson (1973) described experiments testing the toxicity of a range of metals to four green algal species grown in inorganic media. Since copper and nickel are co-contaminants of the Sudbury environment to which the study related, tests were also made of the toxicity of each element at a range of concentrations of the other. Some of the data

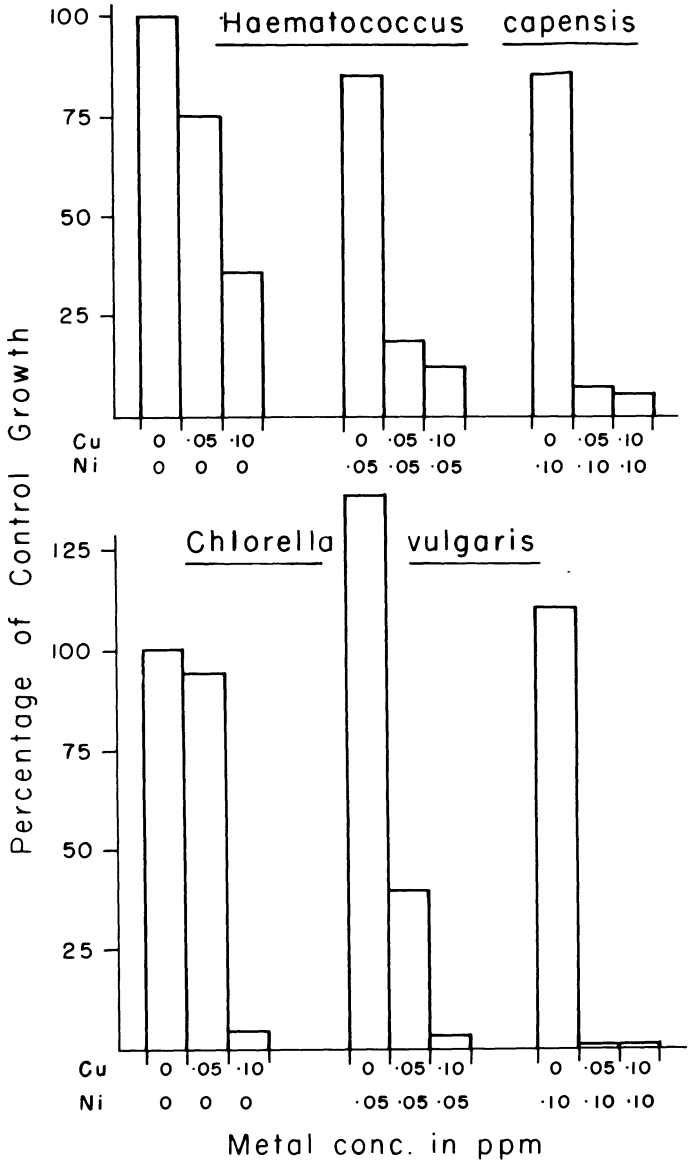

FIG. 6.4 Growth of two laboratory strains of green algae in the presence of 0·05 and 0·10 ppm of both nickel and copper, when present singly and together in a defined medium (BBM). Growth is expressed as percentage of control cultures grown simultaneously. (After Hutchinson, 1973.)

are shown in Fig. 6.4. For *Haematococcus capensis* a concentration of 0·05 ppm Ni caused a reduction in growth compared with control of 17%, while copper at 0·05 ppm caused a reduction of 22%. In combination, the reduction was 82%, which is indicative of a synergistic interaction. A similar situation obtained for *Chlorella vulgaris* was made more dramatic in that 0·05 ppm Ni enhanced growth above that of the control. The degree of synergism appears to relate to the actual concentrations used. *Chlamydomonas eugametos* did not show a synergistic response at 0·05 ppm of the Cu/Ni combination but it did at 0·1 ppm Ni and 0·1 ppm Cu.

In assessing this particular interaction, Stokes (1975*b*) showed that when present together, nickel initially accumulated in the cell walls of *Scenedesmus acuminata* while copper moved readily across the plasmalemma into the cytoplasm. As internal concentrations increased, the copper began to influence the permeability of the plasmalemma, allowing nickel to now flood into the cell. At this point the synergistic response occurred.

A similar nickel–copper synergistic interaction has been described for the floating aquatic fern *Salvinia natans* and for the duckweed *Lemna valdiviana* by Hutchinson and Czyrska (1975).

The amelioration of nickel toxicity by calcium additions have been described for plants growing on serpentine soils, e.g. Crooke and Inkson (1955), though Crooke later (Crooke, 1956) carried out experiments showing that the main effect was that of pH rather than Ca. Proctor and McGowan (1976) found Mg ameliorated Ni toxicity in oats.

In an interesting aside, Foy *et al.* (1978) mention that in higher plants in terrestrial systems, nickel can accentuate sulphur dioxide toxicity, while Ormrod (1977) found experimentally that ozone phytotoxicity was enhanced in solution at nickel concentrations of 10 and 100 μM of $NiSO_4$. These experiments suggest that nickel effects are not only brought about through the root but can take place with gaseous pollutants entering through the stomata.

9. NICKEL IMPACTS FROM SMELTER EMISSIONS

A great deal of research has been carried out on the impacts of nickel emissions from smelters on the local environment, with these studies being especially concentrated on the Sudbury area of Ontario, where very large nickel smelters occur. As in almost all smelter situations, emissions of several metals and gases occur concurrently so that it is difficult to assign

TABLE 6.5

METAL CONTENT OF SUDBURY AREA SOILS COLLECTED AT THREE DEPTHS ALONG TRANSECTS FROM THE CONISTON SMELTER. ANALYSES ARE ON TOTAL ACID DIGESTS, ON SOILS COLLECTED IN 1970

Distance from Coniston (miles)	(km)	Depth (cm)	Nickel (ppm)	Copper (ppm)	Cobalt (ppm)
0·4	0·6	0	1 790	1 370	84
		5	1 040	1 320	52
		10	960	1 450	53
0·5	0·8	0	2 840	1 530	127
		5	2 810	1 240	124
		10	1 520	940	82
0·7	1·0	0	1 170	1 140	59
		5	930	1 200	50
		10	660	950	41
0·95	1·5	0	1 850	2 010	72
		5	1 610	1 860	69
		10	1 160	1 740	6
1·0	1·6	0	2 680	1 290	122
		5	1 710	1 480	88
		10	1 100	1 630	67
1·2	1·9	0	2 160	2 070	85
		5	730	1 770	34
		10	650	1 580	31
2·4	3·8	0	830	1 140	42
		5	840	1 100	58
		10	730	1 170	43
4·6	7·4[a]	0	3 310	1 430	154
		5	2 330	1 620	98
		10	610	940	36
6·5	10·4	0	360	390	33
		5	70	20	24
		10	80	3	24
8·4	13·5	0	100	1 180	6
		5	400	570	6
		10	200	190	6
12·0	19·3[a]	0	650	730	38
		5	550	600	20
		10	200	290	20
31·0	49·8	0	80	30	19
		5	70	30	17
		10	60	20	19

[a] Influenced by more than one smelter. (From Hutchinson and Whitby, 1977.)

particular effects to particular pollutants. At Sudbury, very large quantities of sulphur dioxide have been emitted over the past 80 years, which have devastated the forests of the area. Erosion of soils has also occurred. However, large quantities of particulate emissions have also occurred, these containing especially iron, nickel and copper, as well as smaller quantities of cobalt, lead, arsenic, selenium, silver and zinc (Hutchinson and Whitby, 1974, 1977). Elevations in soil nickel levels and in nickel deposition occur as far as 50–60 km from the three different smelters. Freedman and Hutchinson (1980*a*) calculated that 42 % of the smelter-emitted nickel, 40 % of the copper and 52 % of the iron are deposited within 60 km of the present 340-m-high superstack at Copper Cliff, which went into operation in 1972. Previous to that, emissions were from 210-m and 110-m stacks at the various smelters, and dispersal was less efficient. Consequently, the accumulations in the soils and forest humus layers have been very considerable, and undoubtedly have exceeded toxic concentrations at most sites within about 15 km of the smelters (Costescu and Hutchinson, 1972; Whitby and Hutchinson, 1974; Hutchinson and Whitby, 1974; Freedman and Hutchinson, 1980*a*).

Concentrations in both soils and vegetation decrease with increasing distance from the smelters, and the surface layers of the soil show higher concentrations than those lower in the profile. This is indicative of a surface loading effect from atmospheric separation. The toxicity and indeed the solubility of the three heavy metals: copper, nickel and cobalt, are all accentuated in the Sudbury area by the very strong acidity of the local rainfall, together with the acidifying effects of dry deposition of SO_2. Tables 6.5 and 6.6 show data for 1970 on soil concentrations at distances

TABLE 6.6

REGRESSION AND CORRELATION COEFFICIENTS OF SURFACE SOIL CONCENTRATIONS ON DISTANCE

	Natural log. concentration transformation of regression lines	
Nickel	$y = 2\,199 - 744 \log eX$	$r = 0{\cdot}944$
Copper	$y = 2\,354 - 804 \log eX$	$r = 0{\cdot}777$
Cobalt	$y = 97 - 25 \log eX$	$r = 0{\cdot}655$
Silver	$y = 6 - 1{\cdot}8 \log eX$	$r = 0{\cdot}654$
Lead	$y = 57{\cdot}6 - 10{\cdot}1 \log eX$	$r = 0{\cdot}564$
Iron	$y = 55\,170 - 9\,680 \log eX$	$r = 0{\cdot}526$

All significant at $p < 0{\cdot}05$.

TABLE 6.7

CONCENTRATIONS OF NITRIC–PERCHLORIC EXTRACTABLE NICKEL AND COPPER AND AMMONIUM ACETATE EXTRACTIONS OF SULPHATE, OF SURFACE SOIL ORGANIC HORIZONS (01 + 02 + A1, APPROXIMATE UPPER 7 cm). COLLECTIONS MADE IN 1976 ALONG TRANSECT SSW OF COPPER CLIFF, VALUES ARE THE MEAN ± SD ($n = 16$ REPLICATES PER SITE). DATA FROM FREEDMAN AND HUTCHINSON (1980*a*)

Distance from smelter (km)	Nickel (ppm)	Copper (ppm)	Sulphate (ppm SO_4 sulphur)
3·0	3 000 ± 100	3 700 ± 1 100	150 ± 40
3·5	2 100 ± 1 300	2 700 ± 800	220 ± 20
3·7	2 400 ± 1600	3 200 ± 1 300	170 ± 110
5·5	1 800 ± 800	2 200 ± 600	90 ± 40
6·3	1 300 ± 900	1 400 ± 800	110 ± 40
6·4	680 ± 370	1 400 ± 400	140 ± 30
7·6	1 100 ± 200	1 600 ± 200	120 ± 30
9·5	870 ± 640	1 500 ± 600	100 ± 70
9·8	1 200 ± 500	1 700 ± 400	100 ± 50
10·0	600 ± 200	1 200 ± 500	110 ± 70
12·4	1 400 ± 400	1 600 ± 300	100 ± 60
14·4	960 ± 240	1 000 ± 200	150 ± 60
14·9	690 ± 200	1 100 ± 100	90 ± 40
18·1	680 ± 300	830 ± 330	20 ± 30
21·6	450 ± 200	730 ± 190	10 ± 20
26·5	270 ± 60	320 ± 80	10 ± 10
29·9	320 ± 270	400 ± 400	90 ± 30
34·2	160 ± 40	220 ± 80	30 ± 20
36·5	140 ± 80	170 ± 80	30 ± 30
36·8	240 ± 30	210 ± 50	30 ± 28
39·1	190 ± 70	230 ± 50	20 ± 20
55·0	230 ± 80	140 ± 50	—
63·0	140 ± 30	70 ± 20	—
76·5	100 ± 30	60 ± 10	—

from the now closed Coniston smelter. Data (1976–77) for levels along a transect from the Copper Cliff smelter are given in Tables 6.7 and 6.8. The correlation coefficients in Tables 6.6 and 6.8 show clearly the decline in soil pollution with distance for nickel and copper, while Table 6.8 shows a significant increase in manganese with increasing distance, this being associated with soil organic matter.

The retention of the airborne metals in the upper soil layers is due to metal binding by the humic substances, which act much as an ion exchange column. This has the effect of reducing the rate of loss of toxic metals into the drainage waters but of concentrating the metals in an area of vital

TABLE 6.8

REGRESSIONS AND CORRELATION COEFFICIENTS FOR NICKEL, COPPER, ZINC AND MANGANESE CONCENTRATIONS OF LITTER LAYERS OF FORESTED SITES VERSUS DISTANCE SSE OF THE COPPER CLIFF SMELTER ($n = 24$ SITES). DATA FROM FREEDMAN AND HUTCHINSON (1980*a*)

Site.parameter	Best fit regressions	Correlation coefficient
Nickel	$\log y = -0{\cdot}90 \log x + 3{\cdot}7$	$-0{\cdot}95^a$
	$\log y = -0{\cdot}30\sqrt{x} + 2{\cdot}0$	$-0{\cdot}94^a$
	$\sqrt{y} = -4{\cdot}3 \log x + 16$	$-0{\cdot}94^a$
Copper	$\log y = -0{\cdot}026\sqrt{x} + 2{\cdot}0$	$-0{\cdot}98^a$
	$\log y = -0{\cdot}76 \log x + 3{\cdot}3$	$-0{\cdot}96^a$
Zinc	$y = 0{\cdot}062x + 16$	$+0{\cdot}10$
Manganese	$y = 13x + 210$	$+0{\cdot}62^a$
Sulphate	$y = -3{\cdot}7x + 150$	$-0{\cdot}79^a$

x = Site chemistry, y = distance from smelters in km.
[a] Significant at $p < 0{\cdot}001$.

microbial activity for litter decomposition, nutrient cycling, nitrogen fixation, mycorrhizal formation and seed germination.

The elevation of nickel in the foliage of those plants which have survived the 80 years of fumigations in the inner areas has been reported by various authors (Costescu and Hutchinson, 1972; Hutchinson and Whitby, 1974, 1977; Freedman and Hutchinson, 1980*a*; McGovern and Balsillie, 1973, 1975; McIlveen and Balsillie, 1978). Species differ quite markedly in the extent to which nickel accumulates in the foliage.

The grass *Deschampsia flexuosa* collected at 1·6 km from the Coniston smelter in 1970 had a foliar concentration of 903 ppm Ni, compared with a level of 37 ppm at 49·8 km. At these same two sites, from collections made at the same time, *Betula papyrifera* had nickel concentrations of 148 ppm and 16 ppm, respectively, while the low sweet blueberry (*Vaccinium angustifolium*) had concentrations of 92 ppm and 14 ppm, respectively. Nickel concentrations are quoted as averaging 3 ppm in foliage in unpolluted areas (Bowen, 1966).

Nieboer *et al.* (1975) reported elevated levels of nickel in the two SO_2-tolerant lichens *Stereocaulon paschale* and *Umbilicaria deusta* at sites 8 km from the Sudbury smelters, with levels up to 300 ppm Ni in the former species. The very high nickel levels found by the author in collections of the moss *Pohlia nutans* have also been referred to, while the highest nickel concentration found in the transect survey of Freedman and Hutchinson

(1980*a*) was 620 ppm at a site 5·8 km SSE of Copper Cliff in the moss *Polytrichum commune*.

Despite these high levels, it is clear that foliar levels are generally substantially lower than those of the soils on which the plants grow. The problem of food chain contamination is thus reduced, though small mammals and especially birds may be at risk if feeding substantially in the inner areas around Sudbury.

McIlveen and Balsillie (1977) reported on metal concentrations, including nickel, in fruits and vegetables grown in the Sudbury area. The edible parts of such locally grown vegetables as radish, carrot, beet or potato contained less than 20 ppm Ni. Tomato and cultivated fruits had levels of 9–15 ppm dry weight and < 5 ppm, respectively. At one site near the Falconbridge smelter, washed lettuce foliage collected from a garden in 1971 contained 166 ppm Ni dry weight. In plots established close to the smelters, bean fruits contained from 23 to 96 ppm dry weight, compared with 6 ppm at a control site, radish contained 18 to 74 ppm, compared with 21 ppm at the control site. Although these elevations could be of concern, the great majority of vegetables eaten in the Sudbury area are brought in from elsewhere. No established guidelines or standards for nickel in vegetables exist in Canada.

At other smelters and nickel refineries elevated soil, natural vegetation and crop levels of nickel have been reported. Blauel and Hocking (1974) have studied the decline of the forest and the lichen community at the Thompson nickel smelter in Manitoba. The degree of forest dislocation is very small compared with that at Sudbury. This may relate to a much lower emission of SO_2. They found nickel levels in surface soils (organic layer) of 2030 ppm at 2·4 km compared with 79 ppm at 52 km. The foliar levels in jack pine (*Pinus banksiana*) were 73 ppm Ni at the smelter site and 5 ppm at 52 km. Similar results were reported by Hutchinson (1978).

Temple (1978) studied soils and vegetables near to the Port Colbourne nickel refinery in southern Ontario. Oat crops were known to have shown classical symptoms of nickel toxicity in the 1950s. Nickel concentrations of up to 24 000 ppm were found in organic surface soils 340 m from the refinery. Foliage of *Acer saccharinum* at a site 400 m from the refinery showed acute nickel injury, while Gottfryd (1977) reported up to 600 ppm Ni in foliage of *Solidago canadensis* collected at a site 2·4 km from the refinery. At a muck farm 1 km east of the refinery, surface soils *averaged* 4400 ppm (Temple, 1978). Gottfryd (1977) analysed earthworms (*Lumbricus terrestris*) collected from forest soils near the refinery and found them to contain 2300 ppm Ni dry weight at a site having soil nickel

contents of 3700 ppm. Earthworms from a control site contained 82 ppm Ni in soil containing 92 ppm Ni.

The toxicity of the nickel present in the surface soils at Sudbury to a range of native and cultivated species was established by Whitby and Hutchinson (1974), who both grew the plants on a range of soils under controlled conditions, as well as examining germination and root elongation in distilled water extracts of these same soils. Concentrations of up to 100 ppm in Ni occurred in the water extracts of the most polluted soils. The concentration of copper was also elevated, though to a lesser extent, i.e. up to 49 ppm. Lesser elevations of cobalt also occurred. However, due to the strongly acidifying influence of the smelters the soil pH was as low as 3·0 in the inner areas. This caused high concentrations of water extractable aluminium also to be present, i.e. up to 50 ppm. These Al levels were themselves inhibitory to a wide range of species. Germination was, to a large extent, unaffected by the metal pollution of these soils. When the same species were grown in nutrient solutions with nickel added at concentrations covering the range found in soil extracts, most species were completely inhibited at less than 10 ppm Ni. Levels of only 2 ppm Ni reduced the root elongation of tomato seedlings by 70%. Copper reduced growth by only 30% at 2 ppm, cobalt by 60% at 2 ppm and aluminium by 80% at this concentration. Nickel is the pollutant present at highest concentration in the Sudbury soils and is highly toxic at 1 or 2 ppm. This is the concentration occurring for about 15 km from each of the smelters. The inability of plants to establish in pot experiments utilizing soils from up to 12 km suggests that metal toxicity is a major factor in preventing seedling establishment, while SO_2 phytotoxicity has devastated the mature forest. It was also found that surface soils were the most toxic.

This soil toxicity appears to be due to excessive accumulation of nickel in the roots of the seedlings. In analyses of root and shoot of seedlings of radish, cabbage, tomato and lettuce grown in water extracts of Sudbury soils, whole-seedling nickel concentrations of up to 1050 ppm occurred, and in inorganic nutrient solution, with nickel added at 0, 1, 5, 10, 15 and 20 ppm, root concentrations of 50, 270, 480, 3750, 9800 and 20 580 ppm occurred (Whitby and Hutchinson, 1974). This massive uptake could be anticipated to be lethal, and it was.

The nickel and copper contamination of the area has not been confined to terrestrial systems. Stokes *et al.* (1973) found elevated metal levels in lakes within a few km of the smelter. In some, the metal concentrations were sufficient to have almost eliminated the algal flora. A number of nickel-tolerant algae from these lakes have been referred to previously. In

addition, on the old abandoned Roast Bed near Creighton, Hogan and Rauser (1979) showed clones of the invading grass *Agrostis gigantea* to have nickel-tolerant clones. Cox and Hutchinson (1979, 1980) showed marked nickel tolerance in a number of populations of the grass *Deschampsia cespitosa* which has invaded the Sudbury industrial barrens since 1972. Some of these clones showed stimulation as nickel levels were increased. In recent unpublished studies, Kaiser, Hutchinson and Wright have shown the occurrence of nickel-tolerant ecotypes of *Typha latifolia* and *Phragmites communis*. In the case of *Typha*, the differentiation of populations seems very limited. In both cases, nickel did not inhibit germination.

The spread of the nickel from the Sudbury region through the draining water bodies has also been recorded. In surface sediments of the Wanapitei River, which drains the eastern edge of the Sudbury region, nickel concentrations are elevated to 400 ppm in the upper 15 cm, while elevations can still be noted at the mouth of the French River 65 km away. The water levels of nickel fall from a mean of 42 ppb in the Sudbury area to about 5 ppb at the discharge into Lake Huron. This ongoing nickel and copper contamination is reflected in elevated levels in many of the aquatic macrophytes, periphyton, zooplankton, clams, crayfish and fish (Hutchinson *et al.*, 1975). Sediment concentrations of several thousand ppm were found in lakes close to Sudbury (Allan, 1971).

Nickel is an unusual contaminant of mine tailings and generally is rather low in concentration even in spoil from nickel mines. Levels ranged from 13 to 290 ppm at 10 mine sites examined in the Yukon, NWT and Ontario, with the 290 ppm level occurring at a nickel mine. However, at a recent Yukon site of a now abandoned nickel mine, tailing contained >3000 ppm Ni. The pH of 3·2 also caused a particularly inhospitable location for plant growth (Hutchinson and Kuja, 1979).

10. EFFECTS OF NICKEL ON SOIL MICRO-ORGANISMS: FUNGI

The impact of nickel on soil microbial activity has only been studied to a limited extent, perhaps because elevations in soil nickel levels are somewhat unusual, aside from serpentine soils. Bhuiya and Cornfield (1972) incubated soils with nickel additions alone and with the addition of straw. They found that nickel added at 1000 ppm caused a decrease in the release of CO_2 from the soil. The effect was somewhat greater than an equal

addition of copper. Cornfield (1977) later examined the effect of the addition of 12 different metals separately on CO_2 release during incubation. The soil used was a sandy loam of pH 4·9. At both 10 ppm and 100 ppm Ni, CO_2 evolution was reduced. These are low levels for reduction of microbial activity and do not allow for selection and adaptation of microorganisms in the short time period of incubation. Juma and Tabatabai (1977) found that nickel inhibited the activity of both acid and alkaline soil phosphatases, key enzymes in the mineralization of organic phosphate to plant-available forms.

Other studies have been concerned with impacts of smelter emissions on soil microbial activity. Freedman (1978) and Freedman and Hutchinson (1980*b*) found acid phosphatase activity significantly reduced in soils within 10 km of Sudbury, with activity reduced to less than 50 % of controls at sites closer than 5 km. The phosphatases were extracted from the duff-humus layers. Carbon dioxide evolution was also reduced but not to the same extent as the acid phosphatase activity. The rate of litter decomposition was also reduced at polluted sites. Where regression analyses were performed it appeared that copper accounted for a higher percentage of the CO_2 variability than did nickel, while for acid phosphatase activity, though both metals provided significant correlations, copper was again the best correlated.

Freedman (1978) also looked at the effect of deliberate nickel and/or copper amendments to forest litter collected 35 km from Sudbury. Both metals exerted a negative effect on CO_2 evolution when present in concentrations of 1000 ppm. They also acted synergistically, in that a combined 500 ppm of each metal caused inhibition of CO_2 evolution while neither 500 ppm nickel nor copper separately did so. Fungal isolates were found in even the most polluted soils, though *Penicillium waksmannii* predominated. Carter (1978) also isolated this species from Sudbury soils and showed it to have a high degree of tolerance to nickel specifically.

11. SERPENTINE SOILS AND NICKEL TOXICITY

The overall topic of the ecology of serpentine soils has recently been reviewed in great detail by Proctor and Woodell (1975). They pointed out the complexity of factors which can cause toxicity of serpentine soils and the difficulty of determining specific ones obtained at a particular site. Serpentine soils are extremely diverse, though many do contain elevated levels of nickel, chromium, cobalt and magnesium. One conclusion of

TABLE 6.9

NICKEL CONTENTS OF NATIVE VEGETATION GROWING ON SERPENTINE SOIL (ADAPTED FROM PROCTOR AND WOODELL, 1975). DATA IN ppm DRY WEIGHT

Species	Plant part	Nickel content	Soil nickel	Locality	Reference
Pancheria glabrosa	leaves	86	1 590 total	New Caledonia	Birrell and Wright (1965)
Araucaria mulleri	leaves	20	1 590 total	New Caledonia	Birrell and Wright (1965)
Centaurea paniculata	stems	290	2 600 total	Italy	Minguzzi and Vergnano (1953)
	flowers	42	2 600 total	Italy	Vergnano (1958)
Alyssum bertolonii	stems/leaves	2 264	2 600 total	Italy	Vergnano (1958)
	flowers/fruits	20 405	2 600 total	Italy	Vergnano (1958)
Euphorbia nicaensis	stems/leaves	26	2 600 total	Italy	Vergnano (1958)
Helichrysum italicum	stems/leaves	197	2 600 total	Italy	Vergnano (1958)
Pinus sylvestris	leaves	0–9·2	439 total	Czechoslovakia	Nemec (1951)
Abies alba	leaves	14–15	n.d. total	Czechoslovakia	Nemec (1951)
Pinus sylvestris	leaves	195	260–2 600 total	Czechoslovakia	Nemec (1954)
Betula sp.	leaves	895	260–2 600 total	Czechoslovakia	Nemec (1954)
Salix caprea	leaves	200	260–2 600 total	Czechoslovakia	Nemec (1954)
Sorbus aucuparia	leaves	66	260–2 600 total	Czechoslovakia	Nemec (1954)
Alyssum serpyllifolium subsp. *lusitanicum*	leaves	5 160	245 acetic acid soluble	Portugal	de Sequeira (1969)
Hybanthus floribundus	leaves	10 000	670 total	W. Australia	Severne and Brooks (1972)
Becium abovatum	leaves	261	3 563 total	Rhodesia	Wild (1970)
Becium homblei	leaves	171	5 622 total	Rhodesia	Wild (1970)
Dicoma macrocephala	leaves	1 401	7 373 total	Rhodesia	Wild (1970)
Indigofera setifora	leaves	101	5 750 total	Rhodesia	Wild (1970)

Proctor and Woodell's of relevance to the present subject states: 'There is good evidence for many soils and plants that nickel is an important factor, and equally good evidence in other cases that nickel is of little or no importance'.

Certainly nickel, up to concentrations of several thousand ppm in soil, has been reported from various serpentine areas, e.g. Rhodesia, Scotland, Quebec, Portugal. Much of this nickel is in an unavailable form for plant uptake. Soane and Saunders (1959) reported ammonium acetate-extractable nickel of up to 70 ppm from Rhodesian soils containing up to 4040 ppm Ni, while Spence (1957) and Spence and Millar (1963) reported in excess of 300 ppm Ni in dilute acid extractants of soils from Scotland.

The use of dilute acids for serpentine soils may seriously overestimate the amount of plant-available nickel, as pointed out by Proctor and Woodell (1975). While Spence and Millar (1963) found no toxic symptoms in oats on soils with dilute acid-extracted nickel in excess of 300 ppm, Hunter (1954) reported nickel toxicity symptoms in oats grown in soil containing less than 10 ppm ammonium acetate-extractable nickel.

Soil organic matter plays a crucial role in determining nickel availability on serpentine soils, just as it does on other soils (Halstead, 1968; Halstead *et al.*, 1969). Crooke (1956) found much of the nickel in a peaty serpentine soil to be associated with the organic matter. The role of pH in determining nickel availability is illustrated by the studies of Hunter and Vergnano (1952) who studied the Scottish serpentines of Aberdeen, which are the only serpentines known to cause nickel toxicity. These soils had a pH of 4·5, which is very low for a serpentine and enhances nickel solubility. However, even a high pH cannot always prevent nickel toxicity, as shown by Soane and Saunders (1959) on highly nickeliferous Rhodesian soils which caused toxicity to oats. Mitchell (1945) had earlier deduced from his data that nickel rather than cobalt was the limiting factor for pasture grasses growing on Scottish serpentine soils.

11.1. Nickel Accumulator Species on Serpentine Soils

A representative data set for nickel contents of plants growing on serpentine soils are given in Table 6.9, adapted from Proctor and Woodell (1975). It is apparent that great differences occur between species in their foliar nickel contents. Although soil factors are undoubtedly a major factor, it is also apparent that even on the same soil markedly different plant responses occur. Certain species accumulate nickel levels actually in excess of those of even heavily nickeliferous soils, e.g. *Alyssum bertolonii*, *A. serpyllifolium* subsp. *lusitanicum* and *Hybanthus floribundus*.

Brooks and Crooks (1980) have shown the uptake of nickel, copper and lead by *Lychnis alpina* to be very high, and for nickel a good correlation with soil nickel occurred. *Lychnis alpina* on soils with up to 4100 ppm Ni, had foliar levels of 7000 ppm Ni. Minguzzi and Vergnano (1953) found 10% nickel oxide in the ash of *Alyssum bertolonii* growing on a serpentine soil in Tuscany, while Gambi (1967) showed that large quantities of this nickel accumulate in the epidermis of the stems and in sclerenchyma between the vascular bundles. De Sequeira (1969) identified the closely allied species *A. serpyllifolia* as a nickel accumulator in Portugal, while Malyuga (1964) described a similar situation for *A. murale* from the USSR. This is suggestive of evolution of nickel accumulation and perhaps a nickel requirement rather widely within the genus *Alyssum*.

Hybanthus floribundus from Western Australia is probably the greatest nickel accumulator, with a plant:soil ratio as high as 163 (Severne and Brooks, 1972). Generic evolution of nickel tolerance, accumulation and perhaps requirement is also suggested for the genus *Hybanthus*, in that Brooks *et al.* (1976) also described nickel accumulation in *H. caledonicus* and *H. austro-caledonicus* from New Caledonia, with plant:soil ratios of 16 and 54, respectively. The studies of Severne (1974) were similar to those of Gambi for *Alyssum bertolonii* in that high nickel levels occurred throughout the plant of *H. floribundus*. Jaffre *et al.* (1976) described a New Caledonian species, *Sebertia acuminata*, which is a hyperaccumulator of nickel, containing up to 25% dry weight of nickel in its latex.

While nickel accumulator plants are often also considered to be nickel indicators, this is not always the case. However, from the nickel mineralizations outcropping in Rhodesia, Wild (1970) described the following as specific nickel indicators: *Albizia amara*, *Dicoma macrocephala*, *Barleria aromatica*, *Combretum molle*, *Dalbergia melanoxylon*, *Eminia antennulifera*, *Turraea nilotica* and *Pterocarpus rotundifolius*.

11.2. Population Differentiation to Nickel on Serpentine Soils

Despite the common occurrence of elevated nickel levels in serpentine soils, the evidence of nickel-tolerant ecotypes or races on such soils is rather limited. Such specifically nickel-adapted populations may be the exception rather than the norm. Proctor (1971) using the Wilkins root elongation test, showed that *Agrostis* spp. collected from serpentine soil, nearly all showed some degree of nickel tolerance, though at many of the sites of collection it was not apparent from bioassays, using 'sensitive species' such as oats, that nickel toxicity was a problem. Ernst (1972) used the technique of comparative protoplasmatology to determine the plasmatic resistance of

populations of *Indigofera setifera* in graduated solutions of nickel nitrate. A population from Tipperary Claims, Zimbabwe was highly nickel-resistant but was not found to be tolerant of copper or zinc. However, further evidence from Ernst and from others, suggests that nickel tolerance is not so specific as certain other metal tolerances.

Willett and Batey (1977) determined the effects of metal ions on root acid phosphatase activity of races of six grasses, differing in their tolerance to serpentine soils. While marked differences occurred in the activity of root acid phosphatase to calcium concentrations in the external medium, no differences in response to nickel occurred.

Terlizzi and Karlander (1979) examined algae isolated from serpentine soils in Maryland. A preponderance of blue–green algae, green algae and diatoms occurred, with three of the blue–greens known to be nitrogen fixers. The serpentine soil was unusually low in soil nitrogenase activity due to lack of molybdenum but tolerance to nickel and chromium was shown in these species.

12. NICKEL-TOLERANT ECOTYPES ON NON-SERPENTINE SOILS

A number of examples of nickel tolerance in strains of algae and fungi from the Sudbury smelting area have been referred to already, e.g. Stokes *et al.* (1973), Stokes (1975) and Carter (1978), while the nickel-tolerant populations of serpentine soils have also been discussed. This section will focus on the development of nickel tolerance in higher plants, in response to mine- and smelter-induced elevations of nickel in local soils, and especially the specificity of nickel tolerance in relation to multiple metal tolerances.

In a review of metal tolerance in plants, Antonovics *et al.* (1971) were able to find few examples of evolutionary adaptation of populations with respect to nickel. Part of this is undoubtedly due to the limited number of nickel mines and smelters in the world, compared with, say, copper, zinc or lead mines. It also reflects the relatively low density of professional botanists in many of these locations, such as New Caledonia, Indonesia, Guatemala and even southern Africa.

Populations of *Agrostis tenuis* were collected by Jowett (1958) from a number of mine waste sites in North Wales, at which soil levels of copper, zinc and lead were markedly elevated. In addition, Jowett used a population obtained from Ernst which was collected at a Black Forest site in Germany. At this latter site, the soil analyses showed a nickel concentration of 1150 ppm. These populations were exposed in root elongation tests, by

which clones were allowed to root in calcium nitrate solution containing a range of concentrations of the four metals. Tolerances related to the particular metal elevated at their site of collection were demonstrated. The Black Forest population gave a Tolerance Index of approximately 90 compared with controls with no nickel present. In addition, however, Jowett found that one of the Welsh populations, from Aberllyn, also had a marked but lesser tolerance to nickel. This population was from a zinc spoil heap. Thus, nickel broke the general rule which had been formulated that populations are specifically and separately adapted to different heavy metals.

Gregory and Bradshaw (1965) continued these studies by examining the responses of a much larger number of populations to the four elements lead, zinc, copper and nickel. Though nickel never occurred in higher concentrations than 60 ppm at these collection sites, a number of nickel-tolerant populations were found. In all cases, it was observed that, when nickel tolerance occurred in *A. tenuis*, elevated zinc levels occurred at the collection site. It appears that adaptation to excess zinc in this species confers, as it were 'inadvertently', nickel tolerance. A statistically significant correlation coefficient for nickel–zinc tolerances was reported. It is also apparent that nickel tolerance in the Black Forest population evolved as a result of selection caused by the high nickel levels in the soil.

This rather anomalous position of nickel in terms of the specificity of the mechanism of tolerance, has tended to be confirmed by subsequent studies. Thus Allen and Sheppard (1971) found some elevation in nickel (and lead and zinc) tolerances to be associated with copper-tolerant *Mimulus guttatus*, without these metals being particularly elevated in the soil.

Hogan and Rauser (1979) studied three clones of *Agrostis gigantea* which they have previously shown to be copper tolerant (Hogan *et al.*, 1977). These clones were collected from an old roast bed at Sudbury, which contained high concentrations of both nickel and copper and lesser but elevated levels of cobalt. They found one of the clones to be tolerant to all three metals, while the other two were only tolerant to nickel. One might have anticipated zinc tolerance conferred by the adaptation to nickel, based on the Jowett study, but zinc tolerance was not found in any of the three clones.

To make the evolution of nickel tolerance and the evolution of multiple-metal and co-metal tolerances involving nickel even more intriguing, Cox and Hutchinson (1979, 1980) have reported on responses of Sudbury-area populations of *Deschampsia cespitosa*, and made comparisons with populations from non-polluted areas of Ontario, of the NWT and from

England. The Sudbury populations grow on soils contaminated by aerial deposits of nickel, copper and cobalt, while the acidity of the soil has also mobilized potentially toxic concentrations of aluminium. It was found that a high degree of both nickel and copper tolerance occurred in the Sudbury clones, and that, in addition, aluminium and cobalt co-tolerances occurred, as might have been anticipated. The non-Sudbury populations showed very little copper, nickel or cobalt tolerance, while they also showed significantly less aluminium tolerance than did Sudbury material. However, perhaps in line with Gregory and Bradshaw's observations, the Sudbury population showed significantly enhanced zinc tolerance and, in addition, lead and cadmium tolerance. New data has also shown elevated silver and cobalt tolerances.

In these studies, Cox and Hutchinson (1980) also showed that *D. cespitosa* populations from Sudbury germinate more successfully, grow more vigorously and survive better than does a control population from a non-polluted Ontario site. Hutchinson and Kuja (1979) also reported a superior performance of the Sudbury populations on a wide range of nickel- and copper-contaminated soils and the occurrence of specific nickel toxicity symptoms in the control population on these soils.

Finally, other reports of nickel-tolerant ecotypes have been made. Hutchinson and Wright (1981) have recently shown a positive stimulation to up to 0·5 ppm nickel in a population of *Phragmites communis*, collected from near the Falconbridge nickel smelter, compared with a control population. In this study, nickel did not influence germination. Nickel accumulation was significantly higher in the non-tolerant clones than in the tolerant ones, when nickel was present in solution. Wild and Wiltshire (1971) reported the use of locally adapted populations of *Cynodon dactylon* in revegetating Rhodesian nickel mine dumps, and Peterson (1975) reported but did not present data on nickel ecotypes in *Agrostis stolonifera* and *Silene maritima* from mine sites in Britain.

Clearly, a great deal has been learned about nickel in plants since its initial identification as a plant constituent. The recent upsurge in interest in its possible role in plants, in mechanisms of adaptation to high nickel exposures and the complexities of the co-tolerance role of nickel in multiple-metal exposure for example, ensure that many challenges remain.

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CHAPTER 7

Other Trace Metals

P. J. PETERSON and C. A. GIRLING†

Department of Botany and Biochemistry, University of London, UK

1. INTRODUCTION

There is considerable information published concerning the environmental effects of some of the toxic metals, for example, cadmium, mercury and lead, where their potency has been demonstrated to cause environmental problems. However, there is a growing need to extend our knowledge to encompass the other elements in the Periodic Table which may exhibit an unrecognized toxicity to living organisms. Any element that is biochemically active may be potentially hazardous to crops, livestock, wildlife and man himself. Many factors determine the risk potential of a particular element, whether it is bioaccumulative, mobile, persistent or continually released into the environment. For these reasons it is necessary to provide basic information about the nature of other elements so that adverse effects can be anticipated before they occur.

This chapter aims to outline some of the basic information known concerning a few of the neglected elements, which may be of environmental importance in the future, drawing attention particularly to the factors determining risk potential of the element in terms of the factors outlined above. The elements selected for review include aluminium, barium, beryllium, chromium, cobalt, gold, palladium, platinum, silver, thallium, tin, tungsten, uranium and vanadium. The essential trace elements iron and manganese are briefly discussed for comparative purposes.

† Present Address: Chemistry Division, Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey KT22 7SF, UK.

2. ALUMINIUM

Aluminium is third in order of abundance in the earth's crust but is the most abundant of the metallic elements (Mason, 1966). It is too reactive an element to be found in nature uncombined. The metal was first prepared commercially about 1850 although aluminium-containing minerals such as kaolin (china clay), feldspar, mica, alum, lapis lazuli, as well as complex aluminium oxides and compounds comprising the semi-precious stones, ruby, sapphire, garnet and turquoise, were well known to ancient civilizations.

Bauxite clay is the ore of aluminium and is composed of gibbsite ($8(AlOH_3)$), boehmite ($4(AlO(OH))$), or diaspore ($4(AlO(OH))$). important producers of bauxite are France, Hungary, USA, Guyana, Jamaica, USSR and Australia. Metallic aluminium is produced electrolytically from bauxite and is second only to iron and steel in importance in industry. Aluminium pollution arising from such production is negligible. Aluminium is used commercially in the form of light-weight alloys for aircraft construction, for cooking utensils because of its good thermal conductivity and for transmission lines in view of its good electrical conductivity.

Aluminium, a group 3 element in the Periodic Table, has three electrons in its external quantum shell which are used for forming valency links. It forms many cationic and anionic complexes. No aluminium compound is known in which the valency is less than three. Mason (1966) classes the element geochemically as a lithophile.

Concentrations of aluminium in some coals may range from around 0·4% to 3% (Lim, 1979). Pulverized fuel ash (pfa) arising from the combustion of coal may therefore contain up to 12% aluminium but sufficient soluble aluminium may be released to restrict plant growth (Holliday *et al.*, 1956; Rees and Sidrak, 1956). Under alkaline conditions no aluminium toxicity would be expected; however, indicator studies on clover roots growing in fly ash at pH 8·5 revealed that the root surface was below pH 6, thus giving rise to aluminium toxicity effects (Rees and Sidrak, 1956; Jones, 1961). The concentration of aluminium ions in the aqueous phase of wet pfa depends greatly on pH, which varies from batch to batch and from coal to coal (Collier and Greenwood, 1977*a*,*b*). Under alkaline conditions (pH 9·3) the aluminium concentration was zero, but at pH 4·6 soluble aluminium concentrations reached 18 ppm. Even before this concentration was reached in solution culture experiments, root growth of lettuce was

severely inhibited, indicating that aluminium toxicity may restrict plant growth on pfa dumps.

The average concentration of aluminium in the earth's crust has been calculated to be 81 300 ppm (Mason, 1966). Igneous rocks contain this concentration, shales 80 000 ppm, sandstones 25 000 ppm and limestones 4200 ppm. Soils on average contain 71 000 ppm (Bowen, 1966), high soluble aluminium being recorded in acid soils which is toxic for many plant species. Of the major soil groups, those giving rise to possible toxicities of aluminium are acrisols, ochre and humic andosols, ferralsols, fluvisols and strongly developed planosols (Dudal, 1976). Total soil aluminium ranges from 13·8 % Al_2O_3 for aridisols developed under dry hot climates to 11·7 % Al_2O_3 for oxisols developed under moist hot climates, and to 2·1 % Al_2O_3 for spodosols developed under moist cool climates (Baker, 1976). A recent treatment of the chemistry of aluminium in soils by McLean (1970) is highly recommended as a major reference work.

Reactive aluminium, and thus aluminium toxicity in soil, varies with its form. In aridisols, the aluminium remains predominantly as a part of the primary silicates and silicate clays, while in oxisols much of the aluminium ends up as crystalline gibbsite which is less reactive and becomes toxic to plants only at very low pH (4·0 4·5). Aluminium activity in soils is a function of the soil pH and the amount of exchangeable aluminium in the soil. Aluminium toxicity is particularly severe below pH 5·0 but has been reported at soil pH values as high as 5·5.

Because aluminium is a constituent of soil clay minerals, aluminium toxicity is theoretically possible in most, if not all, soils and can occur when the soil pH decreases to levels low enough to cause the clay mineral structure to decompose (Foy, 1976). When this point is reached, some of the aluminium, formerly a part of the clay particles, migrates to cation-exchange sites on clay surfaces and into the soil solution. Raising the pH of the solution to above 5·2–5·5 usually precipitates aluminium and negates the toxicity. On the basis of total acreage involved, aluminium toxicity is probably more important than manganese toxicity although some soils contain toxic levels of both elements.

The tolerance of plants to aluminium is classified by their growth rates at specified concentrations or activities of aluminium in the soil solution. A method has been developed to screen populations of plants for aluminium response in nutrient culture using $AlK(SO_4)_2 . 12H_2O$ for aluminium treatment at 0–8 ppm level (Reid, 1976; Campbell and Lafever, 1976; Rhue and Grogan, 1976). Solutions are maintained near pH 4–4·5 by daily adjustment

to prevent precipitation of $AlOH_3$. Results obtained from these studies have been compared with screening methods based on acid soils. As aluminium toxicity may not be the only limiting factor in acid soil, and it is difficult to always isolate the aluminium response from manganese, iron, calcium effects, etc., screening methods using soil are not usually precise enough, and the plant parts most directly affected, the roots, are not easily observed.

The most common criterion used to measure aluminium toxicity is to compare either root length or root weight of aluminium-affected plants with control plants grown in the absence of aluminium. Generally, there is a reasonably good relationship between relative root lengths and degree of aluminium insult (Moore *et al.*, 1976). Symptoms also show in the plant tops at a later seedling stage; there is a high correlation between weight of roots and tops of many crops (Reid, 1976). In cereal crops, the root tips turn brown and disintegrate and root growth ceases. These results contrast with the report that aluminium ions at a concentration of 10^{-4} M in nutrient solution at pH 3·6 had a stimulatory effect on root growth of several grasses including *Deschampsia flexuosa* (Hackett, 1962, 1965).

Clarkson and Sanderson (1969) showed that onion root elongation was reduced within 3 h by 10^{-3} M aluminium and stopped completely by 8 h. A cytological examination of the root tip revealed that the number of mitotic figures within the root meristem decreased concomitantly with the decrease in root elongation. Nevertheless, DNA synthesis continued after cell division had halted, but the synthesized DNA had an unusual base composition and was metabolically labile (Sampson *et al.*, 1965). Some effects of aluminium toxicity, particularly root growth, are very similar to symptoms of calcium deficiency (Johnson and Jackson, 1964). Studies on the uptake of calcium by excised wheat roots at pH 4 led to the conclusion that part of the calcium accumulation mechanism was completely inactivated by aluminium at 10^{-4} M or less.

Susceptibility to aluminium toxicity differs widely among plant species. Wallace and Romney (1977) reported that when grown in a nutrient solution containing 8 ppm aluminium, the threshold concentration of aluminium toxicity in rice shoots was about 20 ppm and about 30 ppm in soybeans. Numerous investigators have reported differences in cultivars of various crops in their response to aluminium. Tolerance to aluminium in certain winter barley cultivars was found to be due to a single dominant gene (Reid, 1970). Tolerance was easily transferred and the inheritance was relatively simple. A single dominant gene for aluminium tolerance in wheat was also postulated by Kerridge and Kronstad (1968) but other workers have concluded that tolerance in wheat is not simply inherited.

Most plants contain not more than 200 ppm aluminium but tea (*Camellia sinensis*), an aluminium accumulator, may contain as much as 20 000 ppm in mature leaves (Sivasubramaniam and Talibudeen, 1971). The concentration of aluminium varies considerably with leaf age, maturity and genetics of the plant, rainfall, altitude and soil. Tea leaves accumulate aluminium throughout their life; young leaves contain only about 100 ppm but this increases in old leaves to 5000 16 000 in leaves about to fall. These results indicate that aluminium is not detrimental to this plant but the function, if any, of the large amounts taken up is unknown.

Tolerance of some plant species to aluminium is closely related to their ability to absorb and use phosphorus in the presence of aluminium (Foy and Brown, 1964; Humphreys and Truman, 1964). Sivasubramaniam and Talibudeen (1971) recorded a highly significant relationship between aluminium and phosphorus uptake by tea plants. Since aluminium immobilizes soil phosphorus as insoluble inorganic and organic complexes perhaps such a mechanism is involved in uptake and tolerance within the tea plant. This species also contains appreciable amounts of oxalic acid and polyphenols which form strong complexes with Al^{3+} (which may also be involved in aluminium tolerance) (Jones, 1961).

Another possible mechanism which reduces aluminium toxicity to roots has been reported by Foy *et al.* (1965). An aluminium resistant wheat variety was observed to increase the pH around its roots thereby precipitating soluble aluminium. Foy *et al.* (1967) later reported that aluminium-sensitive wheat and barley cultivars apparently mobilize soil aluminium, and thereby increase its availability by lowering the pH in the root rhizosphere area.

Atriplex and mustard are further examples of aluminium-tolerant plants which are able to accumulate aluminium in their tissues to a concentration of several thousand ppm (Rees and Sidrak, 1961). Members of the families Symplocaceae, Diapensiaceae and most of the Melastomaceae have been known for a good many years to be accumulators of aluminium (Hutchinson, 1943, 1945; Chenery, 1946). The sweat leaf, *Symplocos tinctoria*, can contain over 50% aluminium (ash w.) which is its main inorganic constituent and can be considered as an indicator of high levels of aluminium. Club mosses (*Lycopodium* spp.) and *Ilex aquifolium* have also been reported to be aluminium indicators (Hutchinson, 1943; Malyuga, 1964). Spanish moss (*Tillandsia usneoides*) contains a mean aluminium concentration of 41 000 ppm in the USA but extreme concentrations may exceed 100 000 ppm for plants from certain localities (Shacklette and Connor, 1973).

Despite high levels of aluminium in some plants, such as the sweat leaf, which is browsed by animals, Gough *et al.* (1979) state that there are no reports of animal toxicity owing to naturally occurring aluminium in the environment.

3. BARIUM

Barytes ($4(BaSO_4)$) is an abundant primary barium mineral in some moderate to low temperature veins, with galena, quartz, carbonates and fluorite. It is also found in veins, lenses and as nodules in limestones or marls (Battey, 1972). The rhizopod *Xenophyophora* has been reported to contain barytes in its hard tissues (Bowen, 1966). Barium is concentrated in manganese oxides in the soil and this is in accord with the usually high barium content of sedimentary manganese deposits. Barium can replace potassium, so that barium can be high in micas and potash feldspars, e.g. celsian ($Ba(Al_2Si_2O_8)$), most of them in association with manganese deposits. Witherite ($BaCO_3$), another ore of barium, is rather uncommon but can form deposits in association with barytes (Battey, 1972). Norrish (1975) reports that barytes is soluble enough to cause plants growing on barium-rich soils to be toxic to animals.

Perhaps the most important use of barium is in the manufacture of white pigments for paint and as a filler in paper making. As the industrial uses of barium are relatively few it is not surprising that the barium concentration in municipal sewage sludges show only a small variation (272–1066 ppm dw) (Furr *et al.*, 1976*b*).

Barium at 425 ppm is 14th in order of abundance in the earth's crust and occurs at a higher concentration than most trace elements. Nevertheless barium is apparently not essential for plant or animal growth and many of its soluble salts are relatively non-toxic (Venugopal and Luckey, 1975).

Barium concentrations in coal average around 140 ppm (range 33–750 ppm) (Lim, 1979), and after combustion the element is enriched on the smaller particles of pfa (Campbell *et al.*, 1978). Yellow sweet clover grown on pfa containing 427 ppm barium accumulated only 7·1 ppm barium (dw) which was less than the plants absorbed when growing on soil alone (Furr *et al.*, 1975).

Mean barium concentrations in granite, shales, limestones and sandstones have been reported to be 1220, 580, 120 and 50 ppm,

respectively (Bowen, 1966; Mason, 1966). More detailed analytical studies of barium in rocks in the conterminous USA have been undertaken by Connor and Shacklette (1975). Concentrations of less than 1 ppm were recorded in limestone but a high of 5500 ppm was found in sandstones from the western USA.

Soil barium concentrations have been reported by Bowen (1966) as 500 ppm (range 100–3000 ppm). Similar means have been reported by Connor and Shacklette (1975) for soils in the USA, but extremes of the range have been extended from 15 to 5000 ppm.

The high concentrations of barium in soils have given rise to elevated levels in plants. On a dry weight basis, plants contain about 4–40 ppm barium (Chaudhry *et al.*, 1977). The most famous barium accumulator is the tree *Bertolletia excelsa* which has been reported to contain 0·43% barium (dw) in the seeds, although other trees growing nearby contained 'normal' barium levels (Seaber, 1933; Peterson, 1971). Shacklette and Connor (1973) quote a geometric mean for plants of 390 ppm (ash w.) with a range of 2–70 000 ppm (ash w.). In later work, Connor and Shacklette (1975) summarized barium concentrations in a wide variety of edible crops, cultivated plants and forest species. In general, mean barium concentrations in tree leaves exceeded values for edible crops. Shacklette (1980) has more recently shown that crop species differ in their barium contents, cucumber contains markedly more barium (610 ppm ash w.) than sweet corn (160 ppm ash w.).

Spanish 'moss' (*Tillandsia usneoides*), an epiphyte which must obtain all of its element load from the atmosphere, contains barium concentrations similar to those of ordinary soil-rooted plants (Shacklette and Connor, 1973) which suggests that this element can be readily absorbed by plants. Bowen (1966) quotes higher barium concentrations in 'true' bryophytes compared with other plant orders, but the contribution that soil contamination may make to this total was not assessed.

Although Bowen (1966) and Brooks (1972) mention that barium is moderately toxic to plants there seems little data to support their statements. Chaudhry *et al.* (1977) have examined the effect of soil additions of barium as $Ba(NO_3)_2$ on the growth of crops. They reported that yields were considerably reduced when barium levels in barley leaves and bush leaves reached 1% and 2%, respectively.

In view of the relatively high concentration of barium in plants and soils, and the lack of information about its physiological effects and possible roles at the cellular level, it is suggested that additional research is required on this relatively inert element.

4. BERYLLIUM

Beryllium has one stable nuclide in nature, ^{9}Be, and is classed as a rare element, its scarcity related to the fact that it is unstable in the zone of thermonuclear reactions in stellar interiors. It occurs in silicate minerals, is lithophile or oxyphilic in character, and is concentrated in the upper part of the earth's crust present in pegmatite veins and dykes associated with granite rocks. Beryllium minerals are also found in hydrothermal veins (Day, 1963).

Although beryl has been prized since the times of Ancient Egypt and Greece it is mined in few areas including the Nubian Deserts, parts of India, Brazil and the Congo (Day, 1963).

Beryllium in American coals shows frequent association with vitrain, suggesting that coals derive beryllium from swamp plants or complexes formed between organic matter and beryllium solution (Swaine, 1975). Zubovic (1966) estimates the average organic affinity of beryllium in coal to be 82%. Values of beryllium in Australian bituminous coal range from $<0{\cdot}4$–$8{\cdot}0$ ppm, with a mean concentration of $1{\cdot}5$ ppm (Swaine, 1979). Comparisons with British coal indicate a similar average beryllium concentration of $1{\cdot}8$ ppm, with a range of $0{\cdot}4$–$3{\cdot}0$ ppm (Lim, 1979).

Data for beryllium in plants and soils is very sparse. Brooks (1972) records beryllium in igneous rocks as $4{\cdot}2$ ppm, soil concentrations 6 ppm and plant ash $0{\cdot}7$ ppm. Bowen (1966) provides a value of $<0{\cdot}1$ ppm dry weight for beryllium in terrestrial plants, $0{\cdot}1$–40 ppm in soils and $0{\cdot}0003$ ppm beryllium in fresh waters (Bowen, 1978). Nikonova (1967), during a biogeochemical survey in the southwestern Urals, found levels of up to 10 ppm beryllium in *Vicia sylvatica*, *Aconitum excelsum* and *Calamagrostis arundinacea*.Beryllium was also detected in the wood of birch, larch, aspen and pine. Cannon (1960*b*) determined an average of <2 ppm beryllium in plant ash collected over unmineralized areas from an average of 34 varying types of plant samples. Bryophytes have been analysed for beryllium as biogeochemical indicators of uranium mineralization. Plants collected from streams in mineralized areas on New Zealand were found to contain as much as 109 ppm beryllium (ash w.). Other values ranged from 33 to 65 ppm over known mineralized areas in the watershed (Whitehead and Brooks, 1969).

Although Brooks (1972) has mentioned the severe phytotoxicity of beryllium, beneficial effects on plants have been recorded. Tso *et al.* (1973) noted the 25% increase in yield from *Nicotiana tabacum* grown in solution culture for 2–3 weeks containing 10 mg beryllium at an overall 1 ppm

solution concentration. Tandon and Mishra (1968) found additions of beryllium sulphate to enhance the oxidation of nitrite by *Nitrobacter*. Growth of timothy increased in sandy loam with 10 ppm beryllium added to the soil (Ruhland, 1958). At low beryllium concentrations, germination, nutrient uptake, growth and yield was stimulated, but at concentrations $< 10^{-3}$ M, beryllium was strongly inhibitive. Beryllium was shown to activate both acid phosphatases and inorganic pyrophosphatase and to inhibit alkaline phosphatase at optimum pH values for each enzyme (Horovitz and Petrescu, 1964). Tepper (1972) was able to show that inhibition of alkaline phosphatases was due to replacement of Mg by Be, and also the inhibitory affects of Be on thymidines and DNA polymerase—thereby influencing events leading to DNA synthesis.

The toxic action of beryllium has been demonstrated to depend on soil pH. Beryllium at 1–2 ppm in nutrient solution and acid soils was toxic to crops, but not when crops were grown in soils containing $CaCO_3$; a soluble beryllium salt significantly increased the growth of crops in calcareous soils (pH 7·5–8·0). Beryllium was persistently toxic to crops grown on acid sandy loams (Williams and Riche, 1968). In one calcareous soil addition of 40 ppm beryllium increased the growth of kale, but there was no effect on growth in two other types of calcareous soils, and beryllium treatment damaged plants in light acid soils (Williams and Riche, 1968). Other toxic action of beryllium include effects on root growth; in culture solution 5 ppm beryllium has been shown to decrease the rate of root growth in mustard seedlings by 50% (Williams, 1965). Wallace and Romney (1966) record the inhibition of various enzymes, such as phosphoenol-pyruvate carboxylase in sweet orange leaves, and addition of EDTA, other chelating agents or excess magnesium did not decrease these inhibitory effects. Beryllium showed no evidence of replacing magnesium or manganese in these enzymes, although beryllium did decrease the toxic effects of excess manganese, indicating a beryllium–manganese interaction of some kind. Soluble beryllium inhibited the growth of various crops at concentrations < 2 ppm in nutrient solution and 4% of the cation-exchange capacity of the soil. Beryllium was not readily translocated to the shoot from the roots although leaves accumulated more beryllium than the stems and fruits. Effects of beryllium addition caused an increase in the uptake of phosphorus and decrease in calcium and magnesium in the beryllium-treated plants (Romney and Childress, 1965).

Beryllium and various alloys containing beryllium have a wide usage in industry and commerce. The most significant man-effected output is probably that arising from coal burning where ambient air concentrations

may increase substantially (Schroeder, 1971). In view of the demonstrated phytotoxicity of beryllium and its potentially adverse effects on the respiratory tracts of man and animals, more quantitative data on normal and elevated levels of beryllium in the environment should be collected.

5. CHROMIUM

Chromium, unlike many metals, was unknown to ancient civilizations, possibly because of the scarcity of the various minerals containing it. The only important chromium ore is chromite ($FeCr_2O_4$), found mainly in Russia, Zimbabwe, Turkey and South Africa, although smaller deposits often of little economic importance are found in many other countries. Chromite deposits are known within the USA but these are small and currently not economic to mine.

Chromium is now recognized as an essential trace element to man (Mertz and Schwarz, 1959) but not apparently to plants. The distribution of chromium in the environment and its biological effects have been well reviewed in the National Academy of Sciences, USA publication entitled *Chromium* (Baetjer, 1974). Its biochemistry and physiology have also been the subject of various reviews (Schroeder *et al.*, 1962; Mertz, 1969; Mertz and Roginski, 1971).

Chromium has been used in dyeing, tanning and as a refractory material for the past 100 or so years but it is only within the last two decades that the element has assumed considerable importance, especially in the metallurgical industry. Pollution by chromium and its compounds comes primarily from these industrial processes and product use. Municipal sewage sludges receiving effluent from tanning operations and plating industries may therefore contain elevated levels of the element.

In the earth's crust chromium is fairly abundant, ranking fourth among the 29 elements of biological importance, and is more abundant than many essential elements including cobalt, copper, zinc, molybdenum, nickel and the pollutants lead and cadmium (Baetjer, 1974). Rocks and soils contain varying amounts of chromium. Studies by Shacklette *et al.* (1971) report a soil mean of 37 ppm on the basis of 863 samples. Higher concentrations are found in soils derived from ultramafic igneous rocks, in shales, clays and phosphorites (Bowen, 1966). Spoil derived from an old chromite mine in New Zealand reached 62 000 ppm chromium (ash w.) (Lyon *et al.*, 1970) while mean values for the surrounding serpentine soil were around 10 000 ppm (Lyon *et al.*, 1968).

Serpentine soils throughout the world may contain elevated levels of chromium (Hunter and Vergnano, 1952) but the concentrations are variable, depending on the amounts of chromite present. Soils from various countries contain 1000–3000 ppm (dw) chromium (Shewry and Peterson, 1976) but values of up to 125 000 ppm have been recorded from the Noro chromite mine on the Gt. Dyke in Zimbabwe (Wild, 1974). Values for soluble or extractable chromium are, however, low at around 0·1–1 ppm, i.e. less than 0·15 % of the total chromium. Wild (1974, 1978) concludes that the indigenous vegetation over the highest chromium values does not appear to be different from that on adjacent serpentine soils with relatively low chromium values. Statistical evidence was not produced to substantiate this observation, but if chromium was as toxic as copper or arsenic for example, changes in chromium concentrations would certainly produce very obvious changes in the vegetation and give rise to bare areas. An absence of visible change in the distribution and form of species presumably indicates that the levels of chromium in these plants are not toxic, or that the serpentine flora has evolved chromium tolerance.

Very high amounts of chromium have been recorded in some serpentine plants from New Zealand, e.g. *Leptospermum scoparium* up to 20 000 ppm (ash w.) compared with < 10 ppm for background plants (Lyon *et al.*, 1968, 1971), and in several serpentine endemics from Zimbabwe, e.g. *Sutera fodina* up to 48 000 ppm chromium (ash w.) (Wild, 1974). High concentrations of chromium were not restricted to the leaves but occurred in stems and roots of the plants examined (Wild, 1974). Ratios of mean plant ash concentration to mean soil ash concentrations for six species from New Zealand varied between 0·02 to 0·20, while ratios for Zimbabwean plants were nearer 0·02 on average. Lounamaa (1956) in his work in Finland commented that the chromium contents of soils were about 50 times as high as those in the leaves. Malyuga (1964), on the other hand, showed that the chromium content of Russian plants and soils were approximately similar and near a mean of 1000 ppm (ash w.).

During a biogeochemical survey of several chromium deposits in Russia, Malyuga (1964) showed that the chromium concentration in plants followed fairly well the concentration in soil. Plotting the chromium data on a map clearly indicated the outlines of the ore deposit. Good plant/soil correlations for chromium were also noted by Lyon *et al.* (1968) for some species growing over a serpentine area in New Zealand. The authors concluded that these plants could be useful in biogeochemical prospecting although no significant plant/soil relationship was found in several of the other species examined.

There is evidence for the presence in some soils of chromic (Cr III) and chromate (Cr VI) ions (Soane and Saunder, 1959; Shewry and Peterson, 1976). Chromate is in pH-dependent equilibrium with other forms of Cr VI such as $HCrO_4^-$ and dichromate ($Cr_2O_7^{2-}$), with CrO_4^{2-} being the predominant form at $pH > 6$. Dichromate in soil will tend to be reduced to Cr III by organic matter, making interconversion between the various forms of soil chromium a possibility. Cary *et al.* (1977*b*) concluded on the basis of E_h–pH data that the equilibrium will favour the formation of Cr III rather than Cr VI in most arable soils. Experiments on extraction of chromium-treated soils with different solutions also provided evidence of conversion of Cr VI to Cr III followed by formation of mixtures of hydrated oxides of chromium and iron.

Bartlett and Kimble (1976*a*,*b*) also supported the view that Cr III was the stable form of chromium in soils but their most recent study on fresh field soils, rather than on crushed, dried and stored samples, clearly indicated that Cr III could be readily oxidized to Cr VI (Bartlett and James, 1979). The key to the oxidation appears to be the presence in the soils of oxidized manganese which serves as the electron acceptor in the reaction. They also showed that significant amounts of the chromium oxidized by a soil may remain as Cr VI for several months and can be leached from the soil. It may be concluded that hexavalent chromium is probably the dominant soluble ion in such soils.

The oxidative behaviour of chromium in soils is of environmental importance, since Cr VI is more toxic to plants than Cr III. Landfill disposal of Cr III may therefore present a problem should extensive oxidation take place. Indeed, Bartlett and James (1979) showed that decreased plant growth resulted from Cr VI toxicity following oxidation of Cr III added to a fresh soil.

Hanna and Grant (1962) recorded chromium concentrations in shrubs and trees from 0·2 to 0·6 ppm (dw) which encompasses the mean plant value of 0·23 ppm calculated by Bowen (1966). Schroeder *et al.* (1962) have determined the chromium concentrations in a range of forest and pasture plants. In general these were around 0·4 ppm (dw) but pasture grasses were found to contain more chromium than clover or alfalfa. Acorns contained only 0·02 ppm chromium compared with 0·17 ppm for oak leaves, indicating the poor translocation of chromium to the seed.

Standard kale has been reported to contain 0·308 ppm chromium (dw) (Bowen, 1974). NBS orchard leaves, pine needles and tomato leaves contained higher concentrations at 2·6, 2·6 and 4·5 ppm (dw) respectively, although Renan *et al.* (1979) recorded significantly higher values for these

standard materials. Lichens and mosses have been reported to accumulate more chromium than the leaves of higher plants (Lounamaa, 1956).

Food plants vary considerably in their chromium contents, although the accuracy and reliability of some of the earlier analyses have been questioned (Baetjer, 1974). In general, leaves and stems contain less than 1 ppm (dw) when growing on a normal soil although species differences have been recorded (Cary *et al.*, 1977*a*,*b*). Spinach and maize leaves, for example, contain 4·6 and 10 ppm chromium, respectively (Renan *et al.*, 1979). These latter authors showed that chromium was not distributed uniformly along a maize leaf. In general, the tips contained appreciably more chromium than the mid-section or the stalk area.

Fruits and seeds are especially low in chromium, e.g. fruits 20 ppb and cereal grains 40 ppb (Mertz, 1969). Edible seeds of peas, beans and sweet corn on one site ranged from 10 to 40 ppb (Cary *et al.*, 1977*a*). Buckwheat seed contained only about one-tenth of the chromium in the leaf and stem.

There are conflicting views on the uptake of chromic and chromate ions by plant roots. Bourque *et al.* (1967) working with wheat concluded that chromate but not chromic ions were absorbed by the roots. Myttenaere and Mousny (1974) working with rice have concluded the opposite, and they suggested that chromate may be reduced to chromic before entering the cell. More detailed physiological studies by Shewry and Peterson (1974) and Skeffington *et al.* (1976) have indicated that separate uptake mechanisms exist for the two ions. Chromate uptake was an active process and followed Michaelis–Menten kinetics at low concentration, whereas chromic uptake was passive. Kinetic parameters implied that chromate was being transported via the sulphate carrier.

Transport of chromium up the root is very slow (Skeffington *et al.*, 1976), accounting for the low levels of chromium in the tops of plants. In laboratory experiments, less than 1 % of the accumulated chromium was transported to the shoots (Shewry and Peterson, 1974). Evidently the element enters the vascular tissue with difficulty but once there it can be rapidly transported. However, significantly more chromate than chromic was transported to the shoot. The multiplicity of cation binding sites in cell walls that can absorb chromic ions may well explain why it is not readily transported though apparently readily taken up. This would also explain why Cr–EDTA moves faster than chromic ions in shoots (Myttenaere and Mousny, 1974) as the complex is not retarded by ion-exchange processes.

More chromium was translocated to the tops of wheat seedlings grown in iron-deficient solutions even though they removed much less chromium from the culture solutions (Cary *et al.*, 1977*a*). These authors also found a

relationship between chromium and iron in the tops of field-grown plants and concluded that both elements are translocated by similar or related processes.

Another study on chromium accumulation by crop plants grown in culture solutions also revealed that the roots of each species supplied with chromic ions contained more chromium than those supplied with chromate, but the reverse was found in the shoots (Lahouti and Peterson, 1979). The authors also established that the species differed in their ability to accumulate the element in roots and shoots. Species differences of around 10-fold in the tops and 5-fold in the roots were recorded. Cauliflower seedlings, followed by beetroot seedlings, accumulated the most chromium while mung bean (*Phaseolus aureus*) and barley seedlings accumulated the least. Cary *et al.* (1977*a*) have also noted the pronounced difference in chromium accumulation between species when grown in soil or chromium-supplemented soil. Beetroot plants were again shown to be good accumulators of chromium. Even though the tendency to retain chromium in the roots was common to all species, the leafy vegetables with the highest chromium concentrations in their leaves also contained relatively high concentrations of iron. It is perhaps worth mentioning that during an ultrastructural examination of cauliflower roots supplied with chromate, Lahouti and Peterson (unpublished data) have shown the association of chromium and iron in small electron-dense granules deposited on the cell wall. On the other hand, when roots were grown in chromic solutions, only low concentrations of iron were associated with the chromium-rich granules.

The state of binding of chromium in accumulator plants from serpentine areas is largely unknown. Lyon *et al.* (1969*a*) concluded that the predominant form of soluble chromium in *Leptospermum scoparium* extracts was at the trioxalatochromate III ion. This complex was not present in the xylem sap (Lyon *et al.*, 1969*b*). The transport of chromium as chromate in the xylem sap is therefore analogous to that of sulphate and phosphate which are principally transported as inorganic anions.

The major soluble chromium compound present in extracts of roots and shoots of cauliflower seedlings grown in low levels of chromate or chromic ions was tentatively identified as the trioxalatochromate III ion (Lahouti and Peterson, 1979). Unknown low molecular weight anionic complexes of chromium, not associated with subcellular organelles, have also been reported in leaves of barley (Shewry and Peterson, 1974; Skeffington *et al.*, 1976), lucerne (Blincoe, 1974) and bean (Huffman and Allaway, 1973*a*).

The chemical form of chromium in plants is undoubtedly of considerable

importance in animal and human nutritional studies. The unknown chromium compounds in wheat grains, although present at very low levels, have been reported to be biologically active (Toepfer *et al.*, 1973). Bean leaves, on the other hand, contained higher concentrations of chromium but the element was poorly absorbed by rats (Huffman and Allaway, 1973*a*). The oxalato-complex identified in cauliflower leaves is readily available to animals (Schwarz, 1972; Chen *et al.*, 1973) which may be of considerable nutritional significance for improving the chromium status of animals and man. The form of chromium in the plant may therefore be of greater importance than the total chromium concentration commonly used as a nutritional index.

Whether or not chromium can be considered as an essential element for the growth of plants has been the subject of debate for a number of years. Several early workers (refer Pratt, 1966) have reported that application of potassium dichromate to soil increased the yields of various vegetable crops. More recently, Bertrand and de Wolf (1965, 1968) have found that chrome-alum applications to soil in France increased the yield of potatoes and other vegetables. Improved crop yields following application of chromium have also been reported from Germany, Poland and Russia (Baetjer, 1974). Nevertheless, Huffman and Allaway (1973*b*), in a series of experiments involving growth of five plant species in solutions highly purified to remove chromium contamination, showed that chromium was not required for normal growth and no growth stimulation was measurable with chromium additions. Unfortunately, as they could not reduce the level of contaminant chromium in their solutions below 0·02 ppb and chromium contamination entered their experiments from the atmosphere, the question of essentiality of chromium was not resolved.

Mention has already been made of the greater phytotoxic effects of chromate compared with chromic ions. Perhaps this is related to the higher concentration of chromium found in the tops of plants grown in chromate- rather than chromic-treated soils and solutions (Lahouti and Peterson, 1979). Nevertheless, plants showing visual toxicity symptoms may not contain that much more chromium in their tops than normal plants (Pratt, 1966; Turner and Rust, 1971). Presumably, as chromium is accumulated in the roots, the initial phytotoxic effects take place there rather than in the tops of the plants. Toxic levels in plants have been reviewed by Baetjer (1974) and will not be discussed here.

Elevated concentrations of chromium can be found in several industrial wastes such as pfa, serpentine asbestos mine waste, chromate smelter waste and sewage sludge. The effect on the surrounding vegetation of chromium

discharged into the air from unidentified sources has been observed only rarely (Baetjer, 1974). Leaf necrosis and increased foliar concentrations were noted on vegetables and fruit trees in the neighbourhood of a chromium-plating factory which emitted hexavalent chromium (Desbaumes and Ramaciotti, 1968). The moss *Hypnum cupressiforme* has been used to monitor elevated levels of atmospherically deposited chromium in the region of a Swedish metallurgical factory (Ruhling, 1970). Values as high as 12 000 ppm were recorded, compared with a background value of 10 ppm. No decision was reached on whether the chromium exerted an effect on plant growth.

Pinus ponderosa trees growing on river banks have in fact been used to monitor river concentrations of chromium and other metals (Sheppard and Funk, 1975). Tree ring data appeared to correlate with metals in sediment cores.

Several areas of serpentine tailings from asbestos mines occur in southeast Quebec, Canada; these are largely devoid of vegetation (Moore and Zimmerman, 1977) but the significance of the high concentrations of total chromium present in the material has not been assessed. As the chromium will initially occur as chromite its toxic properties are minimal, but should it be oxidized to hexavalent chromium by processes outlined earlier, the tailings could become very toxic. Chromate smelter waste has been reported to be intensely phytotoxic (Gemmell, 1972).

Chromium may enter sewage treatment works as chromate from the tanning or plating solutions, but is apparently reduced to the chromic form (Baetjer, 1974). Sewage sludge from Britain contained 980 ppm chromium (range 40–8800 ppm) (Berrow and Webber, 1972) and from the USA 3400 ppm (range 200–9100 ppm) (Regan and Peters, 1970) although isolated values up to 14 000 ppm (dw) have been recorded (Furr *et al.*, 1976*b*). The significance of these high chromium levels compared with the other heavy metals present has not been assessed. De Haan (1978), working in the Netherlands, showed that despite high concentrations of chromium in sludge, plant contents were variable. For example, on a sludge containing 1700 ppm chromium, grass contained 67 ppm, while on another sludge containing 1800 ppm chromium the grass contained only 5·6 ppm chromium. Dowdy and Larson (1975) showed that chromium accumulation by barley seedlings was greater on an acid sludge-amended soil than on a calcareous soil.

The concentration of chromium in coal ranges between $< 1{\cdot}5$ to 54 ppm depending on the geographical region, the type of coal, type of mine, etc. (Lim, 1979). Soils around a coal-burning power plant have been reported to

be slightly enriched with chromium compared with the background soil values (Klein and Russell, 1973). The soil enrichments correlated well with wind patterns. Plant materials collected from the contaminated soils were enriched with some elements but no data was presented for chromium as the method of analysis was not sufficiently sensitive to detect the low levels present in the background plants.

The concentration of chromium in pfa has been reported to increase with decreasing particle size (Campbell *et al.*, 1978) but notable differences of view exist in the work of others (Coles *et al.*, 1979). Preferential concentrations on smaller pfa particles implies that chromium is chalcophilic, although Mason (1966) classifies the element as a lithophile. Perhaps the element is best placed in an intermediate group along with elements like barium, beryllium, cobalt, copper, nickel, uranium and vanadium (Coles *et al.*, 1979). Despite the relatively high concentrations of chromium in pfa (167 ppm compared to the control soil of 43 ppm), elevated levels of chromium were not detected in three or more crops grown on pfa-amended soil (Furr *et al.*, 1976*a*). Only slightly elevated levels of chromium (1·8 ppm) were found in yellow sweet clover (*Melilotus officinalis*) harvested from a pfa dump, compared with 0·8 ppm in the control plants. Townsend and Gillham (1975) have reported that the chromium concentrations in barley grain from plants grown on ash were closely similar to concentrations from grain collected from plants grown on soil. Thus, even if considerable quantities of pfa are incorporated into soil, chromium apparently remains largely unavailable to plants. However, long-term leaching and pH changes may affect chromium solubility and hence phytotoxicity, for Phung *et al.* (1979) have shown that chromium solubility from pfa increases with decreasing pH.

6. COBALT

Cobalt has been used to colour glass, pottery and china since about 1450 B.C. when the Egyptians and Babylonians used it extensively. All shades of blue and green can be produced by combining cobalt with nickel, chromium or manganese compounds. It is only in relatively recent times that cobalt has become a metal of importance in industry, especially in the production of alloys used for jet engines, tool steels, magnets and as a catalyst.

On the earth's crust the average concentration of cobalt is 23 ppm

(Mason, 1966). In terms of abundance it is ranked 32nd of all the elements and 19th of the trace elements. The commonest cobalt minerals are smaltite ($CoAs_2$), cobaltite (CoAsS), erythrite ($2(Co_3(AsO_4)_2 8H_2O)$) and lennaeite (Co_3S_4) and the ores are often associated with iron, nickel, copper and silver. Many serpentines are especially rich in cobalt. Principal sources of cobalt are Canada, USSR and especially Zaire.

Cobalt, like iron, forms two normal valence states, cobaltous (Co^{2+}) and cobaltic (Co^{3+}) ions and compounds and a few cobaltites, derivatives of the dioxide CoO_2. The cobaltic ion is a very powerful oxidizing agent, and for this reason its compounds are not very stable, except those complexes which give very small concentrations of the metal ion. The cobaltous ion is therefore the most stable form. Cobalt forms large numbers of chelates with 4- and 6-coordinated structures. Cobalamin or vitamin B_{12} and its derivatives are the only biologically active cobalt complexes where cobalt occurs in the cobaltous state.

Levels of cobalt in coal from the USA range from 1 to 43 ppm with a mean concentration of 0·6 ppm (Lim, 1979). Following combustion, the element is only moderately enriched on smaller fly-ash particles, indicating that cobalt behaves rather more as a chalcophile than a lithophile (Campbell *et al.*, 1978; Coles *et al.*, 1979). Soils around a coal-burning power generating plant have been reported to be enriched in cobalt from atmospheric fallout but the element was not determined in plant materials around the site (Klein and Russell, 1973). Sweet clover, grown on pfa which contains higher concentrations of cobalt than normally found in soil, accumulated twice as much cobalt as control plants grown on soil (Furr *et al.*, 1975).

The essentiality of cobalt for ruminants has been known for many years and in 1948 was shown to be a constituent of vitamin B_{12} which is required by all animals. Holm-Hansen *et al.* (1954) found cobalt to be essential for growth of fresh water blue–green algae and later it was demonstrated that cobalt is required for the symbiotic fixation of nitrogen (Ahmed and Evans, 1960; Reisenauer, 1960). In root nodules the metal requirement is usually associated with the production of cobamide compounds by the rhizobia (Nicholas, 1975). Cobamide co-enzymes are required for metabolic processes of the bacteroides, especially for the normal function of the enzyme methylmalonyl-CoA-isomerase. An inactive isomerase results in failure of the organism to oxidize propionate. It is now known that cobamide compounds are involved in several enzymatic reactions involving group transfer.

When alfalfa was grown in purified nutrient solutions containing

different amounts of radioactive cobalt, the cobalt requirement for nitrogen fixation was met by one part per 10^{11} in the solution, giving plants containing 0·0008 ppm cobalt in their leaves (Wilson and Reisenauer, 1963). The cobalt requirement for nitrogen fixation was only 1/300 of the molybdenum requirement on an atomic basis.

Relatively little is known about the effects of cobalt on non-nodulated plants. Cobalt deficiency has been produced in non-nodulated subterranean clover supplied with nitrate (Wilson and Hallsworth, 1965; Wilson and Nicholas, 1967) and in tomatoes and rubber plants growing in purified sand cultures (Bolle-Jones and Mallikarjuneswara, 1957). Traces of cobamide co-enzymes have been detected by microbiological assay in several plants but it is not known whether these compounds might have originated from microorganisms associated with the plants (Fries, 1962).

The total cobalt content of soils is usually within the range of 1–40 ppm according to Swaine (1955) although Thornton and Webb (1980) quote a wider range of 1–100 ppm for 'normal' soils. Total contents of 20–100 ppm cobalt are found in soils derived from basic igneous rocks or argillaceous sediments, whereas contents below 20 ppm are normal in soils derived from sandstones, limestones and acid igneous rocks (Mitchell, 1945). Mean soil cobalt values reported are 8 ppm (Bowen, 1966) and 10 ppm (Brooks, 1972).

Cobalt deficiency in sheep has variously been reported to occur on pasture soils containing less than 10 ppm total cobalt in the UK (Thornton and Webb, 1980) but Kubota and Allaway (1972) state that 5 ppm appears to separate cobalt-deficient from cobalt-adequate soils in parts of the USA, which is comparable to the value used to identify cobalt-deficient soils in Ireland (Walsh *et al.*, 1956). More detailed discussions on the cobalt contents of rocks and soils of various origins are presented by Mitchell (1945), Latteur (1962), Kubota (1964, 1965), Vanselow (1966) and McKenzie (1975).

Cobalt deficiency in ruminants most commonly occurs on acid, highly leached sandy soils; soils derived from granites; some highly calcareous soils and some peaty soils. Plants on such soils generally contain less than 0·07–0·08 ppm cobalt and serious deficiency conditions occur below 0·04 ppm (Mitchell, 1945; Kubota, 1964). No positive correlation has been shown between total soil cobalt and the amount of cobalt in plants (Alban and Kubota, 1960) but highly significant correlations for EDTA- and acetic-acid-extractable cobalt and the cobalt content of plants have been found over a range of soils series (Mitchell *et al.*, 1957; Alban and Kubota, 1960; Rana and Ouellette, 1968).

The cobalt content of various plant species, when grown under the same

conditions, vary markedly. In general, legumes, cereal forages and most weeds are high in cobalt but grasses and underground portions of vegetables are relatively low in cobalt (Mitchell, 1945; Hill *et al.*, 1953; Kubota and Allaway, 1972). For example, lucerne, clovers and lupins contained 0·3–0·4 ppm cobalt when grown on the same soil with a 17 ppm cobalt content. *Agrostis*, brome grass, timothy and orchard grass contained on average only 0·08 ppm (Latteur, 1962). Range wheat grasses (*Agropyron* spp.) are exceptions and can accumulate up to 23 ppm cobalt (Lambert and Blincoe, 1971).

The swamp black gum (*Nyssa sylvatica*) is another accumulator of cobalt and leaf samples have been reported to contain up to 58·9 ppm cobalt (dw) although other species growing nearby contained less than 1 ppm (Beeson *et al.*, 1955). A more detailed survey revealed cobalt accumulation to 845 ppm while other species again contained less than 1 ppm (Kubota *et al.*, 1960). This cobalt indicator has been useful as a test plant in studying the cobalt status of soils in several geographic regions of the USA (Kubota and Lazar, 1958; Alban and Kubota, 1960). A significant correlation was found between the extractable soil cobalt at a 0–6 or 0–12 in depth and the cobalt concentration in the black gum leaves. Furthermore, the amounts found in this plant, which is easy to collect and identify, have been used as a measure of the cobalt status of other forage species (Lazar and Beeson, 1956).

A variety of factors affect the cobalt status of plants: soil composition, including levels of elements such as manganese and iron; soil moisture and pH; and plant factors such as stage of growth, plant species and type of organ examined are the main factors involved (Mitchell, 1945; Young, 1948; Hill *et al.*, 1953; Latteur, 1962; Kubota *et al.*, 1963; Adams and Honeysett, 1964; Kubota, 1964, 1965; Fleming and Murphy, 1968; Handreck and Riceman, 1969; Whitehead and Jones, 1969; Gille and Graham, 1971). Recent work by McKenzie (1975), Norrish (1975) and others leaves little doubt that the most important factor in the chemistry of soil cobalt and cobalt availability to plants lies in its association with the manganese oxide minerals. Soil pH is probably the next most important single factor in determining levels of soluble cobalt.

Tiffin (1967) reports that cobalt is transported predominantly as the cation (Co^{2+}) in the xylem of tomato plants despite the considerable excess of organic acids in the exudate which bind other metals like iron in an anionic complex. Bowen *et al.* (1962) found that 37% of the cobalt in tomato leaves was soluble in ethanol but its chromatographic properties

were not consistent with its behaviour either as free Co^{2+} or vitamin B_{12}. Water did not leach cobalt from fresh leaves but once dried 75% could readily be leached (Handreck and Riceman, 1969).

The distribution of ^{60}Co in the leaves of five plant species was determined by autoradiographs of pressed dried plants and by measuring the amount of radioactivity in fresh leaves. The most striking feature of the autoradiographs was that certain parts of the plants had considerably more cobalt than others. The element was concentrated in the margins of lucerne, clover and primrose leaves and at the base and extreme tip of the primrose and grass leaves (Handreck and Riceman, 1969). Marginal accumulation might be explained by an increase in the concentration of cobalt in the transpiration stream towards the leaf margins. Accumulation of cobalt at the extreme tips of grass leaves may result from guttation processes since ^{60}Co was determined in the guttation fluid.

Cobalt occurs in high amounts in ultrabasic rocks, where it is associated with olivine minerals. Probably the highest cobalt concentration reported is 2600 ppm in soil from the Shaba province in Zaire (Wild, 1978; Shewry *et al.*, 1979). Serpentine soils in New Zealand can contain up to 1000 ppm cobalt but mean values group around 400 ppm (Lyon *et al.*, 1968; Lyon *et al.*, 1971). Total cobalt concentrations throughout the world are summarized by Proctor and Woodell (1975) who show that serpentine soils generally contain 100 ppm of cobalt or more.

Mean plant accumulation of cobalt varied from species to species in the New Zealand investigation (Lyon *et al.*, 1968, 1971). The ratios of mean plant ash concentration to mean soil concentration ranged from 0·27 for *Myosotis monroi* to 0·87 for *Pimelea suteri*. Furthermore three of the six species examined in detail showed a very highly significant plant/soil correlation for cobalt indicating that the plants could be useful for biogeochemical prospecting. Malyuga (1964) has indeed given several examples in his book of biogeochemical surveys for cobalt/nickel ores in the USSR. Brief exploratory surveys over cobalt anomalies have also been reported from Zimbabwe (Tooms and Jay, 1964).

Duvigneaud (1959*a*,*b*) recognized a number of cobalt endemics, e.g. *Silene cobalticola* and *Crotalaria cobalticola* as well as cobaltophytes, e.g. *Anisopappus davyi* from various cobalt-rich sites in Zaire. *Crotalaria cobalticola*, for example, contained up to 1·8% cobalt (ash w.) in its leaves (Duvigneaud, 1959*a*). Nevertheless, despite high soil cobalt in similar areas in Zaire, Shewry *et al.* (1979) concluded that there was no evidence to support the view that cobalt was an important factor in controlling plant

growth and distribution. Cobalt accumulators such as *Pearsonia metallifera* containing up to 3300 ppm cobalt (ash w.) have also been described from serpentine soils in Zimbabwe (Wild, 1974). What effect these excess levels of cobalt have on the development of endemic plants has not been assessed.

Vanselow (1966) reported that cobalt produces toxicity to plants when the amounts available exceed certain low levels, but that a naturally occurring excess was apparently not toxic. For example, the cobalt endemics and *Nyssa sylvatica* can contain thousands of ppm cobalt in their leaves yet show no toxicity symptoms, whereas, as low a leaf content as 11 ppm in citrus plants produced marked growth depression and chlorosis resembling iron deficiency. Vergnano and Hunter (1952) reported cobalt toxicity in a range of crops growing in nutrient solution and Fujimoto and Sherman (1950) found cobalt toxicity in Sudan grass at 19–32 ppm (dw) when grown on soils fertilized with excess cobalt.

Anderson *et al.* (1973) examined a field crop of oats developed over an ultrabasic area in Australia for cobalt toxicity effects. The concentration of soluble cobalt in the soil solution, centrifuged from the soil at field capacity, ranged from 0·03 to 0·14 ppm and was compared with the concentration known to induce toxicity in oats grown in nutrient solution. At its highest level, cobalt was present at about 1/10 of the level needed to induce toxicity. Analysis of field samples indicated a total cobalt concentration of 14·6 ppm in oat leaves from the centre of the ultrabasic area and Hunter and Vergnano (1952) suggested that concentrations of cobalt greater than 50 ppm in the leaves are needed to induce toxicity. The evidence indicates that despite the high total cobalt levels in the soil, this element was not toxic for the growth of oats.

This element is relatively non-toxic to animals and man, and Gough *et al.* (1979) found no reports of cobalt toxicity attributable to consumption of natural feedstuffs. A deficiency of cobalt is of far greater concern than potentially toxic concentrations in plants.

Cobalt is a biologically active metal directly associated with the health of at least some plants, animals and man. Selective studies are still required to elucidate the role of cobalt in non-nodulated plants and its localization at subcellular levels. Systematic studies are also needed to delineate the role of soil and root rhizosphere microorganisms in the production of vitamin B_{12} and establish whether it is available for plant uptake. This is both a food chain concern and a concern as regards soil productivity. By comparison, present data indicates that environmental hazards associated with cobalt toxicity are minimal.

7. GOLD

Gold has been worked as far back as history records and was probably the first pure metal known to man. Gold mines were plentiful by the first century A.D., occurring in places as far apart as India and Spain. Gold was used for ornamentation, utensils, statues and later as coinage by ancient civilizations. During the mid-1840s, gold was discovered in California and a whole series of 'gold rushes' followed, attracting people from all over the world.

Gold naturally occurs as the native metal, or more usually as telluride ($AuTe_2$), petzite $(AuAg)_2Te$ or sylvanite $(AuAg)Te_2$, normally associated with quartz or pyrites (Goldschmidt, 1958).

Gold deposits occur in belts across the earth's crust in various forms: placers or aluminium quartz veins in sedimentary or igneous formations, banket-pebble beds or conglomerates, or as base metal ore associations. Gold-bearing veins are found in rocks of all compositions and geologic ages, deposited in cavities and associated with rocks such as slates or schists (Goldschmidt, 1958; Mason, 1966).

The greatest gold-bearing regions of the world are the Witwatersrand and the Orange Free State of South Africa. These areas produce over 60% of the total global gold production, although over 75 countries have produced gold since 1952 (Hampel, 1968; Weeks and Leicester, 1968).

Coal deposits are considerably enriched in gold, containing as much as 1 ppm, and recent evidence has suggested that the Witwatersrand gold fields constitute vast populations of pre-Cambrian algae, which were able to concentrate gold from their environment. Pre-Cambrian quartz from the Witwatersrand contains from 3 to 15 ppm gold (Hallbauer, 1975).

Lungwitz (1900) has considered the possibility that plants had a major role in the deposition of gold during geological time. He maintains that periods of dense vegetational growth parallel a decline in gold deposition due to plant gold accumulation; similarly, periods of sparse vegetation coincided with periods of gold deposition. An average gold content for the earth's crust has been given as 1–6 ppb (Jones, 1970). Because gold is siderophilic it is found associated with dibasic rocks rather than granites and is enriched in black shales, deposited in reducing environments rich in organic matter (Goldschmidt, 1958; Mason, 1966).

Values for gold in sea water range between 0·001 and 44 ppb, fresh water concentrations are also within a similar range. Thermal waters arising from great depths where there is a high sulphur content contain comparatively greater gold concentrations (Jones, 1970).

Soil gold concentrations depend upon type and location of the sample, maximum gold occurring in the humic fraction (Goldschmidt, 1937) and 'c' horizon (Razin and Rozhkov, 1963).

Gold has been detected in many plant species in concentrations ranging below detection limits to 36 ppm (ash w.) (Jones, 1970).

Gold can be determined by a variety of analytical methods, choice of method involves consideration of various factors, including range of gold concentration present in the sample, accuracy required, matrix interferences, number of samples, speed of method, availability of equipment and expense. Most methods used for gold analysis are too insensitive to measure gold in samples at the ppb level found in most plant material.

Gold values for the two standard plant reference materials, Bowen's Kale and NBS Orchard Leaves, are 2·4 and 1·6 ppb, respectively (Minski *et al.*, 1977). Warren and Delavault (1950) report values of about 2·5 ppb gold for a range of tree types and other plants in non-auriferous areas of British Columbia, Canada. Analysis of vegetation growing over mineralized areas indicates that plants are able to significantly accumulate gold. Khotomov *et al.* (1966) report a maximum gold concentration of 36 ppm in the ash of *Lagochilus intermedius* from the Kysyl-Kum, USSR. Schiller *et al.* (1973) recorded values of up to 10 ppb dry weight for grass species collected over mineralization in Czechoslovakia, compared to 95 ppb gold on a dry weight basis found in *Festuca* species in mineralized regions of Wales (Girling *et al.*, 1978). *Equisetum* species have been reported to contain 70 ppb gold on a dry weight basis, growing over gold mineralization in parts of British Columbia, Canada (Warren and Delavault, 1950). Girling *et al.* (1978) found high gold concentrations in certain plant species growing in damp or aquatic environments in the gold mineralized regions of the Dolgellau gold belt, Wales, for example, *Mentha aquatica* 50 ppb, *Polygonum amphibium* 47 ppb, *Cirsium palustre* 27 ppb. Aferov *et al.* (1968) reported high gold concentrations in surface waters near to gold mineralization which may explain the observed gold accumulation in these species.

Biogeochemical prospecting (analysis of vegetation in the search for metal deposits) has been used as an alternative to soil analysis with some success (Warren and Delavault, 1950; Warren, 1972; Warren and Delavault, 1957; Warren and Delavault, 1960; Warren and Delavault, 1965). In the case of gold prospecting, high soil arsenic concentrations are often associated with gold deposits and so arsenic has been used as a 'pathfinder' element (Cavender, 1964). In some cases, however, a biogeochemical approach is favoured in the location of anomalies. Preliminary investigations over gold mineralization in Canada have

indicated the potential use of plant gold analysis in the search for anomalies, and its superiority over soil element analysis (Girling *et al.*, 1979). In particular, the cyanogenic plant *Phacelia sericea* has been shown to be an important gold accumulator. Occurring in a widespread distribution in America and Canada, use of this species in the location of new gold deposits could be considerable. Aripova and Talipov (1966) have recorded a specific gold indicator plant from the Tamydynskie Mountains of the USSR. They found *Artemesia terras alba* to be very sensitive to gold anomalies. Several groups of gold dispersion aerosoles were located in the central area of the Kysyl-Kum USSR, where extensive overburden hampered application of ordinary metallometric surveys. Malyuga (1964) determined the amount of gold in the ash of plants growing above mineralized zones to be 125 times greater than the average content in plants for the whole world.

An important factor in natural plant gold accumulation is the ability of certain species to release cyanide into the soil environment, thus rendering normally inert gold metal soluble and available for uptake. Shacklette *et al.* (1970) have reported the effects of flax cyanogenic extracts on plant gold uptake, and other naturally occurring cyanogenic plant species in the field have been shown to contain significantly more gold than non-cyanogenic species growing nearby (Girling, 1978). Addition of *Prunus laurocerasus* extract to gold foil in nutrient solution enabled sufficient gold solubilization to occur after 30 min such that plants grown in the filtrate contained up to 1·3 ppm gold in the shoot on a dry weight basis (Girling, 1978).

Non-cyanogenic plants are also able to accumulate gold and therefore other natural mechanisms must exist to explain this phenomenon. Microorganisms have been considered to play a role in gold solubilization. Korubushkina *et al.* (1974) found certain types of bacteria to be highly active in gold solubilization, suggesting that amino acids, especially histidine, aspartic acid and serine, have a role in gold dissolution. Brokaw (1910) has suggested that a gold chloride complex exists in acidic solutions, $(AuCl_4)^-$, when excess chloride ions and manganese oxides occur. Other chemical mechanisms have been suggested to allow the existence of soluble gold in the natural environment (McCaughey, 1909; Ogryzlo, 1935; Freise, 1931), although all evidence indicates that gold can only exist in solution for short periods of time and cannot migrate substantial distances before it is rendered insoluble.

It has been demonstrated experimentally that gold can exert symptoms of phytotoxicity to *Zea mays* seedlings at solution concentrations of 1 ppm, over a period of several days (Girling, 1978). Leaf wilting occurred during

the early stages of plant growth, followed by leaf discoloration, precipitation of colloidal gold onto the roots and stunted root growth, especially of secondary roots. Plants grown in gold solutions containing less than 0·1 ppm gold for 5 days showed no toxic symptoms. At the toxic threshold of 1 ppm solution gold concentration, root gold concentrations were approximately 100 ppm, dry weight. Gold uptake was demonstrated to be partially dependent on active processes and no significant competitive inhibition of uptake by other selected ions was noted (Girling, 1978). Growth of plants over a range of solution pHs and subsequent harvest showed uptake of gold to be directly dependent on soil solution pH. Parallel addition of equivalent gold species to plant growth solution resulted in maximum plant uptake for the most soluble gold species (Girling, 1978). Once inside the plant, gold becomes mobilized in the transpiration stream, the speed depending on the plant species and gold compound supplied. Maximum gold concentration occurred in the plant root, and radio-tracer gold experiments with *Phacelia* species indicated especial concentration in the leaf tips, with a small percentage of total shoot gold capable of passing from the leaf into the outside environment, a factor of importance in the geochemical cycling of gold. On a cellular basis, the activity of gold has been demonstrated to be dependent upon its chemical nature. For example, gold chloride is readily bound to the cell wall component of the cell, whilst the more soluble cyanide stays largely soluble. Of all the cell organelles, the mitochondria contained the most gold, and some association of gold with nucleic acid components was observed (Girling, 1978). Interaction with pyrimidine nucleotides and inhibition of proteases has been demonstrated (Simkins and Pensack, 1970; Jandl and Simmons, 1957; Davies *et al.*, 1971) and Block *et al.* (1974) found that gold would bind to plasma protein. Accumulation of gold in the mitochondria of kidney cells has been discovered by Stuve and Calle (1969). It has been hypothesized that the inhibitory interaction of gold with those enzymes concerned with breakdown of human cartilage has given rise to preventive gold therapy treatment in rheumatoid arthritis (Simkins and Pensack, 1970). The possibility of gold as an essential element has not been thoroughly explored, as no essentiality for plants or animals has yet been cited.

8. IRON AND MANGANESE

Manganese in group 7 and iron in the transition series of the Periodic Table are two well-known essential elements whose concentrations in soils and

plants and their biological roles have been frequently reviewed. The geochemistry of manganese and iron has been described by McKenzie (1975) and Norrish (1975) and availability, absorption and movement of these elements in plants has been usefully summarized by Loneragan (1975) and Tiffin (1977). Studies on manganese toxicity in crops and in plants on acid soil have been documented by Reid (1976), Devine (1976), Foy (1976), Andrew (1976) and Gough *et al.* (1979). The availability of manganese to plants fits the known solubility of manganese oxides in that it is very dependent on oxidizing conditions and pH. Under acid conditions manganese is sufficiently soluble but deficiencies can occur with a pH of 7–8.

Iron and manganese oxides play an important role in the soil in fixing trace elements such as cobalt, copper, zinc and nickel as well as pollutants like lead (Norrish, 1975). The association of these elements with manganese and iron in soils has important implications for agriculture and plant growth in general. Experiments have shown that the fixation of elements is rapid and that, once fixed, the elements are unavailable to plants. What role these oxides play in regulating the availability of many of the rarer elements is largely unknown.

A continuing supply of iron is required by the plant to maintain proper growth. Any factor which interferes with use of iron, for example, high pH or excess of phosphate, bicarbonate, copper, zinc, cobalt, cadmium, manganese or nickel in the growth medium, may cause iron deficiency (interveinal chlorosis) (Hewitt, 1948, 1953; Chaney and Giordano, 1977). Examples of metal toxic effects are detailed in the relevant sections of this chapter. Probably the most interesting aspect of iron nutrition is that plant species and varieties within species differ in their use of iron. A variety that uses iron under iron stress conditions is called 'iron efficient', a variety that develops iron deficiency is 'iron inefficient'. In iron-efficient plants, iron stress induces changes in metabolism so that hydrogen ions and reductants are released from roots (Brown, 1976). The pH of the root zone is lowered, which favours Fe^{3+} solubility and reduction of Fe^{3+} to Fe^{2+}. These activated factors, in response to iron stress are associated with increased iron uptake by the plant.

The potential use of organic wastes, including sewage sludge and composted refuse, as iron fertilizers has often been postulated as a replacement of inorganic iron or synthetic chelates (Chaney and Giordano, 1977). Many sludges are especially high in iron when $FeCl_3$ is used to precipitate the phosphorus and suspended solids during improved secondary treatment. On the other hand, heavy metals present in the

sewage sludge may eventually accentuate iron deficiency stress even though the deficiency is temporarily corrected (Brown and Jones, 1975). Recent research has provided much information on the waste–soil–plant relationship of various elements including iron and manganese, but little is known about many of the factors controlling these relationships at high levels.

More recently, concern has arisen regarding the effects of an organism's exposure to the more subtle chronic and subchronic concentrations of elements that industrial and other human activities are releasing into the environment. Data on the known toxicity levels of various elements that may reach potentially hazardous concentrations in the environment are presented in this chapter, but the effects on essential iron metabolism are often neglected. It is important that heavy metal pollution effects and trace element requirements be given equal emphasis in studies of element toxicities.

9. PLATINUM AND PALLADIUM

Platinum and palladium are non-essential elements forming compound and complex salts of both a cationic and anionic nature. Platinum has two valency states, divalent and hexavalent; palladium, divalent and tetravalent. There are no reports of these metals involvement in biochemical systems.

Both elements are relatively and absolutely rare, siderophilic in character, and form few compounds in nature with sulphur and metalloids. Background levels of platinum and palladium in igneous rocks average 0·005 ppm and 0·01 ppm, respectively (Bowen, 1966). They are usually found associated with deep-seated magmatic rocks and their weathered counterparts, the serpentines in, for example, South Africa and Zimbabwe. In serpentine areas with chromite mineralization, the platinum content increases from around 0·1 ppm to 2–5 ppm and may reach 50 ppm or more (Yushko-Zakharova *et al.*, 1967). Thiophilic properties of palladium and platinum also lead to their occurrence in sulphide ore bodies, e.g. Sudbury complex, Ontario. In all cases palladium predominates over platinum in these copper–nickel ores; the palladium/platinum ratio ranging from two to three on the average (Yushko-Zakharova *et al.*, 1967). Placer deposits are worked in the USSR and South America, but production is waning from these sites (Day, 1963).

Platinum and palladium have a variety of specialist uses, especially in finely divided forms as catalysts in the chemical industry. In view of their use

as active components of catalytic converters designed to reduce automobile exhaust emissions it can be expected that both metals will assume an increasing importance as contaminants in various ecosystems.

There are few detailed analyses of platinum and palladium concentrations in municipal sewage sludge. Furr *et al.* (1976*b*) report 0·05–0·74 ppm (dw) platinum and 0·32–16·2 ppm (dw) palladium in sludges from 16 American cities. Elevated levels of platinum were notably high in sludges from specific cities but they were not correlated with high palladium localities, presumably indicating different industrial sources for these metals. Availability of these elements to crop plants from sludge-amended soils has apparently not been examined, but in view of the relative concentrations in cow manure, 0·14 ppm platinum and 3·8 ppm palladium, these elements are absorbed by plants to an appreciable extent.

There are few analyses of platinum and palladium concentrations in plants and soils. *Eritrichium chamissonis* plants growing on an ultrabasic complex in North America were reported to contain 4·8 ppm (dw) platinum, and a mixture of *Dryas octopetala* and *Ledum decumbens* contained up to 6·6 ppm (dw) (Rudolph and Moore, 1972). Analyses of the dunite soil show that it contained around 0·4 ppm platinum. If these plant and soil concentrations are typical of the area then plants are capable of concentrating the platinum metal by a factor of about 10.

Platinum has been shown to affect the growth of bean and tomato plants grown in sand culture with added amounts of the element. Bean plants grown in $H_2PtCl_6 . 6H_2O$ at concentrations ranging from 15^{-5} to 3^{-5} M showed inhibited growth, smaller leaf areas, higher osmotic pressure, and have lower transpiration rates, resist wilting for longer than the controls and are less succulent. Tomato plants respond similarly and also show chlorotic lower leaves (Hamner, 1942). Tso *et al.* (1973) showed that platinum and palladium increased the nicotine content of tobacco plants. In neither study were plants analysed for their platinum or palladium contents.

More recently, Pallas and Jones (1978) have studied platinum uptake by nine horticultural crops grown in Hoagland's culture solution containing 0·057, 0·57 or 5·7 ppm platinum at $PtCl_4$. All species accumulated platinum in high amounts in the roots at all concentrations of platinum, e.g. cauliflower and tomato roots contained in excess of 1000 ppm platinum at the 5·7 ppm platinum culture level. Pepper, cauliflower and radish plants significantly accumulated platinum in their tops while the concentration of platinum in the tops of all other plants was below the limit of detection of 1 ppm.

Ridley *et al.* (1977) report the microbiological biomethylation of palladium and platinum although its significance in biogeochemical cycling of these elements and their toxicity to organisms has not been examined. Further studies of the toxicity of these metals to bacteria, fungi and plants, with particular reference to metal species and biotransformations are required.

The results indicate that platinum can enter into food crops that man consumes and in especially high concentrations where root crops are concerned. Where vehicular traffic is heavy and loss from catalytic converters is high, platinum may be introduced into the ecosystem along with lead. Whether such a contamination constitutes a hazard to man remains to be assessed.

10. SILVER

Silver was discovered and recognized as a metal of special merit as early as 3500 B.C. in First Dynasty Egypt. Its value was established as two-fifths that of gold and it has been used as a medium of exchange up to the present day.

The metal silver is widely distributed in nature, a principal component of many minerals, associated with others, as well as occurring as the native metal. Igneous rocks contain on average 0·1 ppm, organic rich shales containing up to 1 ppm. Silver is 63rd in order of abundance in the earth's crust, levels approaching 0·1 g/tonne. Sulphide, telluride and selenide are the most important mineral sources of silver, these are widespread and constitute the most important source of silver to be commercially extracted for the metal. Other minerals contain only small amounts of silver, recovered on extraction of the primary ore in zinc, copper and nickel smelting. Halide minerals are less common sources of silver, found in oxidized zones of ore deposits and at shallow surface depths. Native silver often occurs with halide silver minerals.

Commercial uses for pure silver are limited by its softness. One of the major uses is in the production of very thin films for electroplating. Other uses include manufacture of reflecting mirrors, electrical contacts, alloys, sterling, coinage, jewellery and in dentistry, optics, photography and medicine.

In the natural environment silver normally exists in the lower oxidation state (+ 1), other oxidation states include + 2 and + 3. There are two stable radioisotopes of silver, 107 Ag and 109 Ag in more or less equal proportion

in the natural environment. It is a chalcophilic element (Day, 1963; Laist, 1954).

Brooks (1972) cites a mean soil silver concentration of 1 ppm and a plant ash value of 1 ppm. There is a relatively small amount of analytical data concerning silver in plants. One of the first reports of occurrence of silver in plants was for button mushrooms made by Ramage (1930). He detected between 100 and 500 ppm silver by spectrographic means. Since then other groups have analysed fungi for silver and determined much lower values. Byrne *et al.* (in press) analysed a range of fungi growing in Yugoslavia or Germany on sites free from pollution, by instrumental neutron activation analysis. *Agaricus* species accumulated as much as 133 ppm silver (dw) with a median value of 30 ppm for a range of six species of fungi. *Boletus* and *Lycoperdaceae* also contained significantly elevated concentrations of silver, highest concentrations occurring in the tubules compared to the stalk or upper cap. In a survey conducted in southeast Norway, fungi growing in an unpolluted area contained high levels of silver; *Boletus edulis* containing 2·4 ppm (dw), *Clitopilus prunulus* 5·5 ppm, with higher silver concentrations occurring in the cap compared to other parts of the fungi. Of the fungi analysed there was usually < 1 ppm silver present. A great variation in silver content between different species was noted. Average soil silver concentrations were 1 ppm (Allen and Steinnes, 1978).

The normal range for silver in soils varies from $< 0{\cdot}01$ to 5 ppm with an average of 0·1 ppm, quoted by Boyle (1968), ranging to 0·3 ppm above mineralization. Fresh waters average 0·2 ppb and sea water 0·25 ppb (Boyle, 1968).

Horovitz *et al.* (1974) have reviewed values for silver in various plant species. They cite values ranging from 0·02 to 0·1 ppm (dw) in various fruits and vegetables and cite Kuzina's (1971) work indicating that silver is equally distributed in all parts of the plant. By contrast, Boyle (1968) reports seeds, nuts and fruits to contain greater silver concentrations than other plant parts. Generally, plants contain less than 1 ppm in their ash (Boyle, 1968), although above mineralization this may rise from 10 to 100-fold (Warren and Delavault, 1950). Cannon (1960*b*) reports *Erigonum ovalifolium* to be an indicator plant in geochemical mapping and mineral exploration. Horovitz *et al.* (1974) refer to *Rosa kokanica* and *Lonicera* species as silver accumulators. These authors analysed plants collected from the Botanical Garden of Tubingen University and neighbouring forest areas and determined higher silver concentrations in lower plants, such as the basidiomycete *Clavulina* and the marine alga *Caulerpa*. Two sets of samples analysed by neutron activation analysis in spring and autumn

produced great variation in the silver content of samples, with generally 5–50-fold lower amounts found in September compared to May. Analysis of soil from the same areas of plant collection gave silver values ranging from 0·03 to 0·09 ppm (dw) indicating a high plant:soil concentration factor for some plants; *Clavulina cinerea* containing 16 ppm silver, *Streptomyces arenaca* 7 ppm, *Caulerpa prolifera* 10 ppm and *Sphagnum acutifolium* 2·7 ppm. Some tree species contained high amounts of silver over background areas suggesting a high degree of silver mobility in the soil solution.

Juniperus communis contained silver values ranging from 0·02 to 1·5 ppm growing over soils containing 0·09 ppm. Similarly *Lycopodium circinatum* contained 0·02 to 1·0 ppm where soil values were 0·03 ppm. Bowen (1966) quotes a mean silver value of 0·25 ppm for marine algae and 0·06 ppm for terrestrial plants.

Silver becomes enriched in the humus and organic matter of soils and is bound as humic complexes in silver–organo ligands; for example, silver humates, organic sulphides and other chelated complexes. Silver is bound in coal to organic matter, pyrite and other mineral fractions such as galena, or occurs as the native metal. Swaine (1979) detected a range of values between $<0·2$ and 1·0 ppm silver in coals from New South Wales and Queensland, Australia, levels approaching those of natural vegetation.

Klein and Russell (1973) report values of silver in background soils as 0·247 ppm (mean of 90 samples) compared to 0·272 ppm in enriched soils collected around a power plant on the shore of Lake Michigan. Plants collected from both areas contained no detectable silver. Analysis of a soil profile indicated silver enrichment in the surface 1 cm, but also evidence of leaching into lower depths of 5–10 cm. This indicates formation of soluble silver salts such as sulphates, carbonates and phosphates (Willard-Lindsay, 1979) rather than Ag^+ ion migration, which is considered to bind strongly to organic material.

Ward *et al.* (1977) surveyed soil and plant silver concentrations around mineralized areas of the Maratoto Valley in New Zealand. They found soil silver concentrations ranging from 1·25 to 6·00 ppm over the Silver Queen Reef compared to background concentrations of 0·2 ppm (dw). Around the silver treatment plant values were $<1·0$–3·3 ppm in the soil. Washed leaves of *Beilschmedia tawa* contained up to 1·77 ppm silver and washed twigs 2·37 ppm, compared to slightly lower values from non-mineralized/contaminated areas. Unwashed material from contaminated areas contained higher silver concentrations indicating aerial silver deposition. A range of herbs from the area contained between 0·08 and 0·14 ppm in the

leaves and 0·06–0·12 ppm in the roots. Higher values were found in the leaves of herbs growing near the treatment plant, indicating considerable aerial contamination, which was not completely removed by washing. By contrast, plants analysed from the reef showed higher silver concentrations in the root indicating root absorption as the main means of entry into the plant. Stream sediment values ranged from 0·02 ppm in background areas to 7 ppm in enriched regions.

Smith and Carson (1977*b*) have reviewed silver in the environment and concluded that the highest bio-accumulation factor of 200 occurs by plants in marine and freshwater environments, although plants growing there still generally contain < 1 ppm (dw) silver.

There is no evidence that silver is essential to plants or animals with the single exception quoted by Boyle (1968), that silkworms will not eat mulberry leaves containing no silver.

Silver is considered to be one of the most toxic heavy metal ions, particularly to micro-organisms and fish, however because the silver ion Ag^+ is very easily reduced, it is not readily accessible to living organisms in the natural enviroment (Cooper and Jolly, 1970). Silver sulphide, Ag_2S, is relatively soluble but it has a low ionization potential. AgI is less soluble but biologically more active since it dissociates to form free Ag^+ ions. The toxic action of silver is related to the binding potential of these ions to enzymes and other active molecules at cell surfaces, the lipid phase of cell membranes exerting an important role in absorption of silver ions by living cells (Cooper and Jolly, 1970). Binding of —SH groups in the formation of mercaptides is the principal mechanism of enzyme inhibition (Snodgrass *et al.*, 1960), rather than displacement of metal atoms, although binding is highly reversible. The enzyme yeast alcohol dehydrogenase can be inhibited by addition of small amounts of silver salts, causing irreversible dissociation of the enzyme into four sub-units, and has been used as a basis for detecting silver ions at the 10^{-9} level. The mechanism for the dissociation has been considered to be due to silver replacement by zinc for sites on the enzyme and formation of silver–SH bonds (Townshend and Vaughan, 1970). Other toxic actions of silver ions include complexation with nitrogenous bases on DNA, inhibiting the unwinding and rewinding of DNA during cell replication and transcription *in vitro*. Irreversible binding of Ag^+ to the cell nucleus has also been demonstrated (Witschi and Aldridge, 1968).

Silver has a classic oligodynamic action on bacteria, halting their growth rather than acting as a germicide, but its salts are stimulatory in microbial fermentation (Branham, 1929). Tso *et al.* (1973) have demonstrated a

reduction in yield of *Nicotiana tabacum* on exposure to 1 ppm silver in culture solution after 2–3 weeks. Other authors (Charley and Bull, 1979) have isolated a community of bacteria able to tolerate 100 mM Ag^+. These soil bacteria, particularly *Pseudomonas maltophilia* have a great capacity for silver accumulation, maximum concentrations reaching 3000 mg/g dry weight at an accumulation rate of 21 mg Ag^+ h^{-1}. This tolerance for such high concentrations of silver has been suggested to be due to reduction of the silver ion to metallic silver by enzymatic action. Nielsen and Massey (1940) discuss the toxic action of silver to plants. Silver is especially toxic to seedlings of various plants and retards germination, higher silver concentrations are cited to be fatal to maize (9·8 ppm) and lupins (4·9 ppm). Growth of *Aspergillus niger* was affected by growth in liquid medium silver culture dishes, conidia of *Sclerolinia fructicola* germinated poorly in distilled water containing finely divided silver. The authors' own unpublished work (Girling and Peterson, 1977) with soluble silver salts in water culture has shown that silver can severely affect the growth of 5-day maize and barley seedlings at a solution silver concentration of < 1 ppm in less than 3 days. At the toxic threshold, shoot and root silver concentrations were > 60 ppm and 180 ppm (dw), respectively. Roots appeared short and stunted, with severe necrosis in the leaves. A black precipitate of silver was apparent on the root surfaces and at high silver solution concentrations correspondingly elevated silver levels in the plant roots were attributed to passive silver uptake from the medium.

Ward *et al.* (1979) have carried out experiments with *Lolium perenne* and *Trifolium repens* species. These plants were supplied with silver nitrate in rooting medium at concentrations ranging from 0 to 167 ppm for various intervals of time. Both species accumulated silver in the shoots and roots, root silver concentrations exceeding those of the shoot by a factor of 10. Uptake of silver was shown to be a linear function of substrate silver concentration. *Lolium perenne* accumulated higher silver concentrations than the other species. The authors suggest an exclusion mechanism in the roots of these plants which inhibits movement to the shoot, namely precipitation of silver as phosphate or chloride in the lateral roots as evidenced by the high silver concentrations in these tissues. Lateral root silver levels are more than twice those of the main roots. Previous work by Ward *et al.* (1977) has indicated that these species, when growing adjacent to an operational silver mine, contain considerably more silver in the shoot compared to the root, interpreted as a function of aerial contamination from the treatment plant.

In view of the considerable widespread usage of silver in industry, and the

comparative lack of information about its geochemical cycling, it is suggested that more quantitative and qualitative data is obtained for this rare but biologically toxic metal.

11. THALLIUM

The earth's crust contains approximately 1 g/tonne of the lithophilic element thallium. It is a rare but widely dispersed element, forming monovalent and trivalent compounds and in chemical behaviour resembles the alkali metal cations (Day, 1963; Shaw, 1952). In the natural environment it occurs in isomorphous replacement for K^+ in feldspars and silicates. Thallium is found in all types of hydrothermal sulphide deposits, usually in the disseminated state in low-temperature lead–zinc, antimony–mercury and other deposits.

Deposits high in arsenic are often high in thallium (Velikii *et al.*, 1966). In most natural environments thallium is present as univalent compounds; during rock weathering, ionic absorption on clay sediments produces enrichment in the clay. Thallium becomes enriched in sediments in strong reducing environments where organic matter is accumulated under reducing conditions. In oxidizing conditions thallium Tl^{3+} is precipitated with manganese and iron and removed from solution (Wedepohl, 1972). There are two stable isotopes of this element, ^{203}Tl (29·5%) as well as several short-lived isotopes ^{206}Tl, ^{207}Tl and ^{208}Tl, as decay products in uranium, actinum and thorium series (Day, 1963).

All forms of thallium are sufficiently soluble to cause toxicity to living organisms. Organothallium (3) derivatives are among the most toxic forms. The chemistry, toxicity and pollution potential of thallium is reviewed by Zitko (1975).

Thallium is produced mainly as a by-product of zinc and lead smelting and is used in electrical and electronic applications. Minor uses include as a catalyst in organic synthesis, rodenticide and insecticide (Howe and Smith, 1950; Waggaman *et al.*, 1950).

Coal burning is probably a larger source of thallium loss into the environment than smelting. The average thallium content of bituminous coal has been estimated at 0·7 ppm, occurring in coal in the form of sulphides rather than associated with organic matter (Voskresenskaya, 1968). Davidson *et al.* (1976) quote the highest thallium content in atmospheric emissions from coal burning as 76 ppm present in the finest particulates.

Bowen (1966) gives an average soil thallium concentration of 0·1 ppm although there are few sources of data available. Thallium has been reported to occur in plants at concentrations ranging from below detection limits to several thousand ppm on an ash basis. *Linaria triphylla* is reported to contain 3800 ppm growing in the region of the Alsar ore body in Yugoslavia (Zyka, 1970). Kothny (1969) detected 0·14 ppm (dw) in kale plant material by crystal violet spectrometry, other values of this order for vegetation growing in non-polluted sites are recorded, for example *Melilotus indica* 0·4 ppm, *Papaver escholtszia* 0·8 ppm (ash w.) (King, 1977). Thallium was detected in only 1% of several hundred plant specimens from Colorado, analysed by the US Geological Survey (King, 1977). Values greater than 2 ppm were considered anomalous and ranged from non-detectable up to 100 ppm in mineralized areas. In the Alsar region of Yugoslavia, plants have contained sufficient thallium to prove toxic to animals grazing on them (Zyka, 1970). Other values for thallium in plants are recorded by Smith and Carson (1977*a*).

Thallium demonstrates its toxicity by inhibition of various enzymes in plant and animal systems (Bostian *et al.*, 1975) and also replaces K^+ in activating others (Antia *et al.*, 1972). In seven species of marine algae, L-threonine dehydratase was activated by over 100% with 1% thallium as thallous nitrate.

In some cases, thallium cannot replace potassium, for example in the K–Na pump in frog skin, thallium becomes tightly bound in the membrane and inhibits the action of the pump (Maslova *et al.*, 1971). Various concentrations of thallium have been reported to affect mitosis (Avanzi, 1956), and Von Rosen (1957) has demonstrated that this metal can cause mutagenesis in *Pisum abyssinicum*.

The toxic action of the metal ion is attributable to formation of complexes with phosphate and amino radicals in the chromosome structure, causing it to lose its contractability and ability to enter normal cell division (Von Rosen, 1954). Thallium can bind to the sulphydryl groups on proteins. If high concentrations of sulphur-containing cysteine are administered simultaneously with thallium to plants, cytological damage can be inhibited (Avanzi, 1957), probably because the sulphydryl groups of proteins are protected by formation of thallium disulphide bonds with cysteine. A subsequent dose of sodium selenate after administration of a lethal dose of thallium to rats prevented death (Ganther, 1974), but thallium does not protect against chronic selenosis produced by selenite (Levander and Argrett, 1969).

Stimulatory affects of thallium on micro-organisms have been reported,

thallium at 0·1 ppm concentration in milk (Horn *et al.*, 1936) and 0·08–8·0 ppm thallium in a sugar solution culture promoted growth of *Saccharomyces cerevisiae* (Richards, 1932). Wood (1974) has considered that biomethylation of thallium can occur in the environment, in this event the potential toxicity of thallium may be enhanced.

Thallium normally occurs in very small amounts in the environment, the largest source of pollution arising from coal combustion, where there may be significant enrichment of the element on fly-ash particulates and localized soil contamination. More data is required on the biogeochemical cycle of thallium in order to determine a normal range of values for this element in the environment and hence establish where there is significant contamination.

12. TIN

Tin has played an important role in the development of civilizations, especially economically and culturally, since the beginning of the Bronze Age. The metallurgist who first mixed tin with copper in the proportion of 1:9 to give bronze, made a discovery which changed the course of history. Modern man is conscious of the full importance of tin in present day society; uses including transportation, telecommunications and food preservation. Yet despite its ubiquitous historic use tin has been a neglected element in biological terms. Perhaps this absence of biological interest is related to the fact that most of the industrial nations mine little or no tin. The United States of America, for example, falls into the 'almost no tin' category.

Cassiterite (SnO_2), present in alluvial deposits, is the only tin-bearing mineral of widespread importance. In Bolivia and northeast Siberia, a few tin-bearing sulphides have been recovered in addition to cassiterite (Hosking, 1974). Most of the world's tin is produced in Asia, with Malaysia producing about one-half.

The concentration of tin in shales, igneous rocks and sandstones has been reported by Bowen (1966) to be 6, 2 and 0·5 ppm, respectively. Around 1–10 ppm tin is found in soils (Laycock, 1954; Prince, 1957*b*; Bowen, 1966) and it is strongly absorbed onto the humus fraction. The concentration of tin in coals from various countries ranges from 0·9 to 51 ppm with means around 3–4 ppm (Lim, 1979) which is comparable with the tin concentration in soils. Analytical values for tin in municipal sewage sludges of American cities ranges from 111 to 491 ppm compared with 3·8 ppm in

cow manure (Furr *et al.*, 1976*b*). Although elevated levels were found in specific cities, the importance of this data in terms of plant uptake and movement through food chains seems slight.

Although of the 30 trace metals of possible or probable biological importance tin ranks 21st in the universe, 17th in the geosphere and 12th in the hydrosphere, it is 8th in order of abundance in the body of the American man (Schroeder *et al.*, 1964). Tin was proposed as an essential element for animals by Schwarz *et al.* (1970) yet there is remarkably little information in the literature on the accumulation of tin in plants, microorganisms and animals. Low concentrations of tin, of around 0·01–0·05 ppm, have been reported to stimulate root growth but at concentrations of 5 ppm or more toxic effects have been reported (Cohen, 1940). Several earlier publications have also recorded slight growth stimulations on addition of tin (Cohen, 1940). More recently, Wallihan (1966) has concluded that there is no substantial evidence that tin is essential or beneficial to plants in any way.

Organo–tin compounds have been synthesized by the thousands (Smith and Smith, 1975) because of the strong tendency of tin to make covalent links to carbon. One would therefore, expect tin to occur in all organisms not only in inorganic forms, but also bound through covalent bonds to all of the major carbon compounds such as proteins, nucleic acids, fats and so on.

The two major valence states of tin are stannous (Sn II) and stannic (Sn IV) and with a redox potential of $-0{\cdot}13$ V it places the oxidation–reduction reaction well within the physiological range of enzyme reactions.

Prince (1957*a*,*b*) reported 2·94 ppm tin in corn grain and 6·79 ppm in the cobs compared with 0·4 ppm in the mature leaves. Young leaves and stalks contained 0·82 ppm tin. Schroeder *et al.* (1964) have surveyed the contents of tin in a range of vegetables and cereals grains. They generally found concentrations of the order of 0·1–2 ppm but 9·07 ppm was found in asparagus. Their two soil values were, however, higher than normally reported, 32·71 ppm and 161·94 ppm for the Institute garden and home garden, respectively. They also recorded that tin in tree leaves and herbage varied between 0·5 and 13·2 ppm when collected from soil containing 156·7 ppm tin. Tikhonova and Zore (1968), on the other hand, found lower concentrations, generally less than 0·1 ppm, in vegetables and berries in the USSR. More recently Evans *et al.* (1979) found 0·01 ppm tin in wheat flour and 0·02 ppm in spinach. Bowen (1972) has recorded 0·06 ppm tin in IAEA wheat flour.

Tin in leaves and needles of various trees growing on a sandy loam containing 0·27 ppm tin, varied from 0·26 to 1·6 ppm (Hanna and Grant,

1962). Standard Bowen's kale has been reported to contain 0·16 ppm tin (Evans *et al.*, 1979) but values range up to 0·36 (Bowen, 1974). A series of published values based on a variety of analytical techniques have been summarized by Peterson *et al.* (1979*a*). A concentration of 0·20 ppm would appear to be a reliable value. NBS orchard leaves contain around 0·30 ppm tin (Byrne, 1974).

Lounamaa (1956) studying wild Finnish plants, found that lichens and mosses concentrated tin to higher levels than was commonly found in either the rocks or the other groups of plants examined.

Leaves and twigs were collected from a *Betula* species growing near a mineralized area in southwest England where the soil concentration was 250 ppm and found to contain 1·2 ppm and 0·36 ppm, respectively (Millman, 1957). Lead displayed the opposite relationship, being higher in the twigs than the leaves.

Peterson *et al.* (1976) examined the concentration of tin in a range of plant species collected from tailings of various ages following gravel pump mining operations for cassiterite in Peninsular Malaysia. The tin concentrations in plants from one of the sites showed a difference in species accumulation, while between-site differences were also apparent. The fern *Gleichenia linearis* contained high concentrations, a value of 32 ppm being the highest recorded from a land plant. Another fern species also contained higher concentrations than angiosperm species. Calculated on an ash weight basis *G. linearis* contained 326 ppm tin which exceeds the values of 12–84 ppm (ash w.) tin recorded for six accumulators in the classical study of Sarosiek and Klys (1962). Despite these relatively high values, tin is not accumulated to extreme levels as has been commonly found for some elements such as selenium and arsenic (Peterson, Benson and Zieve, Chapter 8).

Peterson *et al.* (1979*b*) have also examined the concentration of tin in the leaves of mangrove species collected from several rivers in Malaysia. Species concentration differences were again recorded. Samples collected from mangroves growing in waters and muds draining tin-rich areas contained elevated levels of tin compared with plants from background sites. A comparison of the concentration of tin in mangrove leaves compared with the surrounding water gave a concentration factor of approximately 4000. It was suggested that mangroves could be used for biogeochemical prospecting for tin.

Analysis of detritus collected from mangrove mud revealed that it was considerably enriched with tin compared with mangrove leaves (Peterson *et al.*, 1979). Whether this enrichment represents selective adsorption and

chemical complexation by decaying leaves, or whether it illustrates tin accumulation by decomposer microorganisms, was not established. Nevertheless, this finding agrees with the observation that tin enrichment occurs in the humus layer in soils already referred to.

A Sn IV-tolerant bacterium was also isolated from one of the tin-rich rivers in Malaysia (Peterson *et al.*, 1976) and shown to release volatile tin compounds into the atmosphere. Huey *et al.* (1975) have isolated Sn III- and Sn IV-tolerant *Pseudomonas* microorganisms from Chesapeake Bay, USA which have been shown to produce volatile methylated tin compounds.

13. TUNGSTEN

Tungsten, along with chromium and molybdenum, form the subgroup VI B metals. All three metals exhibit tri- and hexavalency and the coordination number in their stable chelates varies from 4 to 6. Scheelite ($CaWO_4$) and wolframite ($(Fe, Mn)WO_4$) are the two principal ores of tungsten. Tungstates are relatively common while molybdenum occurs primarily as the sulphide. The ores are mined mainly in China, Burma, Bolivia and Portugal. Metallic tungsten is used in high-speed tool steels, lamp filaments, spark plugs, etc.

The concentration of tungsten in coal is around 1 ppm and following combustion the element is enriched on the smaller fly-ash particles (Coles *et al.*, 1979). Its chalcophilic behaviour has led to the suggestion that tungsten is associated with the organic phase of coal as a covalently bound organo-metallic complex. Yellow sweet clover grown on pfa has been reported to absorb slightly more tungsten than plants grown on control soil (Furr *et al.*, 1975).

Tungsten concentrations in municipal sewage sludges have been reported to range from 0·9 to 99·6 ppm (dw) (Furr *et al.*, 1976*b*) but few studies have been undertaken to assess its availability to crop plants.

Tungsten is not involved in any mammalian enzyme system (Venugopal and Luckey, 1975) and does not appear to be essential for plant or animal growth (Gough *et al.*, 1979). Recently Gough *et al.* (1979) have reported that tungsten probably does not constitute an important environmental hazard to animals and man.

Bowen (1966) quotes a value of 1·5 ppm for the tungsten concentration in igneous rocks and 0·6 ppm in limestones. Concentrations in soils are around 1 ppm (Bowen, 1966) although Connor and Shacklette (1975) found 1000 ppm tungsten in one of 492 samples of soil from the western USA.

Tungsten availability, as with molybdenum, is increased at high pH ranges in soils due to the formation of double tungstates. The element is precipitated in the pH range 3–5 (Brooks, 1972).

The mean concentration of tungsten in plants has been reported as 0·07 ppm (dw) (Bowen, 1966) or 0·5 ppm (ash w.) (Brooks, 1972). Connor and Shacklette (1975) however found around 50 ppm tungsten in leaf and stem ash from various trees species. De Kate (1967) noted that plants growing in a mineralized zone contained from 2 to 18 times the concentration of tungsten compared with their background value of 2·7 ppm.

The work of Kovalevskii (1966, 1979) demonstrates that tungsten is concentrated by plants to a significant degree and that its concentration in plant ash may exceed the values for tungsten in soil ash. The older woody twigs, rather than the leaves, are the principal sites of accumulation. The concentration of tungsten in 2- or 3-year-old twigs has been reported to correlate with the tungsten concentration in the 'B' and 'C' horizons of the soil. Kovalevskii (1966) has shown that the amount of tungsten in the leaves of several plants, e.g. *Pinus sibirica*, *Ledum palustre*, reaches a certain level only, which is characteristic of the species, whereas the leaves of *Salix* spp. apparently have no limit to their accumulation.

Little work has been undertaken on the plant physiology of tungsten. The tungstate ion has a marked antagonistic effect on the metabolism of molybdate by lower organisms (Higgins *et al.*, 1956; Keeler and Varner, 1957). Its effects on higher plants such as barley and tobacco have been studied by Heimer *et al.* (1969) who showed that tungstate has an effect on nitrate assimilation. When tungsten was substituted for molybdenum in the nitrate reductase enzyme, it had no catalytic activity, presumably because W VI is too stable to undergo a valency change (Nicholas, 1975).

Experimental studies on the effects of tungsten on organisms are few and in the main are related to the function of molybdenum in cellular processes. Judged from the data presented here, tungsten probably does not constitute an environmental hazard, although further studies on plants are necessary as the element can be accumulated to high levels under some conditions.

14. URANIUM

Uranium has no stable nuclides, several isotopes are sufficiently long-lived to occur in various proportions in the environment: ^{234}U (0·006 %), ^{235}U (0·7 %) and ^{238}U (99 %). It exists in two valency states, tetravalent and

hexavalent, the crustal abundance is about 2 g/ton, the hexavalent form occurring in the upper layers of the earth as the very stable UO_2^{2+} ion and at lower depths, where the oxidation potential is lower, as the U_4^+ ion. Uranyl compounds have a higher solubility than the tetravalent series and so are more mobile in the environment. Uranium is present in 1 % concentration in at least 100 minerals.

Uranium is mined mainly in the Congo, Canada, USA and Czechoslovakia. Its uses include in nuclear energy plants as fuel, and also as catalysts and staining pigments (Day, 1963).

Brooks (1972) quotes uranium levels in igneous rocks to be 2·6 ppm, and concentrations in soils as 1 ppm. Szalay (1964) accounts for high uranium contents in coals to be due to enrichment by humic acids. Insoluble humic acids, derived from humified plant lignin, are able to bind uranium by cation-exchange processes from very dilute waters to an enrichment factor of 10 000:1. British values for uranium in coal average 1·3 ppm, with a range of 0·5–2·3 ppm (Lim, 1979). Australian bituminous coals contain a mean of 2 ppm uranium, ranging from 0·4 to 3·5 ppm (Swaine, 1979).

Fresh waters rarely contain more than 0·001 ppm uranium, in ocean waters the value is around 0·003 ppm (Day, 1963). Bowen (1966) records uranium accumulation in soil humus, especially in alkaline soils. Szekeley (1960) reports a range of 0–3 ppm uranium in soils investigated, 5–10 ppm occurring in organic soils correlating with the geological and geomorphological characteristics of the soil. Manskaya and Drozdova (1951) noted that uranium binds to humic acids incorporating calcium to form calcium uranyl humates, uranium becoming less mobile in the soil profile. Brucher (1961) demonstrated that tetravalent uranium was retained by humus as $U(SO_4)_2$; upon strong acid leaching through the soil column, uranium was released as U_4^+.

Raikov *et al.* (1966) carried out experiments with soils containing 60 ppm uranium and noted that on application of nitrogen, phosphorus and potassium there was decreased uranium uptake in the shoots of the plants used by a factor of 100. Uranium contents were 100 times higher in the roots than other plant organs, and lowest in seeds. In field experiments with crop plants such as rye, maize, lucerne, sugar beet and wheat, farmyard manure or inorganic fertilizers caused a reduction in the uranium content of the plants. Uranium was mainly located in the roots of wheat, maize and rye. Lucerne and sugar beet uranium contents were 10 times higher than in the other species, sugar beet also accumulated more uranium in the leaves than in the roots.

Golikova (1963) found uranium to enter young plants and accumulate in

different organs over the vegetative period, highest uranium concentrations occurring in the old leaves of sugar beet and wheat and least in the grain. Cannon (1964) analysed the uranium content of juniper needles and leaves of shrubs and found 0·5 ppm in the ash of material collected in a normal area, compared to 2 ppm over an area of mineralization. Bowen (1966) quotes an average of 0·038 ppm uranium for land plants. Whitehead and Brooks (1969) collected bryophytes from streams in New Zealand and measured their uranium contents. Values between 0·7 and 19·5 ppm were recorded in areas of no known mineralization. Values up to 86 ppm were found where uranium ores were present. These aquatic bryophytes, although containing no substantial rooting organ, are able to integrate long-term values of the uranium content of the water they grow in. Other terrestrial indigenous plants growing in the vicinity of uranium ore deposits in New Zealand contained as much as 0·5 % dry weight uranium. *Coprosma australis* contained a mean uranium concentration of 46 ppm (ash w.) over mineralized soil, the mean soil uranium concentration equalling 628 ppm (ash w.) (Whitehead *et al.*, 1971); a good correlation between soil and plant uranium was noted.

Cannon (1971) has noted that uranium is often found associated with high concentrations of selenium and so selenium indicator plants can be used in uranium prospecting. The two most useful plant species in uranium prospecting are *Astragalus preussi* and *A. pattersoni*, although there are others; for example, 7400 ppm uranium has been found in the roots of *Sarcobatus* spp. (Hewitt and Smith, 1975) over enriched areas. Morphological changes can occur due to irradiation effects from the uranium ore body (Shacklette, 1962; Shacklette, 1964). Shacklette has described the unusually shaped fruits of *Vaccinium uliginosum* and the genetic changes in *Epilobium angustifolium* that cause whitening of flowers in a higher than normal frequency. The flower *Stanleya pinnata* growing over uranium deposits is unable to tolerate the radiation produced from the ore and produces no petals or stamens, the sepals are thickened and enlarged and the pistil eventually produces a small new plant asexually (Cannon, 1971). In general, uranium is absorbed best by plants with a fairly acid cell sap and a high cation-exchange capacity in the root. Usually, large amounts of calcium, selenium and sulphur are absorbed along with the uranium, but very little potassium. Plants used in biogeochemical prospecting usually belong to the pine or rose family (Cannon, 1957).

Some plants have been demonstrated to become partially adapted for conditions of irradiation. Mewissen *et al.* (1959) germinated seeds of *Andropogon filifolius* collected from uraniferous and non-uraniferous soils

from the Belgian Congo and during soaking for 12 h, irradiated both sets. Seeds from the uraniferous soils showed a high percentage of germination and developed longer stalks and roots than seeds from non-uraniferous soils. Generally, uranium in elevated concentrations is toxic to plants, although as mentioned some plants may adapt; a further example is illustrated by Cannon (1960*a*) who notes that growth of some plants is stimulated by uranium and early flowering is induced. Singer *et al.* (1947) demonstrated the toxic inhibition of uranium on several enzyme systems. Uranium salts are used in staining tissues for electron microscopy studies, revealing its capacity to bind to nucleic acid components. Uranyl ions bind to adenine $N_{(3)}$ atoms in AMP, ADP and ATP *in vivo* (Feldman *et al.*, 1967). At acidic or alkaline pH, dinuclear complex uranyl nitrate is formed and uranium bonds to the phosphate and ribose groups of AMP, ADP and ATP (Agarwal and Feldman, 1968).

The distribution of uranium in the leaves of *Coprosma australis* was studied by Whitehead *et al.* (1971). Uranium was found to be in the water-soluble fraction (7%), probably as the simple uranyl ion. The remainder of the uranium was thought to be associated with the cellular particles, as much as 50% binding to protein material in the cell wall.

Uranium has not been considered as an essential element or to be generally beneficial to living organisms. Ruhland (1958) has demonstrated that growth of timothy in sandy loam is improved by addition of 500 ppm uranium, but generally effects of uranium are detrimental to the plant.

Because uranium emits radiation during decay it presents a potentially radiotoxic hazard as well as metal toxic. In areas of uranium mining there may be localized pollution problems, although the element is usually in sufficiently low concentrations to offer no environmental hazard.

15. VANADIUM

Vanadium is a lithophilic element, widely distributed in nature, having a crustal abundance of 110 g/ton. It has two stable isotopes, ^{50}V (0·24%) and ^{51}V (99·76%), and is fairly common in rocks and a variety of independent minerals, the most important being the following: roscoelite $KV_3Si_3O_{10}(OH)_2$, vanadinite $Pb_5(VO_4)_3Cl$ and patronite VS_4. Substantial amounts of vanadium occur in crude oils and petroleum ash, the element is also found in certain ores and oil shales. Vanadium has four oxidation states (2, 3, 4 and 5) and readily forms complexes with oxygen and sometimes sulphur. In nature it occurs as vanadates and organically

complexed forms. The ionic radius of trivalent vanadium is close to that of the ferric ion, so it may replace iron in iron minerals, particularly magnetite, Fe_3O_4, associated with gabbroic magmas which may contain 2000 ppm vanadium. During weathering, vanadium becomes oxidized to the vanadate ion (VO_4) which is soluble over a wide range of pH and is hence quite mobile in the soil. Vanadium forms cationic and anionic water-soluble salts, the pentavalent forms being the most stable. Labile and coordination chelates with ligands such as hydroxy, nitro and cyanogen groupings commonly occur. The stability of vanadium and hence availability to plants depends on the geochemical and physical environment: in reducing or very alkaline conditions containing carbonate it is soluble, when oxidizing in the presence of potassium of calcium or reducing conditions with organic matter it is precipitated.

Metallic vanadium is used to produce steel alloys for making high-speed tools and rust-resistant springs, in ceramics, dyes and paints, and as the pentoxide as an important catalyst in several chemical processes. Deposits are mined mainly in the USA, Zimbabwe and West Africa (Bengtsson and Tyler, 1976; Day, 1963; Cannon, 1963).

Levels of vanadium in Australian bituminous coals range from 4 to 90 ppm, with a mean concentration of 20 ppm, most values ranging between 10 and 60 ppm (Swaine, 1977). Ascidians and holothurians notably accumulate vanadium, using it in their circulatory systems in respiration. These organisms have been considered to give rise to the very enriched vanadium levels found in petroleum and oils (Bertrand, 1950). Normal concentrations of vanadium in vegetation are much lower and do not account for such enrichment.

Szalay and Szilagyi (1967) account for the association between vanadium and fossil debris in the following way. Their experiments demonstrate the immobility of vanadium in certain conditions. Insoluble humic acid prepared by purification of peat was shown to reduce mobile metavanadate anions $(VO_3)^-$ to vanadyl cations $(VO)^{2+}$ which can be firmly bound to humic acids by cation exchange, the geochemical enrichment factor equalling at least 50 000:1. Crude oils have been found to contain up to 1400 ppm vanadium: Venezuelan oils are noted to be particularly enriched in the metal (Bengtsson and Tyler, 1976).

Schroeder (1973) considers there is only presumptive evidence for the essentiality of vanadium, but slight stimulatory effects on various biological systems have been noted. Arnon and Wessell (1953) reported the beneficial effects of vanadium on the growth of green algae. Vanadium stimulated the growth of *Scenedesmus obliquus* at concentrations up to 100 ppm in

solution, higher concentrations proved to be toxic. Low concentrations of vanadium were observed to stimulate nitrogen fixation in *Anabaena circinalis*, but elevated levels of vanadium were thought to decrease the fixation rate, probably by competing with molybdenum, a better enzyme activator than vanadium (Sahay and Sankaram, 1968).

Other authors demonstrated an increase in nitrogen fixation in legumes, vanadium substituting for molybdenum in the nitrogenase enzyme in *Azotobacter* and *Clostridium butyricum* (Bové, *et al.*, 1957; Buczek, 1973; Jensen and Spencer, 1946–47; Buck and Horner, 1935). Vanadium has the ability to influence soil enzyme systems which affect mineralization processes and release of plant nutrients, thus indirectly affecting primary production (Bengtsson and Tyler, 1976). Tyler (1976) records the influence of vanadium on soil phosphatase activity. Acid phosphatases of soils from a conifer forest are significantly inhibited by as much as 20–30% on addition of various vanadium salts, $VOSO_4$, $NaVO_3$, Na_3VO_4 and V_2O_5 at a rate of 50–100 ppm vanadium of the dry weight of the soil. Singh and Wort (1970) monitored biochemical changes in the sugar beet by spraying foliage of young plants with 0·01 M $VOSO_4$. Growth of leaves was markedly reduced, although the net carbon dioxide assimilation rate increased. The activity of nitrate reductase, phosphatase, glutamic–pyruvic transaminase and invertase were partially inhibited, whereas activity of sucrose synthetase increased, higher concentrations of sucrose and nitrate in the storage roots were produced, but lower concentrations of reducing sugar, amino acids and protein resulted. The overall effect was regarded as non-desirable for plants growing in normal conditions. In rats, vanadium has been shown to inhibit glucose metabolism (Meekes *et al.*, 1971) by involvement with thiol-containing co-factors such as co-enzyme A.

The biological availability of vanadium is poorly understood; most plants accumulate vanadium in very small amounts from the soil in relation to the total soil content. The average vanadium content for the world's soils is taken as 100 ppm (Vinogradov, 1959). Organic matter in the soils of coniferous woodland in Sweden has been reported to contain only 4–11 ppm vanadium (Bengtsson and Tyler, 1976), although other authors regard vanadium to combine readily with humic material (Szalay and Szilagyi, 1967). Urban topsoils are considered to contain higher vanadium levels than rural soils, soils near metallurgical industry containing several hundreds of ppm vanadium (Bengtsson and Tyler, 1976). More extensive data concerning vanadium in soils of polluted areas is sparse. Vanadium levels in fresh water are usually between 0·1 and 1·0 ppb, 2·0 ppb and above are indicative of pollution. Concentrations of 0·1–0·3 ppb vanadium occur

in the open sea, and 0·5–2·0 ppb around coastal areas in industrialized regions (Bengtsson and Tyler, 1976). Analytical data for vanadium in waters is variable, but may reflect differences in the accuracy of the analytical methodology rather than real variations.

There are few detailed analyses of vanadium concentrations in plants from natural soils, average values for unpolluted sites range from 0·5 to 2·0 ppm (dw). Vegetable crops generally contain less than 0·5 ppm (Bertrand, 1950; Schroeder *et al.*, 1963; Smith, 1973). Bertrand (1950) reports 0·66 ppm (dw) for the average vanadium concentration of basidiomycete fungi, although the species *Amanita muscaria* has been found to contain between 61 and 181 ppm (Bertrand, 1950). Recently, work cited by Byrne *et al.* (1976) has described how a water–alcohol-extractable compound of vanadium from *Amanita muscaria* was isolated and named Amavadin. The compound has a low molecular weight, formula $C_{12}H_{20}N_2VO_{11}$. Mosses such as *Hypnum cupressiforme* from forest areas in Sweden have up to 10 ppm vanadium in their tissues, much higher than other plant species from similar sites, possibly indicating the significance of aerial deposition in vanadium accumulation by mosses. In urban areas of Sweden, 50–250 ppm vanadium are recorded in mosses—sometimes even higher levels are detected in areas of iron and ferro-alloy industry or in the vicinity of large oil-fired power stations (Bengtsson and Tyler, 1976). Bertrand (1950) quotes an average of 1 ppm vanadium (dw) in vegetation from unmineralized areas and <1–8·6 ppm for lichens.

Warington (1956) has studied the toxic effects of vanadium on the growth of plants in culture solution. Concentrations between 10 and 20 ppm vanadium were toxic to the sorghum plants used in the experiments. High amounts of vanadium in solution caused colour deepening in the shoots followed by apical iron deficiency and chlorosis. Other experiments demonstrated a reddening effect of leaf tips and stems at vanadium concentrations of 10 ppm in solution. At 100 ppm, stunting and death of plants occurred. When similar experiments were carried out with *Astragalus preussi* no toxic effects were noted at vanadium concentrations of 100 ppm. Cannon (1963) has noted that *Astragalus* species are able to tolerate very high amounts of vanadium which they utilize in nitrogen fixation. In areas of mineralization these plants are used as indicators of vanadium deposits. Other vanadium indicator plants include *Allium macropetalum*, *Castillega angustifolia* and *Chrysothamnus viscidiflorus* (Cannon, 1963).

Present evidence indicates that tolerance of high amounts of vanadium in plants is due to immobilization of the element as an insoluble product in the

root, partial exclusion mechanisms also preventing uptake of the element from enriched soils. Two species of *Astragalus*, *A. pattersoni* and *A. preussi* accumulate variable amounts of vanadium depending on their calcium content. *Astragalus preussi* contains only 50% of the calcium content of *A. pattersoni*, suggesting that *A. preussi* has its vanadium in a more soluble form compared to the other species, since there is less calcium to precipitate out as calcium vanadate in the root. Insoluble calcium vanadate is tolerated in the plants much more readily than the soluble phytotoxic forms of vanadium, hence the ability of *A. pattersoni* to accumulate significantly larger concentrations of vanadium. Samples of Juniper trees were analysed from mineralized areas of the Thomson uranium/vanadium district in Utah, USA. Branch tips averaged only 55 ppm (ash w.), with greatest (110 ppm) vanadium concentrations occurring in the roots. Peeled Juniper roots growing over the ore deposit at a depth of 3 m contained over 2200 ppm, indicating that vanadium becomes quickly immobilized in the plant, probably forming insoluble calcium vanadate. Mosses growing in mineralized streams in the area contained up to 108 ppm vanadium, other species growing over the mineralized area contained between 10 and 44 ppm vanadium (dw) (Cannon, 1963).

Absorption of vanadium depends upon soil type, high plant:soil accumulation ratios occurring in seleniferous soils or river alluvium, whilst high calcium soils restrict movement of vanadium into the plant and localize the element in the plant root. A direct correlation between selenium and vanadium absorption and translocation to the shoot was noted in the ash of various species (Cannon, 1963). Watkinson (1964) analysed *Amanita muscaria* and found different sections of the toadstool to accumulate vanadium and selenium in inverse proportion, although a direct correlation between vanadium and selenium concentrations in the total plant was observed.

Amanita species were noted to be one of the first colonizers on experimental vanadium-enriched soil which contained up to 200 ppm vanadium. Control samples of *Amanita* growing on normal vanadium soils contained only < 15 ppm (ash w.). Some plants containing high levels of calcium such as *Verbesina* were able to tolerate water-soluble soil vanadium concentrations of up to 560 ppm and exclude vanadium or prevent significant translocation to the leaves, total vanadium in the harvested plant portion equalling only 40 ppm (ash w.) (Cannon, 1963).

Vanadium has been demonstrated to be a biologically active metal, effecting primary production and other biochemical processes, its increased man-effected output indicates that these effects may become increasingly

important and therefore further studies into the environmental behaviour of this metal are warranted.

16. DISCUSSION

From consideration of the data drawn together in this chapter, certain non-essential elements warrant particular attention for further research. These can be listed as thallium, beryllium, silver, uranium, and vanadium.

Isolated instances of thallium poisoning have been reported in the past as a result of industrial pollution. In the case of beryllium and silver, although there is evidence of adverse health effects as a result of occupational exposure, particularly beryllium as the oxide, there is some circumstantial evidence which indicates that these elements could become environmentally hazardous in the future. For example, use of these elements in industry and subsequent loss/discharge into waste disposal systems. More localized sources of pollution include smelting of ores and disused mine tailings, where silver in particular may be present at phytotoxic concentrations.

Two elements which appear to warrant more definitive study are uranium and vanadium. Vanadium has been demonstrated to have both beneficial and detrimental effects on living organisms, but whether these effects have far-reaching and significant consequences to the ecosystem cannot be ascertained at present.

In view of the expected expansion of the nuclear energy programme there will be an increased mobilization and dispersion of uranium during mining, refining, transport and preparation of the metal for use in the industry. This element demands an appraisal of an additional factor as an environmental pollutant due to its radioactive nature, its radiotoxicity as an alpha emitter and its chemical toxicity. This element has been shown to exert direct and indirect changes on chromosomal material in plants and animals.

In this chapter, each element has been considered as a single agent in its interaction with the environment. However, it is becoming increasingly necessary to examine the interrelated effects of multi-elements as they occur in living organisms. For example, selenium has been shown to ameliorate the effects of mercury at concentrations which would otherwise prove to be toxic. Alternatively, in cases where animals graze on pastures containing high levels of molybdenum, copper uptake is inhibited resulting in hypocuprosis in the animals. This antagonistic action of molybdenum upon the utilization of dietary copper is also synergized by both inorganic and organic dietary sources of sulphur, which react within the digestive

tract to form thiomolybdates, which in turn form stable complexes with copper, thus greatly decreasing levels of available copper.

From the elements discussed in this chapter, those of most significant concern are also those which are absolutely and relatively rare in the earth's crust. Throughout the period where living organisms have evolved, they have done so in geochemical environments to which they are now well adapted. In the case where elements are rare and localized then to introduce them into the general environment, even in minute quantities, may give rise to significant toxicity to organisms which are unadapted to these elements. It is on this criterion that the elements thallium, beryllium and uranium warrant especial attention.

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CHAPTER 8

Metalloids

P. J. PETERSON, L. M. BENSON and R. ZIEVE
Department of Botany and Biochemistry, University of London, UK

1. INTRODUCTION

Plant and animal nutritionists, veterinarians, toxicologists and physicians have long been aware of the benefits of certain elements and the hazards of others to the health of plants, livestock and man. This chapter is concerned with three elements, arsenic, selenium and antimony, which are commonly identified with the toxic or potentially toxic metals, but in view of their chemical and physical properties they are more suitably termed metalloids. Mason (1966) includes these elements in the chalcophile group, for they normally occur in sulphide minerals and form volatile species upon combustion. They are also referred to as atmophile elements by some authors because of their volatility.

Arsenic and antimony are in group VA of the periodic table and are found primarily in the $-3(AsH_3)$, $+3(As_2O_3)$ and $+5(As_2O_5)$ oxidation states as well as the metal. The chemistry of arsenic displays many similarities to that of phosphorus. Selenium is in group VIA with possible valencies of $-2(H_2Se)$, $+4(H_2SeO_3)$ and $+6(H_2SeO_4)$ as well as the elemental form. The chemical behaviour of selenium is similar in many respects to sulphur.

Both arsenic and selenium have attracted considerable scientific interest but antimony has been largely neglected and is usually overlooked as an element of nutritional or environmental concern. Before the impact of these elements on plants can be assessed, the major steps in the biogeochemical pathway must be outlined. This chapter is concerned with the occurrence, transport and biological effects of these elements in rock–soil–plant

systems. Although industrial or agricultural operations are, or have been, important sources of contamination, especially of arsenic, the major sources of input into the ecosystem are the rocks from which the soil has developed. The concentration and form of arsenic, selenium and antimony in rocks and soils will therefore be outlined and related to the accumulation of these elements by indigenous plants as well as crops. Background information and baseline studies will also be mentioned to help distinguish environmental pollution effects from natural variation.

Arsenic, selenium and antimony are ubiquitous in all soils and plants and can be accumulated to high levels by some tolerant plant species and ecotypes. It was established many years ago that ingestion of plants containing elevated concentrations of selenium and arsenic could give rise to ill health and toxic conditions in animals. However, it was not until comparatively recently that their essential roles in animal nutrition were established. Antimony has attracted little attention and is not considered essential for plants or animals. Although animals largely obtain their trace metals via plant ingestion, relatively little information is available on plant requirements. There are no well documented plant growth effects attributable to a deficiency of these elements, instead the research emphasis has focused on element toxicity. An early report by Trelease and Trelease (1938) on a selenium-induced growth response in selenium-accumulating *Astragalus* plants was not upheld by a recent and more extensive survey (Broyer *et al.*, 1972*a*,*b*). Instead the authors concluded that selenium additions did not have a direct effect on plant growth, rather they corrected a phosphate-induced growth depression caused by high culture phosphate levels. In a further attempt to show a direct selenium-induced growth response, Broyer *et al.* (1966) rigorously purified nutrient solutions and examined the growth of crop plants in solutions supplemented with selenium. Again no growth increases were noted; only toxicity effects were measured at elevated selenium concentrations.

In view of the marked differences in the chemistry, biochemistry and physiology of selenium, arsenic and antimony, each element will be considered separately. But to illustrate comparative concentrations of these elements in plants and soils, abbreviated analytical data is tabulated in Table 8.1. Three standard plant materials have been listed but they do not correspond with the soils data. It is evident that the arsenic concentration is high in orchard leaves, which presumably reflects the early use of arsenical pesticide sprays. In pine needles the antimony concentration is higher than the arsenic concentration, whereas the converse is true for kale, perhaps indicating the relative concentrations of these elements in the soils.

TABLE 8.1
MEAN ARSENIC, SELENIUM AND ANTIMONY CONCENTRATIONS IN SOILS AND PLANTS

Material	Arsenic (ppm)	Antimony (ppm)	Selenium (ppm)	Author
Soil	6	2–10	0·2	Bowen (1966)
Soil	5	0·5	0·5	Brooks (1972)
Plants				
Kale	0·141	0·069	0·121	Bowen (1974)
NBS Orchard leaves	10·0	2·9	0·08	Peterson *et al.* (1979*b*) NBS Certified Values
NBS Pine needles	0·21	1·14	—	Renan *et al.* (1979)

In preparing this chapter the authors have not attempted to include references to all of the work carried out in recent years but have selected the information to illustrate the main aspects of the subject. More detailed reviews have been prepared on related topics by the National Academy of Sciences (USA) with their volumes on arsenic (Levander, 1977) and selenium (Oldfield, 1971). The books on selenium by Trelease and Beath (1949), Rosenfeld and Beath (1964) and the symposium volume *Selenium in Biomedicine* (Muth, 1967) contain much useful data relating to early investigations. Selenium biochemistry has been recently reviewed by Downes *et al.* (1979). There are several relevant reviews which deal with the use of arsenical pesticides and herbicides in the volume edited by Woolson (1975). Antimony in the environment has seldom been reviewed, perhaps the most recent and useful account is by Valente (1978).

2. SELENIUM

2.1. Selenium in the Environment

2.1.1. Background concentrations

A range of selenium concentrations in plants and soils from non-seleniferous areas are presented in Tables 8.2 and 8.3 respectively. Low selenium soils producing plants containing less than 0·1 ppm (dw) (dry weight) occur in broad areas throughout the world and have given rise to ill health problems and deficiency symptoms in a range of farm animals and poultry. Igneous rocks, being inherently low in the element, constitute the parent material of a great many selenium-deficient soils (Sharman, 1960; Kubota *et al.*, 1967; Gardiner, 1969). In New Zealand where selenium

TABLE 8.2
SELENIUM CONCENTRATIONS IN NON-SELENIFEROUS SOILS

Area	Range (ppm)	Mean (ppm)	Author
Global	—	0·2	Bowen (1966)
Global	—	0·5	Brooks (1972)
Global	0·1–2·0	—	Swaine (1955)
Russia	<0·01	—	Vinogradov (1959)
USA	0·1–4·32	0·31	Shacklette *et al.* (1974)
Sweden	0·16–0·98	0·39	Lindberg and Bingefors (1970)
Norway	0·068–1·70	0·50	Låg and Steinnes (1978)
Denmark	0·20–1·44	0·57	Bisbjerg (1972)
India	0·158–0·710	0·46	Misra and Tripathi (1972)
New Zealand	—	0·60	Wells (1967*b*)

deficiency problems are of serious concern, very low values of selenium have been found in granites, limestones, ultrabasics and schists and in the soils derived from them (Wells, 1967*a*,*b*).

Total selenium concentrations in soil parent material have been reported to influence, at least to some extent, selenium concentrations in wheat plants (Doyle and Fletcher, 1977). Furthermore, they indicate that soil parent material maps could form a suitable sampling base for designing plant-sampling programmes to outline problem areas.

As the minimum dietary level required to prevent white muscle disease in sheep is from 0·03 to 0·1 ppm selenium, depending upon various nutritional factors (Allaway *et al.*, 1967), many areas in the USA, Australia, Denmark, New Zealand and Great Britain must be considered to be deficient or

TABLE 8.3
SELENIUM CONCENTRATIONS IN NON-SELENIFEROUS PLANTS

Area	Plants	Range (ppm)	Mean (ppm)	Author
Global	—	—	0·2	Bowen (1966)
USA	Forage medians	0·02–0·26	—	Kubota *et al.* (1967)
USA	Alfalfa	0·01–0·09	—	Allaway *et al.* (1966)
Canada	Alfalfa	0·013–0·217	0·079	Walker (1971)
Canada	Red clover	0·004–0·023	0·007	Walker (1971)
Australia	Pasture	0·050–0·620	0·226	Gardiner and Gorman (1963)
Finland	Various	<0·010–0·420	0·141	Koljonen (1974)
Denmark	Cereals	0·002–0·110	0·018	Gissel-Nielsen (1975)
Denmark	Grass	—	0·032	Gissel-Nielsen (1975)

potentially deficient (refer to Table 8.3). Of the 1788 forage and grain samples analyzed by Kubota *et al.* (1967) from sites throughout the USA, 36% contained less than 0·1 ppm selenium. In a further study, Carter *et al.* (1968) reported the selenium contents of forage and hay crops in the Pacific Northwest. These deficient locations have been mapped to delineate the problem areas where selenium supplementation will be required. As selenium is not essential for plant growth, foliar deficiency symptoms cannot be observed on plants containing low concentrations of selenium. Foliar analysis is therefore required to establish the nutritional state of the plants.

There is some evidence that variation in selenium contents may occur in forage plants when grown on soils of low selenium contents or low availability (refer to Table 8.3). Marked differences in selenium concentrations were also found in several species of pasture plants collected from field sites in New Zealand (Davies and Watkinson, 1966*a*). Grasses accumulated two to four times more selenium than white clover (*Trifolium repens*), with *Agrostis tenuis* consistently showing the highest concentration. This latter species was also shown to accumulate more selenium during pot trials than either ryegrass (*Lolium perenne*) or red clover (*T. pratense*) (Peterson and Butler, 1966). Red clover accumulated only low concentrations of selenium compared with alfalfa in a Canadian study (Walker, 1971). Similarly, Goodwin (1968) reported that native grasses grown on lateritic soils contained higher concentrations of selenium than clovers. It is noteworthy that the highest incidences of selenium-responsive diseases of livestock in New Zealand are associated with improved pastures especially rich in clovers (Cousins and Cairney, 1961).

2.1.2. Baseline studies

Many plant species growing on selenium-enriched soils have given rise to serious animal ill-health problems throughout the world. In a great many cases the parent materials especially concentrated in selenium are of sedimentary origin, with the shales being of particular importance. Shales containing unusually high amounts of selenium have been imported from the USA (Rosenfeld and Beath, 1964), Canada (Rosenfeld and Beath, 1964; Fletcher *et al.*, 1973), South Africa (Brown and de Wet, 1962), Australia (McCray and Hurwood, 1963), Ireland (Walsh *et al.*. 1951), Great Britain (Webb *et al.*, 1966) while the selenium toxic soils in Israel are derived from limestone alluvium (Ravikovitch and Margolin, 1957) and those in South America from black slate alluvium (Rosenfeld and Beath, 1964). High levels of selenium are also encountered in the lateritic soils of Hawaii and Puerto

Rico (Rosenfeld and Beath, 1964). The selenium content of some shales is elevated only in small localities (e.g. Ireland; Walsh *et al.*, 1951), or may remain high and relatively constant in a particular strata for several hundred miles (e.g. USA; Cannon, 1957).

In its geochemistry selenium resembles sulphur and is therefore a component of sulphide minerals. Koljonen (1977) has suggested that selenium can be used as an indicator element in geochemical exploration of sulphide ores and that it gives an indication of the ore type. Because uranium and vanadium ores commonly contain sulphides, they are also enriched with selenium. The ore carnotite, for example, contains appreciable concentrations of selenium which *Astragalus* spp. are able to absorb in concentrations reaching several thousands of ppm. *Stanleya* on the other hand is not capable of extracting large amounts of selenium from this ore, but concentrates the element from the small quantities present in gypsum (Cannon, 1957, 1971). Species differences can be very marked, e.g. *S. pinnata* has been reported to contain 2380 ppm selenium, whereas *Artemesia canadensis* growing in close proximity on the same geological formation contained only 6·8 ppm (Moxon *et al.*, 1950).

Volcanic emanations are a further primary source of selenium and volcanic ash flows and lava may be especially high. The data reported by Wells (1967*a*,*b*) for rocks and soils in New Zealand illustrates this aspect.

2.1.3. Industrial contamination

Biological processes, together with geological and physico-chemical factors, have also resulted in local concentrations of selenium in coal, lignite, fuel oil and natural gas. Coal, in particular, is highly enriched with selenium with a mean for USA coals of 2·08 ppm (range 0·45–7·7), for British coals of 2·8 (range 1·8–4·4) and Australian coals of 0·79 (range 0·21–2·5) (Swaine, 1977; Lim, 1979). The selenium in fossil fuels may enter the environment either by atmospheric discharge from coal-fired electrical generating plants (Bertine and Goldberg, 1971; Klein *et al.*, 1975; Mackenzie *et al.*, 1979) because of the relatively low boiling point of the element and its compounds, or by leaching from pulverized fuel ash (pfa) disposed of on land (Gutenmann *et al.*, 1976). The pfa had total selenium contents ranging from 1·2 to 16·5 ppm (mean 8·0) and mature white sweet clover (*Melilotus alba*) growing on a landfill site contained in excess of 200 ppm (dw) in top-most portions of the plant. Lower concentrations were found when the whole plant was analyzed. The yellow sweet clover (*M. officinalis*) has also been reported to accumulate selenium when growing on pfa tips (Furr *et al.*, 1975).

New England asters (*Aster novae-angliae*) growing voluntarily on pfa dumps also accumulate selenium, for the element was detected in pollen gathered by bees foraging on the site (De Jong *et al.*, 1977). Presumably the concentrations in the plant must have been high for the pollen values were 14·1 ppm selenium (fw) (fresh weight) compared with the controls where selenium could not be detected ($< 0{\cdot}1$ ppm). *Astragalus racemosus*, the well known selenium accumulator, has also been shown to accumulate over 100 ppm selenium when grown on pfa (Gutenmann and Lisk, 1979).

Vegetables grown in pots on soils containing pfa have been reported to accumulate up to 1 ppm selenium compared with 0·02 ppm selenium for plants grown on control soil (Gutenmann *et al.*, 1976). Cabbages grown on soils amended with 7% w/w pfa from various sources were shown to accumulate selenium in proportion to its concentration in the particular ash (Gutenmann *et al.*, 1976).

Johnson (1970) found 1·6–19 ppm selenium in newspaper, cardboard and laboratory tissues. As large amounts of papers are incinerated, appreciable quantities of selenium can be expected in stack emissions which will disperse into the environment. Selenium also occurs in petroleum, motor oils and automobile tyres (Hashimoto *et al.*, 1970). These authors reported that the selenium concentration in soils in the Tokyo area were high and approached toxic levels.

Selenium is present in phosphate rocks and in superphosphate produced from them (Robbins and Carter, 1970). Superphosphate can contain around 20 ppm or more selenium which may well lead to a build up of selenium in some soils subject to heavy applications of fertilizers. Gregers-Hansen (1967) found 5 ppm selenium in superphosphate but concluded it had no discernible effect on the selenium status of Danish loam. As a result, plant uptake from such sources has been found to be very low (Gissel-Nielsen, 1971*a*,*b*).

The commercial source of selenium is the anodic slime from electrolytic copper refining. Elevated levels of atmospheric selenium have been reported in the immediate neighbourhood of a copper refinery but this decreased with distance from the source (Selyankina and Alekseeva, 1970). Soil profiles, grass analysis and aerosol enrichments indicated that selenium and other toxic elements, in addition to lead, have contaminated the environment around a lead smelter (Ragaini *et al.*, 1977). The top soil and grass in the valley near the smelter were enriched by factors of 100 and 64 respectively when compared with background concentrations and average forage values compiled from the literature. Nevertheless, the selenium pollution arising from smelting processes would appear to be of relatively

minor importance compared with possible effects from elevated arsenic emissions (refer Section 3.1.2).

Sewage sludges contain all of the elements used in modern industry and of these zinc, copper, nickel, cadmium and perhaps arsenic and chromium can give rise to potential agronomic problems (Chaney and Giordano, 1977). Selenium, however, has largely been neglected in these investigations probably because of the inherent analytical difficulties. Furr *et al.* (1976*b*), in an analytical survey of various municipal sludges in the USA, reported elevated concentrations of selenium throughout, but no assessment was made of its availability to plants on disposal sites.

The selenium content of wheat grain was shown to significantly increase from a mean on control plots of 0·29 to a mean of 0·42 ppm (dw) in plots receiving sludge applications.

2.2. Selenium Cycling

A global biogeochemical selenium cycle has been constructed in detail by Mackenzie *et al.* (1979) and used to assess the relative magnitudes of some of the rates of transfer (fluxes) of the element at the surface of the earth. The authors concluded that anthropogenic emissions (the sum of fossil fuel and particulate fluxes) greatly exceed the natural emissions (the sum of continental and volcanic dust fluxes). This is borne out by the large normalised selenium-enrichment factors reported in air sampled near industrial locations in the northern hemisphere, but the significantly lower values from Nigeria and South Africa where smaller degrees of industrialization occur (Peirson and Cawse, 1979). However, the importance of biologically methylated compounds released as volatiles from soils and plants has yet to be accurately determined. A value of 30×10^8 g selenium yr^{-1} was computed as the amount of selenium released to the atmosphere from soils (Mackenzie *et al.*, 1979). This is an important source of atmospheric selenium (cf. $119{\cdot}8 \times 10^8$ g selenium yr^{-1} from coal–lignite, oil and gas combustions and from roasting of sulphide ores), but no account was taken of the volatile selenium compounds given off from plants which will greatly increase the amounts of selenium released into the atmosphere by biological processes.

There is little published data about the input of selenium from the atmosphere back into soils and plants, although a mechanism (or mechanisms) for its return must exist. Soils are capable of reabsorbing volatile selenium compounds and the process is largely or entirely a chemical reaction rather than biological (Zieve and Peterson, pers. comm.). Organic matter, clay and other factors affect the ability of soil to reabsorb

the volatiles from the atmosphere although the magnitude of these processes has not been quantified. Similarly, there is evidence that plants are capable of absorbing selenium from the air (Asher *et al.*, 1967) but further research is needed to assess the importance of these processes in the selenium cycle.

Låg and Steinnes (1978) have produced one of the most interesting papers in recent years on the importance of atmospheric selenium input into a terrestrial ecosystem. During a regional geochemical survey of trace elements in Norway they discovered that the selenium concentration in the humus layers of forest soils decreased with increasing distance from the ocean and was shown to be highly significantly correlated with the annual rainfall. Thus, much of this element is supplied to the soils through precipitation. Låg and Steinnes concluded that anthropogenic selenium was not the major source of the atmospheric input and that natural mechanisms must be responsible for this process.

The Se/S ratios of various geochemical materials vary greatly between the two extremes of sea water ($0{\cdot}001 \times 10^4$) and magmatic sulphides (1×10^4) (Hashimoto and Winchester, 1967). This latter value supersedes the widely quoted S/Se ratio of 6000 calculated earlier (Goldschmidt, 1954). The selenium content of glacial material of different ages has been shown to be relatively constant, presumably indicating that selenium is not very stable in the atmosphere after its input from fossil fuel burning (Weiss *et al.*, 1971). However, the sulphur content of the ice increased markedly in recent times, indicating the stability of sulphur dioxide and its potential for long-range transport. Thus Se/S ratios varied from $5{\cdot}5 \times 10^4$ for glacial samples, dated at 1724, to $1{\cdot}1 \times 10^4$ for samples collected in 1965.

The geochemistry of selenium resembles only in part that of sulphur. During weathering, selenium behaves differently to sulphur mainly because of the formation of selenite ions and its fixation as ferric selenite or adsorption on hydrous iron oxides (Swaine, 1978). The stability of the selenite ion is a feature of selenium chemistry whereas selenate unlike sulphate, is probably only formed under strongly oxidizing alkaline conditions (refer to Section 2.3.1). Conditions favour the retention of selenium in most soils and although some migration to rivers takes place, much of the selenium will be adsorbed on particulate matter. Thus sea water contains only very low levels of soluble selenium.

2.3. Selenium Chemistry of Soils

2.3.1. Soil chemistry and available selenium

It is evident that the forms of selenium in the soil are the key to its

availability to plants. Although several studies have shown a correlation of total selenium content of specific soils, or pfa-amended soils, with the selenium content of vegetation (Nye and Peterson, 1975; Gutenmann *et al.*, 1976) most studies covering a wide range of soil types illustrate lack of any such correlation (Allaway, 1968). Several attempts have been made to find a correlation between the plant content of selenium and a certain fraction of soil selenium. Considering the great differences in the total selenium content of soils it is not surprising that few correlations have been found. Nye and Peterson (1975) working on soils derived from marine black shales in Ireland, found linear relationships between the selenium concentrations for six plant species and the total soil selenium or water-soluble selenite in soil extracts. Williams and Thornton (1972) also working with soils containing elevated levels of selenium, suggested that EDTA-extractable selenium estimated reasonably well the selenium available to plants in a greenhouse trial. However, within low-level selenium areas, neither total, nor water-soluble selenium seemed to be correlated with plant accumulation (Lindberg and Bingefors, 1970; Lévesque, 1974; Hamdy and Gissel-Nielsen, 1976). On the basis of isotope dilution studies, Gissel-Nielsen (1977) concluded that a significant ryegrass/soil correlation occurred only with the selenium in the isotopically exchangeable fraction of soil.

Plots of calculated ion activity values at various pHs have indicated that the chemical form of selenium present in soil is largely a function of soil pH and redox potential (Geering *et al.*, 1968). These workers concluded that the concentration of selenium in soil solution was governed primarily by a ferric oxide–selenite adsorption complex (selenium oxidation state +4). Selenium would therefore be expected to concentrate largely in clay and iron hydroxide sediments (Watkinson, 1962). Some recent investigations by Howard (1977) and Hamdy and Gissel-Nielsen (1977) support the concept that the geochemistry of selenium is largely controlled by that of iron, with which selenium is closely affiliated in both oxidizing and reducing environments. In aerated acid soils (pH 4·5–6·5) selenium is usually bound to a basic ferric selenite of extremely low solubility and is essentially unavailable to plants (Geering *et al.*, 1968). In waterlogged acid soils, elemental selenium and selenides form from added selenite at least, but the extent to which elemental selenium is a naturally occurring soil constituent is not well known (Oldfield, 1972). Elemental selenium has, however, been reported to occur in sandstones near a uranium deposit (Howard, 1977).

In alkaline soils (pH 7·5–8·5) selenium may be oxidized to the selenate ion. Thus selenates are favoured in alkaline environments of the semi-arid regions and can be removed by leaching, for they are not sorbed on the

reactive iron oxides. However, Howard (1977) concluded that atmospheric oxidation will not be completely attained under natural conditions of weathering. Nye and Peterson (1975) and Peterson (1979), using high voltage paper electrophoretic techniques to separate soluble selenium compounds, showed the predominance of selenate in an alkaline selenium toxic soil from South Dakota and the predominance of selenite in an acidic selenium toxic soil from Ireland.

Geering *et al.* (1968) stated that the rate of transformation of selenite to selenate, and the reverse reaction, are relatively slow. The rate of reduction of selenite to elemental selenium varies from soil to soil but is apparently independent of pH (Cary *et al.*, 1967). This reduction of selenium would have important short-term effects, since re-oxidation to plant-available forms is very slow. Elemental selenium is also relatively stable in soils but can be slowly oxidized by microbiological and chemical processes, especially at high pH (Geering *et al.*, 1968).

Soil organic matter and microorganisms also play an important role in the chemistry of selenium in soils (Gissel-Nielsen, 1977). Nye and Peterson (1975), during their characterization of the highly seleniferous soils of Ireland, concluded on the basis of enzyme digest experiments that much of the selenium is complexed by organic matter. This interesting technique does not appear to have been extended to other soils. The role of soil microorganisms including actinomycetes, bacteria and yeasts in accumulating the element and in the conversion of soluble inorganic selenium to volatile forms is an important one in the selenium cycle and in possibly detoxifying seleniferous areas.

2.3.2. Microbial transformations

Microorganisms can be expected to play an important role in selenium transformations in soil. Many are capable of reducing selenite to red colloidal selenium in pure culture but the relevance of the data to soil conditions has not been established (Brenner, 1916; Levine, 1925; Falcone and Dickenson, 1960, 1963). Several bacteria and fungi have also been reported to oxidize colloidal selenium, or selenite, to selenate (Lipman and Waksman, 1923; Sapozhnikov, 1937; Bird *et al.*, 1948). Higher plant extracts are also capable of this latter oxidative process (refer to Section 2.4.2).

Reduction and methylation of inorganic selenium by several genera of common soil fungi and a coryneform bacterium in pure culture have been reported by several workers (Fleming and Alexander, 1972; Cox and Alexander, 1974; Barkes and Fleming, 1974). These or related organisms

are certainly active within the soil and release volatile compounds into the atmosphere, thus explaining the loss of added selenium in some experiments and the loss of selenium from some stored samples (Abu-Errish *et al.*, 1968; Francis *et al.*, 1974; Doran and Alexander, 1977*a*,*b*). The major volatile compound has been identified as dimethylselenide (Francis *et al.*, 1974; Doran and Alexander, 1977*a*,*b*) but dimethyldiselenide has been reported from soil amended with selenomethionine (Doran and Alexander, 1977*a*). Hydrogen selenide has also been tentatively identified (Doran and Alexander, 1977*a*).

The methylation of selenium is an important transformation in the soil for it leads to a change in both mobility and toxicity of the element, but from a plant physiological standpoint the relevance of the data has yet to be assessed.

2.4. Accumulation of Selenium by Plants

2.4.1. Improvement of forage selenium levels

Strategies available for the prevention of selenium-responsive diseases among grazing animals are either the amendment of low selenium soils with the deficient element or by direct foliar application of selenium during crop growth.

Pasture top-dressing with selenium-enriched fertilizers has been tested in many countries (Cary and Allaway, 1973; Grant, 1965; Davies and Watkinson, 1966*b*; Gissel-Nielsen, 1975). Selenate was initially shown to be the most effective form for raising the selenium content of pasture and crop plants, but the effect was only short-lived (Grant, 1965). Selenite had a more lasting effect and seems stable during fertilizer manufacture (Gissel-Nielsen, 1975, 197*6*). Elemental selenium was ineffective in increasing levels of plant selenium.

Fertilizer anions, such as sulphate, phosphate and nitrate, have been reported to influence the accumulation of selenium by plants in field and pot experiments. Sulphate has been commonly found to reduce uptake of selenate (Hurd-Karrer, 1938) but the effect may be attributed to a sulphate stimulation of growth and thereby a dilution of the absorbed selenium (Davies and Watkinson, 1966*b*). Other authors consider that the sulphate–selenate interaction is a direct effect at the uptake stage (Gissel-Nielsen, 1974). Fleming (1962) found that increasing amounts of phosphate decreased the plant selenium concentrations, whereas Carter *et al.* (1972) reported the opposite effect. In more detailed experiments, Gissel-Nielsen (1974) showed that selenium concentrations in plants decreased with increasing additions of both nitrogen and sulphur, while the effect of

phosphorus was dependent upon the nitrogen and sulphur levels. The application of selenium to the soil is, however, an inefficient method of meeting the selenium requirements of animals (Allaway *et al.*, 1966).

Foliar spraying with selenium avoids the complicated soil interactions and the variability of uptake due to different soil conditions. Selenium is taken up readily through the leaves of pasture plants and cereals, translocated to the roots and retained by the plant (Watkinson and Davies, 1967; Gissel-Nielsen, 1975). Spray applications require only small concentrations of selenium, compared with pasture fertilizers, to achieve the same selenium content of the crops (Gissel-Nielsen, 1975). Rigid control procedures will undoubtedly be required to prevent selenium toxicity to crops and animals should this method be considered of practical use in selenium-deficient areas.

2.4.2. Selenium uptake and transport

Many plants have been shown to concentrate selenium to levels in excess of the level initially present in the external medium, though the degree of concentration depends on the form of the selenium supplied and on the species under investigation. In general, a higher concentration of selenium has been found in plants when selenate has been added to the soil than when selenite was supplied, but this effect was probably caused by the rapid adsorption of selenite to clays as discussed in Section 2.3.1. Conflicting results have been obtained from solution culture experiments where such adsorption effects are eliminated. Butler and Peterson (1967) working with sterile cultures found that selenite was more readily absorbed than selenate, whereas Ulrich and Shrift (1968), Shrift and Ulrich (1969) and Gissel-Nielsen (1973) reported that selenite uptake was slower than selenate uptake.

Studies with excised barley roots and *Astragalus* species strongly suggest active absorption of selenate by the root, and competitive experiments with sulphate indicate that both ions have a common binding site (Epstein, 1955; Leggett and Epstein, 1956; Ulrich and Shrift, 1968; Shrift and Ulrich, 1969; Ferrari and Renosto, 1972). A common binding site for sulphate and selenate has also been found in several fungi (Yamamoto and Segal, 1966; Tweedie and Segal, 1970). An active transport mechanism was clearly shown in the excised root experiments of Ulrich and Shrift (1968), for uptake was reduced by respiratory inhibitors, low temperature and sulphate. At the end of a 1 h uptake period a 40-fold concentration of unchanged selenate had been accumulated by one of the species investigated.

Uptake of selenite is, however, more complicated. The results of Ulrich and Shrift (1968) show that while selenite absorption was less affected by metabolic inhibitors than was that of selenate, substantial inhibition did occur, suggesting that at least a portion of the uptake was metabolically mediated within the root. Selenite may enter the root by diffusion, followed by a continuous metabolic assimilation to other compounds via energy-dependent reactions.

Long-distance transport of selenium has received little attention. Gissel-Nielsen (1973) showed that barley plants grown in nutrient solutions containing radioactive selenite or selenate, contained concentration ratios of root/top of from 2·5 to 3·6 for the selenite treatment, and from 1·5 to 1·9 for plants supplied with selenate, indicating that selenite is less readily transported to the shoots than selenate. Selenium transport through intact tomato roots and into the xylem exudate has been examined in detail by Asher *et al.* (1977). With radioactive selenate additions to the culture solution, selenium concentrations in the exudate were six to thirteen times higher than in the external solution. Existence of values in excess of one is consistent with the view that selenate transport is an active process, especially since the xylem exudate is likely to be electrically negative with respect to the solution. The observation that selenate was the only detectable selenium species in the exudate provides further evidence for the active uptake hypothesis. In a comparable study, Gissel-Nielsen (1979) also recovered selenate in the xylem sap of corn seedlings supplied with selenate.

With selenite additions, xylem exudate concentration factors were always less than unity, i.e. lower than the external solution (Asher *et al.*, 1977). Analyses of exudates showed that negligible amounts of selenium were transported as selenite at physiological concentrations. Most of the selenium transported in selenite-fed plants was a selenate, or, as an unknown selenium compound. This observation is consistent with the view that selenite has been metabolized in the root to selenate. Perhaps this component corresponds with the inhibitor-sensitive compound of selenite uptake reported by Ulrich and Shrift (1968). Biological oxidation of selenite to selenate was also indicated in the recovery of selenate in extracts of *Astragalus* root tips incubated with selenite (Ulrich and Shrift, 1968). Thus oxidation of selenite to selenate would seem to be an essential feature of the absorption and long-distance transport of selenium by roots.

2.4.3. Metabolism of selenium

There are many gaps in our knowledge of the intermediary steps by which selenate and selenite are assimilated in plants. Nevertheless, it is clear that

seleno-amino acids are synthesized from inorganic selenium although the details are still in doubt. The first step is generally agreed to be the reaction of selenate with ATP to yield adenosine 5′-phosphoselenate although it has

$$ATP + SeO_4^{-} \overset{\text{ATP sulphurylase}}{\rightleftharpoons} APSe + PP_i$$

not specifically been isolated from higher plants (Shrift, 1973). The second step is generally considered to be the reaction of APSe with another molecule of ATP to produce phosphoadenosine 5′-phosphoselenate.

$$APSe + ATP \overset{\text{APS kinase}}{\rightleftharpoons} PAPSe + ADP$$

This compound has not been isolated from plants and efforts to demonstrate its presence have been unsuccessful (Ellis, 1969). Whether PAPSe was unstable or whether the enzyme did not synthesize the product was not established (Wilson and Bandurski, 1958).

There is evidence, based on the use of radioactive precursors, that serine serves as an intermediate in the synthesis of selenocysteine in plants (Chen *et al.*, 1970). Indeed, cysteine synthases have been partially purified from a variety of plants and selenocysteine isolated and characterized (Ng and Anderson, 1978, 1979).

$$Se^{2-} + O\text{-acetylserine} \xrightarrow{\text{cysteine synthase}} \text{selenocysteine} + \text{acetate}$$

Selenocysteine has also been shown to be used as a substrate by a partially purified cysteinyl-tRNA synthetase from the mung bean *Phaseolus aureus*, although detailed experimental evidence to support the concept of its incorporation into polypeptides appears to be lacking (Shrift *et al.*, 1976).

The mechanism for the reduction of inorganic selenium remains controversial (Shaw and Anderson, 1974; Dilworth and Bandurski, 1977) but presumably involves glutathione reductase (Ng and Anderson, 1978). Se^{2-} inhibited the synthesis of cysteine and S^{2-} inhibited the synthesis of selenocysteine, implying competition between the two ions for the enzyme.

Selenocysteine has not been isolated and characterized from plants (Shrift, 1973), but labelling studies indicate that it is the precursor of Se-methylselenocysteine found at high levels in the selenium accumulator *Astragalus bisulcatus* (Chen *et al.*, 1970; Chow *et al.*, 1972). Selenocystine and selenocysteic acid have been shown by chromatographic and electrophoretic techniques to be present in enzymatically hydrolyzed proteins, but insufficient material was isolated to permit detailed chemical characterization (Peterson and Butler, 1962; Butler and Peterson, 1967).

TABLE 8.4
SELENIUM COMPOUNDS IN HIGHER PLANTS

Compound	Plants	Author
[a]Selenocystathionine	*Neptunia amplexicaulis*	Peterson and Butler (1967) Peterson and Robinson (1972)
	Morinda reticulata	Peterson and Butler (1971)
	Lecythis ollaria	Aronow and Kerdel-Vegas (1965)
	Stanleya pinnata	Martin *et al.* (1971)
	Astragalus spp.	Martin and Gerlach (1969) Nigam and McConnell (1972)
	Oxytropis spp.	Martin *et al.* (1971)
[a]Se-methylselenocysteine	*Astragalus* spp.	Shrift and Virupaksha (1965) Virupaksha and Shrift (1965) Martin and Gerlach (1969) Martin *et al.* (1971) Nigam and McConnell (1972)
	Oonopsis condensata	Trelease *et al.* (1960)
	Stanleya pinnata	Shrift and Virupaksha (1963) Chen *et al.* (1970)
[b]Selenohomocystine	*A. crotalariae*	Virupaksha *et al.* (1966)
[b]Selenomethionine and its oxide	Various pasture plants	Peterson and Butler (1962)
	Spirodela oligorrhiza	Butler and Peterson (1967)
	Bromegrass	Jenkins and Hidiroglou (1967)
	Wheat	Olson *et al.* (1970)
[b]Se-methylselenomethionine	Pasture plants	Peterson and Butler (1962)
	Non-accumulator *Astragalus* spp.	Virupaksha and Shrift (1965)
[b]Selenocystine and	Various pasture plants	Peterson and Butler (1962)
[b]Selenocysteic acid	*Spirodela oligorrhiza*	Butler and Peterson (1967)
	Bromegrass	Jenkins and Hidiroglou (1967)
[b]Se-propenylselenocysteine and its oxide	*Allium cepa*	Spare and Virtanen (1964)

[a] Compounds isolated and chemically characterized.
[b] Compounds tentatively identified by chromatographic and electrophoretic behaviour.

A range of seleno-amino acids have been isolated from various plants and these are listed in Table 8.4. Accumulators synthesize selenocystathionine, Se-methylselenocysteine and its γ-glutamyl peptide, whereas non-accumulators synthesize selenomethionine and Se-methylselenomethionine. It has been reported that species may have evolved mechanisms to exclude selenocysteine and selenomethionine from their proteins, whereas non-accumulator species incorporate these amino acids into their proteins (Peterson and Butler, 1967).

Only traces of cystathionine were found in selenocystathionine isolated from *Neptunia amplexicaulis* extracts (Peterson and Robinson, 1972) and Se-methylselenocysteine predominated over the sulphur analogue in *Astragalus* species (Martin *et al.*, 1971). Detailed studies of the biosynthetic pathways of these compounds are lacking.

A seleniferous leaf wax has been reported from the accumulator *Stanleya pinnata* (McColloch *et al.*, 1963), and a seleno-lipid and a neutral seleno-sugar isolated from *Astragalus racemosus* (Zingaro *et al.*, 1977).

2.4.4. Localization of selenium

A study of the distribution of selenium absorbed by the roots reveals that it is translocated to all parts of the plant including the seeds. With the selenium accumulators *Astragalus bisulcatus* and *A. crotolariae*, higher concentrations were found in the leaves and stems than in the roots, irrespective of the concentration and form of selenium (Rosenfeld and Eppson, 1962; Broyer *et al.*, 1972*a*,*b*). The opposite has been reported for alfalfa, subterranean clover and other crops (Broyer *et al.*, 1966). Newly formed leaves of accumulators contained considerably more selenium than older leaves, but autoradiographic studies of the distribution of radioactive selenium indicated that it is exported from the leaves as they age, and when they drop from the plant they contain very little selenium (Rosenfeld and Eppson, 1962). Midribs of leaflets and rachides of leaves contained the most selenium. This high mobility of selenium is consistent with its presence in one or more low molecular weight compounds (refer to Table 8.4), which are considered by Rosenfeld (1962) to be localized in the cytoplasmic fraction of the cell. Rosenfeld (1962) isolated chloroplasts from *A. bisulcatus* leaves which apparently contained only low levels of radioactive selenium, but as they were largely disrupted by the extraction technique employed, the results were not conclusive. Trace amounts of selenium only were found associated with the chloroplast pigments in a variety of plants (Peterson and Butler, 1962; Peterson and Butler, 1967), but this conclusion has no bearing on the presence or absence of the selenium in the intact chloroplast.

Now that Ng and Anderson (1978) have isolated intact chloroplasts which can catalyze the synthesis of selenocysteine at a high rate, the localization of seleno-amino acids in cell organelles from accumulator plants requires further attention. The high concentration of seleno-amino acids in such plants also poses the question whether the amino acids are stored in the cytoplasm or in the vacuole.

2.4.5. Essentiality of selenium

Essentiality of selenium for growth of selenium accumulators was first put forward by Trelease and Trelease (1938) when they demonstrated a selenium-induced growth stimulation in greenhouse trials. Nine accumulators were reported to have been stimulated and six non-accumulators to have been severely poisoned, but no attempt was made to examine if it was possible for the plants to complete their life cycle in the absence of the element. Broyer *et al.* (1972*a*,*b*) working with two accumulators and two non-accumulators, grown hydroponically in selenium-free and selenium-supplemented solutions, have now demonstrated that the selenium-induced growth increases noted by Trelease and Trelease (1938) were probably related to an amelioration of the phosphate toxicity present in the hydroponic solutions. Zieber and Shrift (1971) have also described the lack of evidence for the essentiality of selenium for growth of callus tissue from either a selenium-accumulator or non-accumulator *Astragalus* species.

No beneficial effects of selenium supplementation were demonstrated on the growth of either alfalfa or subterranean clover in hydroponics where the solutions had been rigorously purified to remove traces of the element (Broyer *et al.*, 1966). Despite the special purification scheme adopted, control plants after harvest contained higher concentrations of selenium than could be accounted for by the water and nutrient salts used, indicating that substantial amounts of selenium must have been absorbed from the atmosphere during the experiment. The authors estimated that if these plants require selenium, the level is probably less than 0·079 ppm (dw). As the selenium concentrations in the control plants were above those found on some selenium-deficient soils (see Table 8.3), further work is required to resolve this problem.

Selenium has been proposed as an essential element for growth of certain bacteria, where it is required in enzyme systems, e.g. formate dehydrogenase in *E. coli* and *Clostridium thermoacetium* (Pinsent, 1954; Enoch and Lester, 1975), and glycine reductase in *C. stricklandii* (Turner and Stadtman, 1973). Although comparable enzyme studies in plants are lacking, a specific rôle for the element is probably doubtful. Perhaps this

could be expected, as selenium is apparently not essential for plant growth and development.

2.4.6. Selenium toxicity

There are many reports in the literature on the comparable toxicities of selenate, selenite and other seleno compounds towards plants, animals and microorganisms and of the amelioration of these toxic effects by other ions (Rosenfeld and Beath, 1964; Shrift, 1973). Some of these early reports are conflicting, and no useful purpose can be served by detailing these effects yet again. Selenium toxicities are not confined to whole plants. Stewart *et al.* (1978) report that selenium inhibits organogenesis in detached leaves grown in sterile culture.

The mechanism whereby the element exerts its toxic effect is not known, but it is believed to involve competition with sulphur metabolism (Ferrari and Renosto, 1972; Stewart *et al.*, 1978). For example, the presence of seleno-amino acids completely inhibited the incorporation of sulphur amino acids into proteins, but the biochemical effects are not known in detail. However, it seems reasonable to suppose that the indiscriminate substitution of selenium for sulphur in biopolymers such as proteins, nucleic acids and derivatives of complex carbohydrates would have marked deleterious effects on the plant.

2.4.7. Selenium accumulators

Ordinarily, plants growing on soils containing high concentrations of selenium exhibit selenosis, but selenium accumulator species are able to successfully tolerate the element as well as accumulate it to high levels—even up to 1% (dw). Many early studies have shown that species differ markedly in their ability to accumulate selenium. Miller and Byers (1937) reported that the selenium-accumulating plants *Astragalus pectinatus* and *Stanleya pinnata* contained 4000 and 330 ppm selenium respectively, while the non-accumulator grasses *Munroa squarrosa* and *Bouteloua gracilis* growing in the same soil nearby, contained only 4 and 2 ppm selenium respectively.

Beath and his co-workers classified American selenium-tolerant plants according to their selenium contents (Trelease and Beath, 1949):

Group 1: Primary accumulators which grow only on seleniferous soils and accumulate selenium to very high levels. The four major genera are *Astragalus* (Leguminosae), *Xylorhiza* (*Machaeranthera*, Compositae), *Conopsis* (*Haplopapus*, Compositae) and *Stanleya* (Cruciferae). Presumably these species are

selenium endemics. These plants accumulate selenium in the thousands of ppm.

Group 2: Secondary accumulators are plants which accumulate selenium to lower levels but are not restricted to seleniferous areas. Some important genera are *Aster* (Compositae), *Atriplex* (Chenopodiaceae), *Castillega* (Scrophulariaceae), *Grindelia* (Compositae). Plants in this group accumulate selenium in the hundreds of ppm.

Group 3: Weed and grass species which occasionally grow on these areas. These plants contain relatively low levels of selenium—up to 30 ppm.

About 25 out of the 500 or so *Astragalus* species classified in North America are selenium accumulators, and these are spread across several sections of the genus (Trelease and Beath, 1949). They accumulate selenium either as the selenium analogues of the non-protein amino acids, cystathionine S-methylcysteine or occasionally both, whereas non-accumulating *Astragalus* species synthesize the selenium analogues of protein amino acids including S-methylmethionine. The accumulators from Australia, *Neptunia amplexicaulis* and *Morinda reticulata* as well as the accumulator from South America, *Lecythis ollaria* also synthesize selenocystathionine (refer to Table 8.4). The latter two species accumulate the element from low selenium soils but *N. amplexicaulis* grows on high selenium soils. Presumably the accumulators survive by excluding selenium from their enzymes and other proteins (Peterson and Butler, 1967).

The seleniferous soils of the Great Plains area, which support the primary accumulators, contain around 2 ppm soluble selenium (total selenium about 4 ppm), whereas the Irish seleniferous areas contain less than 0·2 ppm soluble selenium and often only 0·02 ppm despite their very high total selenium concentrations (Nye and Peterson, 1975). At these low soluble selenium concentrations, plants are not subjected to environmental stress and species do not need tolerance mechanisms to exclude this element. This geochemical difference between the two soils probably helps explain the absence of a selenium-specific flora in Ireland. There is also a metabolic difference between Se^{+4} and Se^{+6} which is probably involved in the evolutionary development of tolerance. As was mentioned in Section 2.4.2 above, selenate, which is the principal ion in the American toxic soils, is accumulated metabolically, and a tolerance mechanism would definitely be required, whereas selenite, the principal ion in the Irish soils, is largely accumulated by passive processes—and to lower levels. Geochemistry thus

helps to explain why a seleniferous endemic flora has developed on one site and not on another.

3. ARSENIC

3.1. Arsenic in the Environment

3.1.1. Baseline and background studies

Arsenic concentrations in igneous rocks average 2–3 ppm (dw) but shales, clays and phosphate rocks are generally enriched in the element (Levander, 1977). The average arsenic content of soils is 5–6 ppm (dw) but it varies considerably among geographic regions. Soils derived from shales and those from rocks within the metamorphic aureole around the Dartmoor granites (UK) are significantly enriched up to 250 ppm (dw) (Colbourn *et al.*, 1975). Herbage samples from the latter sites contained 2 ppm arsenic (dw) compared with $<0{\cdot}5$ ppm on control sites. Soils derived from quartzite containing a number of mineral veins, near Brisbane, Australia, can contain 100–200 ppm arsenic and have given rise to reduced growth and toxicity symptoms on the leaves of banana palms (Fergus, 1955). Affected leaves contained up to 2 ppm arsenic (dw).

Soils overlying sulphide ore deposits may contain several hundred ppm arsenic; the reported maximum was 8000 ppm (Levander, 1977). Naturally occurring arsenic anomalies occur in Rhodesia often associated with gold and accompanied by antimony (refer to Section 4.3). The values usually vary between 300–5000 ppm arsenic (Wild, 1974*a*) rarely reaching 20 000 ppm. They have given rise to characteristic arsenic-tolerant flora, similar in aspect to that produced by high levels of nickel and copper. These arsenic anomalies produce vegetation anomalies with bare areas and stunting of woody vegetation in what is usually a wooded country. Wild considers that there are no endemic arsenic-tolerant species despite the great age of the anomalies. Perhaps this is related to the relatively low arsenic concentrations encountered in the plants? Although little plant data is available, values up to 240 ppm (ash weight) or approximately 24 ppm (dw) have been reported.

Warren *et al.* (1964) showed that in the neighbourhood of some mineralized veins in British Columbia, Canada, the arsenic content of the A_2 horizon of the soil reached 4600 ppm (dw). These authors reported that the Douglas Fir (*Pseudotsuga menziesii*) commonly accumulated from 10 to 100 times more arsenic than any of the other trees and plants growing in association with it. First-year Douglas Fir stems contained the highest concentration of arsenic with values being reported of up to 10 000 ppm

(ash weight) corresponding to approximately 200 ppm (dw). First-year stems contained higher concentrations than second-year stems and first-year needles contained higher concentrations of arsenic than second-year needles. Corresponding values for other plants in the same geochemical area extended up to 20 ppm (dw) (Girling *et al.*, 1979).

The high concentrations of arsenic in areas of geothermal activity is notable (Lancaster *et al.*, 1971; Reay, 1972; Fowler, 1977). Geothermal mud, fumarole gases, ground water and the surrounding soils have been reported to contain such high concentrations of arsenic that the health of animals grazing the area is seriously affected (Grimmett, 1939; Grimmett and MacIntosh, 1939). Submerged aquatic species growing in geothermal water contained up to almost 1000 ppm arsenic (dw) with lesser amounts in emergent aquatics and terrestrial plants (Reay, 1972). The release of considerable amounts of arsenic into effluent water from geothermal power plants in New Zealand has been reported (Axtmann, 1975; Sabadell and Axtmann, 1975) but its effects on the local arsenic cycle has not been completely studied.

Higher than average contents of arsenic have been found in sandstones and shales associated with uranium–selenium mineralizations in South Dakota and elsewhere in the Great Plains area, USA (Olson *et al.*, 1940). The arsenic content of the seleniferous soils varied from 7–18 ppm and there was no correlation between the arsenic and selenium contents of soils and associated plants. The arsenic concentrations of indigenous plants varied from 1–4 ppm and in general they contained much less arsenic than selenium.

3.1.2. Industrial contamination

There are three major sources of arsenic contamination in the environment; smelting of metals, burning of coal and the use of arsenical pesticides. Smaller amounts of arsenic are released into the atmosphere from the burning of refuse, petroleum and oil burning and a future problem may arise from the production of oil from shale which contains elevated arsenic concentrations (Chappell, 1979). Local contamination may arise from the production and use of arsenic-containing wood preservatives (Grant and Dobbs, 1977).

Mining and smelting of ores: The occurrence of arsenic in lead–zinc and copper ores and its presence in most pyrites causes arsenic contamination of the environment in the vicinity of smelters treating such ores, since some arsenic compounds volatilize during the smelting process (Crecelius *et al.*, 1974; Ragaini *et al.*, 1977). The majority of the arsenic lost in the emissions

is in the trioxide form (white arsenic) which forms arsenous acid when dissolved in water. Gold ores also contain high concentrations of arsenic, mainly as arsenopyrite, leading to pollution problems near gold mines and refining operations (O'Toole *et al.*, 1971; Rosehart and Lee, 1973; Jervis and Tiefenbach, 1979). Arsenic can also be emitted from secondary smelters again giving rise to elevated arsenic levels in the vicinity (Temple *et al.*, 1977). The arsenic content of grass and crops in general increases with soil content, but may be small compared with the large amounts in some contaminated soils (Thornton *et al.*, 1979).

Stack dust and flue gases from smelter operations have contaminated soils and plants to varying degrees depending on the distance of sample collection from the smelter and the efficiency of precipitators and baghouses, stack height, etc. Crecelius *et al.* (1974) report that within 5 km of a large copper smelter, arsenic concentrations in the surface soil reached 380 ppm (dw) while concentrations up to 260 ppm (dw) were recorded in surface soils near a lead smelter (Ragaini *et al.*, 1977). In this latter case, grass samples reached 59 ppm (dw) one quarter of a mile from the smelter, while grass samples 1·8 miles from a gold refining site reached 89 ppm (O'Toole *et al.*, 1971). Concentrations of up to 2000 ppm arsenic were recorded in top soil by Temple *et al.* (1977) a concentration which is known to markedly inhibit growth of white spruce trees (Rosehart and Lee, 1973).

Arsenical mine dumps and smelter slag tips containing large concentrations of arsenic and associated metals can be found throughout the world. The most widely studied examples are located in the UK (Porter and Peterson, 1975, 1977*a*,*b*) and Rhodesia (Hill and Nothard, 1973; Wild, 1974*b*) where concentrations of arsenic commonly reach 1–3 % in the spoil material. The major geochemical difference between the two environments lies in the pH of the spoil; values of 8–9 being typical of Rhodesian spoil, while the UK samples range between 2 to 4. As a result, the Rhodesian plants contained low concentrations of arsenic while the UK plants contained high concentrations. The predominant role of acidity is illustrated by the work of Hutchinson and Kuja (1979) where *Deschampsia cespitosa* grown on spoil containing 5200 ppm arsenic (pH 1·8–2·0) contained 3200 ppm arsenic, while the same species on a neutral spoil (72 000 ppm arsenic, pH 6·9–7·0) contained only 300 ppm.

Mature *Agrostis tenuis* leaves collected from UK sites accumulated up to 1 % arsenic (dw) but mean values of around 1000–3000 ppm (dw) were commonly found for grasses and other arsenic-tolerant plants collected from various tips in the UK (Porter and Peterson, 1975). These tips support an impoverished and restricted flora which has evolved arsenic tolerance in

recent times. In Rhodesia perhaps the tops have been colonized by migration from the natural arsenic anomalies nearby. The extent of vegetational cover is largely dependent upon the arsenic concentration in the spoil, but some tips, containing the highest levels of arsenic, are quite bare (Wild and Wiltshire, 1971*a*,*b*).

Coal fired power generation: Arsenic concentration in coal from the USA, Australia and the UK range from around 0·5–93 ppm (dw) (Swaine, 1977; Lim, 1979) with coal from the USA containing the highest concentrations. Brown coals from Czechoslovakia can contain up to 1500 ppm arsenic (dw) (Bencko and Symon, 1977). Arsenic exists largely as arsenopyrite in coal (Duck and Himus, 1952; Swaine, 1975) and occurs as arsenic trioxide in the emissions from power plants (Bencko and Symon, 1977). Fly ash particles retained in the plant are enriched with arsenic, the highest concentration being recorded on the smallest particles (Campbell *et al.*, 1978; Lim, 1979; Coles *et al.*, 1979). The very small airborne fly ash particles can contain up to 1700 ppm arsenic. Elevated levels of arsenic were found in soil collected around a generating station (Temple *et al.*, 1977).

Analysis of seven species of crop plants grown on soil amended with pfa showed that the arsenic concentrations were higher than in the control plants grown in soil alone (Furr *et al.*, 1976*a*). Yields were variable, but no attempt was made to correlate them with the concentration of potentially toxic metals present. At acid pH, Collier and Greenwood (1977*a*) concluded that the concentrations of soluble arsenic were low in pfa, while at alkaline values, arsenic concentrations were high and a potential cause of phytotoxicity. These authors then showed that the concentration of arsenic in solution would seriously restrict root growth although the phytotoxicity was influenced also by the concentration of phosphate present (Collier and Greenwood, 1977*b*). They concluded that arsenic may well be a long-term source of phytotoxicity in pfa.

3.1.3. Herbicides, insecticides and desiccants

From the late nineteenth century to the middle of the twentieth century inorganic arsenicals were used as general pesticides in orchards and potato fields. Lead arsenate was used for insect control in orchards and calcium arsenate in cotton, tobacco and blueberry fields (Small and McCants, 1961, 1962; Chisholm, 1972; Anastasia and Kender, 1973). Paris green (copper acetoarsenite) magnesium arsenate, zinc arsenate, zinc arsenite and many others have been used from time to time as specific insecticides (Levander, 1977). Sodium arsenite has been widely used as a weedkiller and a non-selective soil sterilant, while arsenic acid has been used extensively as a

cotton desiccant (Woo, 1965; Tammes and DeLint, 1969; Steevans *et al.*, 1972; Levander, 1977). More recently the organic arsenicals, monosodium methanearsonate (MSMA), disodium methanearsonate (DSMA) and cacodylic acid ((CA) hydroxydimethylarsine oxide) have been introduced for use as herbicides, silvicides and as a desiccant (Levander, 1977).

Arsenic residues have been found in soils receiving inorganic and organic arsenicals but these are especially high in old orchard soils which received lead arsenate (Woolson *et al.*, 1971*a*; Chisholm, 1972; Hess and Blanchar, 1977). In general, these orchard soils containing up to 2500 ppm arsenic (Woolson *et al.*, 1971*a*) are phytotoxic to various crops (Vandecaveye *et al.*, 1936; Jones and Hatch, 1937; Crafts and Rosenfels, 1939; Kardos *et al.*, 1941) but the degree of toxicity depended on pH and the concentration of phosphate, iron, aluminium and the amount of organic matter (Woolson *et al.*, 1971*b*). Phytotoxicity is also dependent upon the sensitivity of the crop (Deuel and Swoboda, 1972*a*; Woolson, 1973), therefore total soil arsenic does not accurately reflect the form that is available to plants or its degree of phytotoxicity.

Regardless of the form in which the arsenical is applied, it is eventually oxidized and metabolized to arsenate (Woolson, 1973). Extractable arsenic at 5 ppm is toxic to sensitive species while at 50 ppm, growth of less sensitive crops (containing up to 15 ppm (dw)) may be reduced by up to 50 %. Yield-limiting arsenic concentrations in plants were calculated to be 1 ppm and greater in soybeans (a sensitive crop) and 4·4 ppm and greater in cotton (Deuel and Swoboda, 1972*a*). Even greater differences in crop sensitivity were recorded by Woolson (1973). Plant arsenic concentrations corresponding to a 50 % growth reduction were 0·7 ppm in the tomato fruit, 10 ppm in spinach leaf and up to 76 ppm in radish root. Grain and edible seeds like peas and sweet corn accumulate less arsenic than other parts of the plant (MacPhee *et al.*, 1960; Jacobs *et al.*, 1970*a*).

Arsenic has not been one of the elements of major concern in sewage sludge and it has generally been neglected in analytical surveys. Haan (1978) quotes values from 5·8 ppm to 45·9 ppm in sludges from Holland, and Furr *et al.* (1976*b*) recorded values from 3 to 30 ppm in sludge from the USA. Temple *et al.* (1977) found 4·2–19·8 ppm arsenic in top soil collected from a sewage treatment plant in Canada. The corresponding concentration of arsenic in the surrounding vegetation was from 0·3 to 1·2 ppm.

3.2. The Arsenic Cycle

Various arsenic cycles, either on a global basis for agronomic ecosystems, or in natural waters, have been proposed in recent years (Frost, 1967; Allaway,

1968; Ferguson and Gavis, 1972; Wood, 1974; Sandberg and Allen, 1975; Levander, 1977; Mackenzie *et al.*, 1979). Arsenic can be emitted into the atmosphere in significant quantities from high-temperature sources such as vapour from coal-fired power generating plants, burning of vegetation and vulcanism. Estimates of inputs into the environment reveal that industrial and fossil fuel emissions are high (780×10^8 g arsenic yr^{-1}) compared with mining (460×10^8 g arsenic yr^{-1}) and continental and volcanic dust fluxes (28×10^8 g arsenic yr^{-1}) (Mackenzie *et al.*, 1979). Natural low-temperature biological methylation also releases arsenic into the atmosphere as volatile compounds (refer to 3.3.2).

Wood (1974) suggested that in reduced environments such as sediments, arsenate is reduced to arsenite and methylated to methylarsonic acid or dimethylarsenic acid. These compounds may be further methylated or reduced to trimethylarsine or dimethylarsine and lost to the atmosphere. Mackenzie *et al.* (1979) calculated that 210×10^8 g arsenic are vaporized annually from the land surface by these processes. The continental vapour flux is about eight times the continental dust flux, pointing to the important role of organisms in the cycling of arsenic. Whether plants can release volatile arsenic compounds by enzymatic processes analogous to the release of volatile selenium compounds does not appear to have been established. Perhaps naturally occurring volatile arsenic compounds are not as important as volatile selenium compounds in geochemical cycles, for Låg and Steinnes (1978) reported that arsenic concentrations in the humus layers of Norwegian forest soils depended on local geology rather than on the supply of the element from rainfall. The selenium concentration however distinctly decreased with increasing distance from the ocean (refer to Section 2.2).

The activities of society have given rise to a gain of arsenic on the land from industrial and pesticide operations, and in the oceans from present-day denudation rates of the continents. Mackenzie *et al.* (1979) calculated that the arsenic content of sea water will rise substantially compared with the predicted accumulation rate for selenium. They concluded that 'concern over a rapid build-up of arsenic concentration to a toxic level in these major global reservoirs is probably unwarranted'. Nevertheless, man's activity does cause high environmental concentrations at some locations.

3.3. Arsenic in Soils

3.3.1. Introduction

The physical and chemical characteristics of soil are important controls of its adsorptive capacities and therefore will affect the availability of

arsenic to plants. A wide variety of arsenicals can be absorbed by plants, but they have different toxic effects. The transformations occurring in the soil and the availability of the arsenicals are two important factors to be considered when studying the plant–soil system.

Bohn (1976) calculated that under the E_h and pH conditions likely to be encountered in soils, inorganic arsenic could be present in solution as either arsenate or arsenite. Under reducing conditions, arsenic–sulphur complex ions may be present and arsenite would be likely to be the dominant form (Deuel and Swoboda, 1972*b*). Elemental arsenic and arsine can also exist in strongly reducing environments (Walsh and Keeney, 1975). However, arsenate would be the stable oxidation state in oxygenated environments, with $H_2AsO_4^-$ predominating under acidic conditions and $HAsO_4^{2-}$ predominating under alkaline conditions.

In the last decade, experimental techniques have been developed to separate the inorganic and organic species of arsenic found in the environment (Braman and Foreback, 1973; Andreae, 1977). These have been utilized by some researchers to investigate the arsenic species in the soils. Braman (1975) shows that dimethylarsinic acid (cacodylic acid) may be a ubiquitous arsenic compound found in all soils and predominant in many. Porter and Peterson (1977*a*,*b*), however, found that arsenate was the predominant water-soluble form in mine spoils, although dimethylarsinic compounds were occasionally detected. Woolson (1973) states that regardless of the form in which the arsenical is applied, it is eventually oxidized and metabolized to arsenate. In later work, when more analytical techniques were available, he suggests that trimethylarsine (TMA) may also be an important compound (Woolson, 1977).

Methanearsonic acid and methanearsonates differ from arsenate by having a methyl-group substitute for a hydroxyl group linked to the arsenic atom. The relative proportions of ionic and molecular forms of organoarsenicals are determined primarily by pH of the solution (Hiltbold, 1975). In most agricultural soils (pH 5·0–7·0) the univalent ion of methanearsonates predominates, but both the cacodylate ion and acid may occur in these soils.

3.3.2. Microbiological transformations

During studies of the persistence of inorganic and organic arsenicals the importance of microbial transformations has become apparent. Both oxidative and reductive changes in methanearsonates and cacodylic acid occur in soil (Hiltbold *et al.*, 1974). Oxidation of the methyl carbon of disodium methanearsonic acid (DSMA) to carbon dioxide occurs under

aerobic soil conditions and is directly affected by the amount of organic matter available for microbial activity (Dickens and Hiltbold, 1967). Von Endt *et al.* (1968) found that 20% of monosodium methanearsonic acid (MSMA) added to a soil bacterial culture was degraded to carbon dioxide and arsenate after three days. No arsenite could be detected. Cacodylic acid (CA) has also been shown to be demethylated by microbial activity (Woolson and Kearney, 1973). Sachs and Michael (1971), however, did not find any proof that methanearsonates added to soils were demethylated.

Several studies on the loss of volatile arsines have been reported. Reed and Sturgis (1936) suggested that arsenate was reduced to arsenite and lost as arsine from flooded rice soils, although later this was found to be only a small proportion of the total arsenic. In similar reduced environments, Deuel and Swoboda (1972*b*) found no loss of arsine from arsenate treated soils. Recently, gaseous arsenic evolution studies on soils and sediments have been carried out using radioactive tracers. Extensive volatilization of arsenic (60%) from CA treated soils occurred under anaerobic conditions, while only 35% was lost under aerobic conditions (Woolson and Kearney, 1973). Akins and Lewis (1976) also found arsenic losses from soils treated with DSMA which were a function of moisture content as well as organic matter content. In later work, Woolson (1977) found alkylarsines being generated more rapidly and in greater quantities from aerobic soils than from anaerobic soils, regardless of the arsenical treatment.

Both TMA and DMA have been isolated and identified in air above grass treated with sodium arsenite or CA, while only TMA was identified in air above grass treated with methanearsonic acid (MAA) (Braman, 1975). Air above soils treated with sodium arsenate, MSMA or CA, was also found to contain TMA and DMA, but not methylarsine (Woolson, 1977). Cheng and Focht (1979), however, found no indication of DMA or TMA evolution from arsenate-treated soils and have identified the volatiles emitted from the soils as arsine and methylarsine.

3.3.3. Soil chemistry

Arsenic can be added to soils in many forms, including inorganic arsenite and arsenate. Arsenite will be oxidized in normal agricultural soils to arsenate. Compounds of methanearsonic acids or CA are also used as herbicides and hence arsenic is present in these forms in soils. These organic forms may be slowly oxidized to inorganic arsenate and carbon dioxide (Dickens and Hiltbold, 1967) in the presence or absence of microorganisms or may be volatilized and lost from the soils. It is also apparent that the methyl substituent of the methanearsonate ion does not interfere with its

adsorption and it reacts as arsenate in soil systems (Braman, 1975). Wauchope (1975) suggests that all the arsenical herbicides, even those with organic substituents, act like their inorganic analogues. It is only recently that the importance of organic arsenic species in long term studies has been shown (Woolson, 1977; Braman, 1975). However, the majority of work carried out on persistence and absorption of arsenic in soils has been on inorganic arsenate.

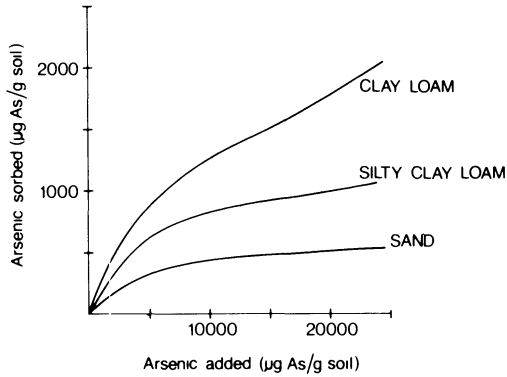

FIG. 8.1 The sorption of arsenic by different soils. (Modified from Jacobs *et al.*, 1970*b*.)

Arsenic persistence is controlled by the sorption capacity of the soil and by leaching losses. It is also likely that a significant proportion of added arsenic will be lost as a gas from soil, as already discussed.

The sorption capacity of a soil is affected by the texture, sesquioxide content and the presence of other interfering elements. Sand and silt fractions show little arsenic sorption capacity due to the low surface area and the predominance of quartz (Dickens and Hiltbold, 1967) while the clay fraction is the main sorber (Johnson and Hiltbold, 1969; Jacobs *et al.*, 1970*b*). This is because arsenate, as with phosphate, is adsorbed by minerals possessing exposed hydroxyl groups, such as kaolinite and iron and aluminium oxides: montmorillonite and vermiculite have a much lower absorptive capacity (Dickens and Hiltbold, 1967). The influence of the clay fraction on arsenic sorption can be seen in Fig. 8.1.

The presence of iron and aluminium oxides in soil is often correlated with the clay minerals present (Wauchope, 1975) and hence to soil texture. The importance of iron, aluminium and other cations on arsenic sorption can be ascertained by partial soil extractants. A chemical extraction procedure

(Williams *et al.*, 1967) similar to that used to characterize the phosphorus components in soil (Chang and Jackson, 1957) has been used by many workers to elucidate the manner in which arsenic is held in soils (Johnson and Hiltbold, 1969; Woolson *et al.*, 1971*a*,*b*; Jacobs *et al.*, 1970*b*). Much of the arsenic in soils is sorbed by amorphous iron and aluminium compounds (Wauchope, 1975). Woolson *et al.* (1971*a*,*b*) found that soils with high reactive iron (Schwertmann, 1964) had predominantly iron-bound arsenic.

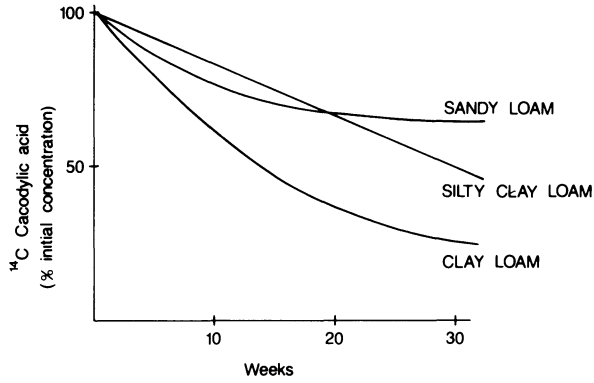

FIG. 8.2 Persistence of ^{14}C-Cacodylic acid in different soil types. (Modified from Woolson and Kearney, 1973.)

If reactive iron was low, then aluminium–arsenic or calcium–arsenic compounds were the predominant forms of arsenic. Woolson and Kearney (1973) investigated the distribution of CA in the various soil fractions. They found that, in contrast to inorganic arsenic which was mainly present in the iron and aluminium forms, CA was distributed thus: water soluble > Al > Fe > Ca.

Soil pH influences the activity of aluminium in clays and hydrous oxides of soil colloids, but the amount of these colloids, rather than the pH effect, is the governing factor in their adsorptive capacity (Hiltbold, 1975).

The sorption of arsenic is time dependent (Walsh and Keeney, 1975); equilibration of arsenic additions to soils is reached in less time with coarse-textured soils or with low levels of added arsenic (Jacobs *et al.*, 1970*b*). Less arsenic is present in the available fraction in fine-textured soils and this means that arsenic residues are less phytotoxic in these soils (Jacobs *et al.*, 1970*b*).

CA persistence is also affected by soil type. Figure 8.2 shows that after 32 weeks in an enclosed container more C^{14} remained in a sandy loam than

in a clay loam; the losses would primarily be through microbial transformations. There is a lack of residual phytotoxicity from CA which is advantageous when using organo-arsenical herbicides (Hiltbold, 1975). (Refer to Section 2.4.2.)

Wauchope (1975) in his studies of the sorption of the arsenicals and phosphate, concludes that sorption increases in the order $P < CA <$ arsenate $\simeq$ methylarsonate. Phosphate is a relatively immobile chemical and it is predicted that the arsenical herbicides should pose little threat to ground waters by leaching, although CA is more likely to be lost by leaching than inorganic arsenic.

Dickens and Hiltbold (1967) found a considerable difference in rates of movement of arsenic, as DSMA, through columns of clay loam and loamy sand. Fifty-two percent was lost from the loamy sand column, while none was leached through the clay loam with 51 cm of added water. About half of the added DSMA remained in the top 2 cm of the clay loam. Schweizer (1967) in complementary work on the phytotoxic effects of DSMA found a marked reduction with time after DSMA addition to soil.

Leaching of arsenic is particularly significant in soils of low adsorptive capacity (Hiltbold *et al.*, 1974). In clay soils, arsenic will be maintained in the iron and aluminium complexes and leaching will occur much more gradually (Tammes and De Lint, 1969). Arsenic concentrations in soil, as arsenate and MSMA, have been shown to continuously decline at rates directly related to the concentration present (Neiswander, 1951; Tammes and De Lint, 1969; Hiltbold *et al.*, 1974). In studies of methanearsonate movement over four and six years, essentially all of the applied arsenic was recovered in the plough layer (Hiltbold *et al.*, 1974) or top 30 cm of the soil (Johnson and Hiltbold, 1969).

3.4. Agricultural Effects

3.4.1. Introduction

Arsenicals have been used in agriculture as pesticides or plant defoliants for many years, as mentioned in Section 3.1.3. Organic arsenicals have largely replaced inorganic forms as selective or general herbicides. They are applied at much lower rates than the inorganic arsenicals thus reducing the problems associated with arsenic accumulations in agricultural and horticultural soils (Robinson, 1975; Walsh and Keeney, 1975).

3.4.2. Phytotoxicity

The phytotoxicity of arsenic is affected considerably by the form in which it occurs in the soil. Arsenite is more phytotoxic than arsenate and both are

much more phytotoxic than MSMA and CA when present in soil. If the arsenicals are foliarly applied then CA is the most phytotoxic (Sachs and Michael, 1971). The following discussion will concentrate on the effects of arsenic residues in the soil and not on foliar applications.

The phytotoxicity of arsenic residues is influenced more by their chemical forms than by their amount in a given soil (Woolson *et al.*, 1971*a*). It is rare that a yield decrease can be correlated with total soil arsenic in all soils (Sandberg and Allen, 1975). The different forms vary in their availability and hence toxicity; water-soluble arsenic is more phytotoxic than other more firmly bound forms. Woolson *et al.* (1971*b*) showed a highly significant correlation between the logarithm of total soil arsenic and growth reduction in corn ($r = 0{\cdot}74$), but when the sum of arsenic contained in the water soluble Ca–As, Al–As and Fe–As fractions was correlated with growth the correlation coefficient (r) was 0·82. Vandecaveye *et al.* (1936) concluded that poor growth of alfalfa and barley was due to the readily soluble arsenic in orchard soils. Woolson *et al.* (1971*b*) showed significant correlations between growth reduction and mixed acid and bicarbonate extractable arsenic. Walsh and Keeney (1975) suggest that the use of Bray P-1, sodium bicarbonate or the mixed acid extractant to predict arsenic phytotoxicity will be most convenient, because they are routinely used for available phosphorus.

Soil texture is an important factor in determining the phytotoxicity of arsenic added to soil. High rates of calcium arsenate application lead to arsenate residues in coarse-textured cotton coils. These residues caused reduction in the growth of several plants including cotton, soybeans and rice. Similar application rates to fine-textured soils did not produce toxic problems to these plants (Reed and Sturgis, 1936). Woolson (1973) also observed similar effects of texture, as are shown in Tables 8.5 and 8.6. Yields indicated that arsenic was more phytotoxic to all crops in the Lakeland soil, while residues in Hagerstown soil were least phytotoxic. In comparable studies on the effect of arsenic on corn growth, a much more pronounced toxicity was observed on Plainfield sand (7% clay) than on Waupun silt loam (29% clay) with the addition of 20 or 80μg arsenic/g soil (Jacobs and Keeney, 1970). The yield reductions were directly related to the soil-extractable arsenic levels measured in Bray P-1 or ammonium acetate. Woolson (1973) found correlations accounting for 64–83% of the variation between available arsenic levels and plant growth. He used regression equations to determine levels of available arsenic necessary to reduce plant growth by 50%. The order of crop sensitivity is the order in which the plants are placed in Table 8.6.

TABLE 8.5

SELECTED SOIL CHARACTERISTICS AND THE EFFECT OF ARSENIC ADDITIONS ON ARSENIC AVAILABILITY (MODIFIED FROM WOOLSON, 1973)

	pH	Clay content (%)	Available arsenic[a]			
			50[b]		500[b]	
			0 months	19 months	0 months	19 months
Lakeland loamy sand	6·2	10·5	20	11·6	384	440
Hagerstown silty clay	5·5	30	6·0	5·3	18·3	11·8
Christiana clay	4·4	24·4	18·3	11·3	429	256

[a] Arsenic extracted using mixed acids.
[b] Arsenate applied (μg/g).

The reducing conditions under which rice is grown may make arsenic more available (Lockard and McWalter, 1956) or may reduce arsenate to arsenite which is a more toxic form. Reed and Sturgis (1936) reported that farmers in the southern USA had difficulty in growing rice in fields previously used for cotton which had been treated with calcium arsenate, perhaps due to these reasons. There is also evidence that rice is more susceptible to arsenic toxicity than are dryland crops.

Organic arsenicals are applied at considerably lower single and annual

TABLE 8.6

THE EFFECT OF ARSENIC ADDITIONS ON CROP YIELDS (MODIFIED FROM WOOLSON, 1973)

Crop	Arsenate applied (kg/ha)	Plant growth as % of control in each soil		
		Lakeland loamy sand	Hagerstown silty clay	Christiana clay
Green beans	50	6	71	78
	500	NG	3	<1
Radish	50	128	75	44
	500	NG	10	4
Tomato	50	58	94	79
	500	NG	23	3
Cabbage	50	98	109	78
	500	NG	27	1

NG = no growth.

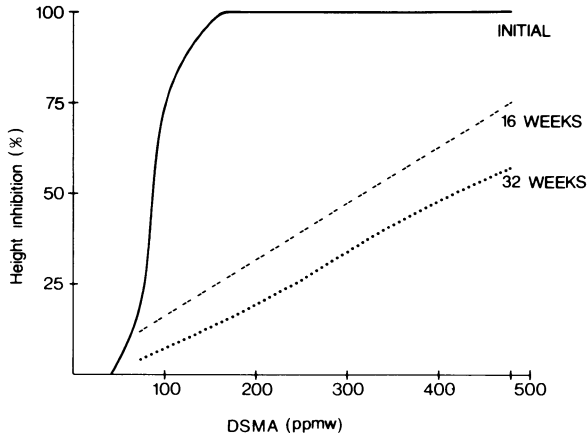

FIG. 8.3 The effect of DSMA on cotton growth after 0, 16 and 32 weeks from application. (Modified from Schweizer, 1967.)

rates than inorganic forms and therefore there should be less phytotoxicity to crops from soil residues. MSMA or CA herbicides are applied to certain orchard crops up to three times a season at rates not to exceed 4·5 kg MSMA/ha or 5·6 kg CA/ha (Sandberg and Allen, 1975). They calculated the greatest input of elemental arsenic/ha/yr as 5495 g from MSMA or 9122 g from CA. Normally the rates required for most agricultural crops and for weed control would not be this large. Schweizer (1967) found only small growth effects in soybeans or oats from DSMA residues in cotton soils. Based on his greenhouse studies, cotton tolerated 50 ppm arsenic. He calculated, with no correction for arsenic losses, that it would take more than 40 years for DSMA levels to build up to this concentration at present recommended application rates. However, the crops grown in rotation with cotton were more susceptible. Due to the sensitivity of rice to DSMA, further studies were carried out to investigate the effects of different levels of DSMA additions to different soil types. The importance of soil texture in determining the phytotoxicity of arsenic additions was demonstrated again. The addition of DSMA at levels between 2·5 and 100 ppm only significantly affected rice growth in a silt loam (39% sand, 57% silt, 4% clay: pH 7·9), but not a clay soil (10% sand, 23% silt, 67% clay: pH 6·3), or another silt loam soil (21% sand, 57% silt, 22% clay: pH 6·4):

Incorporation of DSMA reduced cotton growth initially but toxicity decreased with time as is shown in Fig. 8.3. Similar results have been reported by other workers (Sandberg & Allen, 1975).

3.5. Arsenic in Plants

3.5.1. Introduction

Plant accumulation of arsenic can be affected by many factors, including plant species, type of compound and method of application, soil conditions and fertilizer application. It is rare that arsenic accumulations in plants reach levels that are harmful to animals and man because invariably, growth is reduced before the content will reach toxic levels. This was shown by Woolson (1973) who calculated the values in crops that would occur

TABLE 8.7
ARSENIC CONTENT OF CROPS AT A GROWTH REDUCTION OF 50% (GR^{50})[a]

Crop	Arsenic at GR^{50} (ppm)	
	Edible dry plant	Whole dry plant
Radish	76·0	43·8
Spinach	10·0	10·0
Green bean	4·2	3·7
Cabbage	1·5	3·4
Lima bean	1·0	1·7
Tomato	0·7	4·5

[a] Data obtained from crops grown immediately after sodium arsenic additions. (Modified from Woolson, 1973.)

when their growth had been reduced by 50% (GR^{50}). The contents (Table 8.7) were calculated from regression equations of available arsenic in the soil on crop arsenic content and growth. It is unlikely that farmers could economically harvest a crop with such a reduction in yield; therefore levels of this kind are infrequent.

3.5.2. Arsenic in crops

Average values for arsenic contents in crops are shown in Table 8.8. The highest residues of arsenic are found in plant roots (e.g. sugar beet and radish), with intermediate values in the vegetative top growth (e.g. spinach, grasses), and edible seeds and fruits containing the lowest levels of arsenic (Liebig, 1966; Woolson, 1973; Walsh and Keeney, 1975). The limit set for arsenic content in fruits, crops and vegetables is 2·6 ppm (fw) (US Public Health Service). Most data are now presented in terms of dry matter, which does not facilitate comparison with this limit. It is possible that data given

TABLE 8.8
AVERAGE ARSENIC CONTENT OF CROPS

Crop	Arsenic content (ppm)	Author
Sugar beet	2·83	Sandberg and Allen (1975)
Sorghum fodder	2·15	
Sorghum seed	0·17	
Cotton lint	1·27	
Cotton seed	0·06	
Bermuda grass	1·23	
Oats	0·80	
Sugar cane	0·30	
Alfalfa hay	0·29	
Barley	0·13	
Soybeans	0·12	
Corn fodder	0·22	
Corn seed	0·10	
Wheat	0·07	
Pasture	9·6	Thoresby and Thornton (1979)
Lettuce	1·4	
Strawberries	0·7	
Barley grain	0·4	
Vegetables	0·26	Schroeder and Balassa (1966)
Grain	0·70	

are affected by various contaminations which might account for the elevated levels quoted, e.g. potato peelings (Jacobs *et al.*, 1970*a*) (Table 8.9) and radish (Woolson, 1973) (Table 8.7), where soil particles might be firmly adhered to the outer skin. Jacobs *et al.* (1970*a*) also reported a problem of aerial dust contamination which might give elevated levels for whole-plant crops.

The effects of inorganic and organo-arsenical treatments on crop content have been investigated under normal, as well as exceptionally high applications (Table 8.9). Ehman (1966) found no arsenic residues in crops rotated with cotton or cotton after DSMA additions of 5·6 kg/ha and 11·2 kg/ha. No increases in cotton content were noted by Baker *et al.* (1969) after similar organo-arsenical treatments. In comparison, Johnson and Hiltbold (1969) found that additions of all methanearsonates at similar levels although not affecting the yield, did increase the arsenic content of cotton, soybeans, corn, oats, vetch, clover and sorghum. A difference in uptake dependent on the herbicide was noted for some crops; cotton

TABLE 8.9
ARSENIC CONTENTS OF CROPS AFTER SELECTED ARSENICAL TREATMENTS

Crop	Arsenic content (ppm)		Treatment
	After 1 year	After 2 years	
Corn fodder	3·53	0·89	Sandberg and Allen (1975). After 10 years normal CA treatment in one application
Corn seed	0·16	—	
Soybean seed	1·6	0·55	
Wheat	0·38	0·21	
Cotton seed		5·2	Johnson and Hiltbold (1969). After normal MA treatments
Soybean seed		4·5	
Sorghum		3·1	
Corn grain		2·5	
Crimson clover		1·9	
Oats		1·7	
Vetch		1·6	
Potato peelings		84·0	Jacobs *et al.* (1970*a*). 5–85 years arsenite treatment in one addition
Potato tubers		0·5	
Snap bean seeds		1·0	
Peas		ND	
Sweetcorn		ND	

ND = not detected.

showed more uptake from MSMA and DSMA than from MAMA, while no difference was found in soybeans. Treatments equivalent to between 5 and 85 years application increased the content of potatoes and snapbeans, but arsenic was below detection limits in the edible portion of peas and sweetcorn (Jacobs *et al.*, 1970*a*). Johnson and Hiltbold (1969) from their studies of the effects of normal DSMA treatments suggest that only sustained use of DSMA may increase the soil arsenic residue sufficiently to be manifest in the crop arsenic content. The values they quote (Table 8.9) for crops, however, are much higher than those cited by Sandberg and Allen (1975) after treatments equivalent to ten times the normal application.

Arsenic residues in plants can be related to soil arsenic levels, but there are many barriers to its movement in plants and the effect of soil parameters on arsenic availability does not allow good correlations. Woolson's (1973) results support this statement when he found that available soil arsenic correlated better with whole-plant arsenic content than with the edible plant part. Increasing the pH of grassland soils has been shown to decrease

plant uptake (Everett, 1962). The arsenic content of corn grown on sand, rather than on a silt loam, was increased by ten fold. The latter values were reflected in the available soil arsenic levels (Bray P-1) (Jacobs and Keeney, 1970). These results emphasize the importance of soil effects on the toxicity of added and residual arsenical compounds.

The data given in Tables 8.7, 8.8 and 8.9 show the species difference in arsenic uptake and the variations in plant part concentrations. There is also a difference in uptake between winter and summer crops, even with the same available arsenic levels (Benson, 1953; Johnson and Hiltbold, 1969). A suggested explanation has been that the cool, humid winter season keeps transpiration and absorption of water and arsenic at much lower levels than prevails with summer crops (Johnson and Hiltbold, 1969).

Even when elevated applications have been used in experiments, the arsenic content in crops has rarely been increased above normal levels, and certainly not above the recommended limits (Johnson and Hiltbold, 1969; Jacobs *et al.*, 1970*a*; Sandberg and Allen, 1975). It can be concluded that the major danger of present arsenical pesticides is that of decreased productivity rather than high arsenic uptake by the consumers of such crops (Jacobs *et al.*, 1970*a*).

3.5.3. Effects of fertilizers on arsenic in crops

Nutrient addition to a soil may cause competition between elements for fixation sites in the soil and for root uptake. Fertilizer additions can significantly affect available soil arsenic when values are high (100–500 μg As/g soil) with levels increasing with addition of N and P or N, P and K. Levels were significantly reduced by calcium carbonate addition (Woolson, 1972). It was concluded that the addition of NH_4NO_3 and $Ca(H_2PO_4)$ was the best fertilizer treatment giving the lowest available arsenic levels, arsenic residues and least effect on plant growth regardless of arsenic treatment. The addition of N, N and P or N, P and K has increased arsenic residues in corn (Woolson, 1972).

Phosphorus is the most influential of fertilizer materials on arsenic toxicity (Woolson, 1972). Arsenate, but not arsenite, uptake is affected by phosphate (Clements and Munson, 1947). This is because arsenate and phosphate are chemically similar and compete for the same sites in soils or transport systems (Dean and Rubins, 1947). Phosphorus has been used to ameliorate arsenic toxicity (Benson, 1953) and several authors have noted a reduction in effectiveness of arsenic, as phosphorus levels increase, on a wide variety of crops (Benson, 1953; Hurd-Karrer, 1939; Woolson *et al.*, 1973).

In solution culture experiments, increasing the ratio of phosphorus to arsenic caused reduced arsenic uptake (Rumberg *et al.*, 1960; Hurd-Karrer, 1939). At phosphorus/arsenic molar ratios of 4:1 or greater, arsenic toxicity on wheat was substantially reduced (Hurd-Karrer, 1939). Rumberg *et al.* (1960) found similar results when the solutions contained sufficient arsenic to be toxic.

Soil phosphorus/arsenic ratios do not, however, appear to be a reliable indicator of their interactions and effects on plant growth. Walsh and Keeney (1975) suggest that this is partly due to the difficulty of evaluating 'available' phosphorus and arsenic in soils. Another suggestion is that the ratio response seems to be valid only when the potential arsenic concentrations in solutions or soil extracts are large enough to be toxic (Sandberg and Allen, 1975). Woolson *et al.* (1973) observed a reduction in arsenic toxicity due to phosphorus/arsenic ratios of 0·7:1 and 42:1. They found no influence, however, at a ratio of 6·8:1, with high levels of added arsenic (1000 μg As/g) which does not support the latter hypothesis.

On naturally low phosphorus soils, phosphorus additions caused a significant reduction in arsenic toxicity in some grasses. However, no effect was found at the higher P treatments (Carrow *et al.*, 1975). On a soil with high phosphorus treatments, the grasses exhibited no significant effect of phosphorus on arsenic toxicity.

At comparable soil arsenic levels, addition of phosphorus has significantly increased the arsenic content of the plant, In contrast, however, the effect of arsenic on phosphorus was not consistent. The amount of phosphorus in the soil solution appeared to govern the uptake of arsenic into the plant because when solution phosphorus was high, the effect of arsenic was reduced (Woolson *et al.*, 1973). Everett (1962) also found that applications of phosphorus could increase arsenic uptake, but found that the expected injury was eliminated by the high phosphorus content in the plant.

Albert (1934) and other workers have recorded that the addition of phosphorus increased arsenic toxicity to several crops. Jacobs and Keeney (1970) found that corn yields were reduced similarly regardless of levels of applied phosphorus. At 80 μg arsenic/g soil, phosphorus did not influence arsenic toxicity on a silt loam soil, but enhanced the toxic effects on Plainfield sand. Similar results were obtained when phosphorus additions were made to a sandy soil by Woolson *et al.* (1973) as is shown in Fig. 8.4. Woolson found a significant increase in plant growth with both arsenic and phosphorus additions at 100 μg/g soil, but at higher additions phosphorus decreased yield when corn was grown on a silt loam. In the silty clay loam,

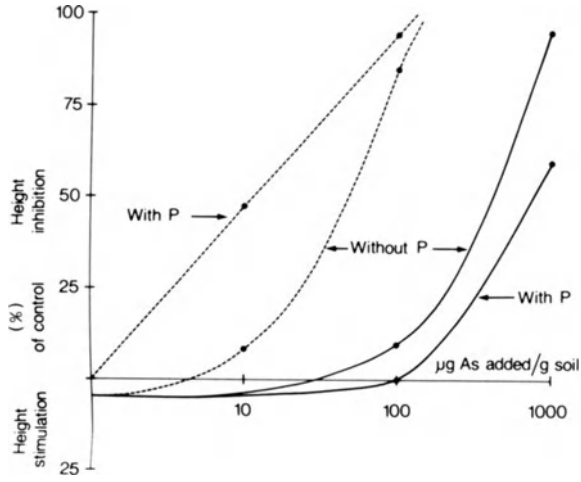

FIG. 8.4 Corn growth responses to arsenic and arsenic plus phosphorus (added in equimolar amounts) to two soils. (Modified from Woolson *et al.*, 1973.) ----, Lakeland sandy loam; ——, Hagerstown silty clay loam.

arsenic was fixed more readily than phosphorus and therefore arsenic became less available for plant uptake.

However, these results suggest that in sandy soils, phosphorus displaces arsenic from the soil system (Dean and Rubins, 1947; Jacobs and Keeney, 1970). Enhanced phytotoxicity to cotton from DSMA additions occurred when increased increments of phosphorus were added to a silt loam, but the effect was reduced on another silt loam. This was possibly because of the higher clay content increasing the fixing capacity in the latter (Schweizer, 1967).

Woolson (1975) (cited by Sandberg and Allen, 1975) found that low levels of phosphorus added to an arsenic toxic soil will displace arsenic from soil particles to increase the toxicity to plants, but larger applications of phosphorus will compete with arsenic at the root surface and decrease toxicity.

3.5.4. Arsenic uptake and translocation

Arsenate uptake by barley seedlings was found by Asher and Reay (1979) to consist of a rapid initial uptake phase followed by a 'steady state' uptake phase lasting for at least 40 min. Both the initial and 'steady state' phases were markedly affected by temperature. Thus a reduction in temperature from 20·5 °C to 2·5 °C caused a 10-fold reduction in the 'steady state' uptake

rate and a 19-fold reduction in the magnitude of the initial uptake step, which suggests that both may be linked with root metabolism.

Phosphate was found to be a powerful inhibitor of arsenate uptake with both the initial and 'steady state' phases being affected (Asher and Reay, 1979). Arsenate, on the other hand, at equivalent concentrations was a mild inhibitor of 'steady state' phosphate uptake. These results are consistent with the view that both ions are transported by the same carrier mechanism—which has a higher affinity for phosphate than for arsenate. Increasing phosphate levels in nutrient solutions therefore directly affects the uptake of arsenate and explains many earlier results (Rumberg *et al.*, 1960). A reversal of arsenate inhibition of seedling growth by phosphate has also been reported by various workers (Speer, 1973).

When arsenate and arsenite were supplied to intact plants, both phases of uptake were apparent but were three to four times higher for arsenate than arsenite (Asher and Reay, 1979). It would appear that arsenite uptake is also a metabolic process, but whether it enters the plants via the phosphate carrier was not established.

Although organo-arsenicals can be absorbed from nutrient solutions through the root and to a lesser extent from soil, their primary pathway of entry is through the leaves and stems (Hiltbold, 1975). Surfactants are routinely added in spray solutions to enhance foliar absorption. There is evidence of greater foliar absorption of MAA and MSMA than of DSMA, but this effect is dependent to some extent on temperature. Plant species differ in their response to methanearsonates, probably due in part to differences of permeability of leaf surfaces.

Translocation of the organo-arsenicals out of the treated tissue is essential if the herbicide is to have a metabolic function. The superiority of methanearsonates over sodium arsenite lies in part with their more extensive translocation (Hiltbold, 1975) which has both acropetal and basipetal components (Rumberg *et al.*, 1960). Analytical studies have shown that arsenic accumulates in the meristematic tissue which is the major area of herbicidal activity. Rumberg *et al.* (1960) suggested that the rapid injury from sodium arsenite treatment may be responsible for the lesser transport; the arsenite usually produced injury symptoms within a few hours of treatment, whereas DSMA requires many hours or even days to produce chlorosis. The authors were only able to recover 30–40 % of the labelled arsenic from DSMA in the foliage whereas 85 % of the arsenite was recovered. They assumed that the remainder would have gone into the roots.

Cacodylic acid is considered to be a general contact toxicant. Apparently

its only translocation is apoplastic (Levander, 1977). DSMA translocation is considered to take place both apoplastically and symplastically. Apoplastic movement of MAA was reported to be more rapid than symplastic movement (Sckerl and Frans, 1969). Symplastic movement of MSMA has also been reported. A more detailed discussion of organo-arsenical translocation has been presented by Levander (1977).

3.5.5. Arsenic metabolism

As arsenic is chemically similar to phosphorus it seems likely that it participates in may cell reactions. Specific organo-arsenical compounds, e.g. arsenobetaine, arsenocholine or arsenolipids have been found in some organisms and arsenic has been reported to replace phosphorus in the phosphate groups of DNA. However, arsenic incorporation into metabolic processes in higher plants has received little attention.

Trivalent arsenic probably reacts with sulphydryl enzymes leading to the production of complexes, membrane degradation and cell death. Arsenate action is, however, more subtle. It is known to uncouple phosphorylation and to have a profound effect on enzyme systems (Dixon and Webb, 1958).

When one considers the variety of reactions in plants that involve sulphydryl groups and phosphorus, it is easy to appreciate the number of ways in which arsenites and arsenates may interfere with the physiological and biochemical processes that constitute growth.

The mechanism of phytotoxicity of the organo-arsenicals is not known (Hiltbold, 1975). Organo-arsenicals can apparently be metabolized, but whether such compounds are effective as herbicides is not clear. For example, Sckerl and Frans (1969) found an MAA metabolite with a positive ninhydrin reaction in Johnson grass extracts suggestive of a complex with histidine. Sachs and Michael (1971) also reported the presence of a ninhydrin-positive complex of MSMA. Duble *et al.* (1968) noted that DSMA was almost completely complexed in the plant and that the complex was the mobile form of the herbicide. Cacodylic acid, on the other hand, showed little if any association with cell constituents (Sachs and Michael, 1971). The carbon–arsenic bond is apparently stable in plants but is rapidly broken down in soils (Von Endt *et al.*, 1968).

3.5.6. Essentiality of arsenic

Arsenic has not been shown to be an essential plant nutrient although it has been recently proved to be essential for animal metabolism. However,

stimulation of growth by arsenic additions has been reported by several workers. Stewart and Smith (1922) found that arsenic additions were beneficial to the growth of peas, wheat and potatoes, but not to beans. Jacobs *et al.* (1970*a*) found a slight growth improvement in potatoes and corn, but not in peas and snapbeans, with the addition of 45 kg sodium arsenite/ha. Increased yields have also been observed in wheat, rye, corn, soybean and cotton when arsenic, in the form of calcium arsenate, has been added at levels of 1200 and 500 μg/g (Cooper *et al.*, 1932). It is possible that arsenate additions may displace phosphate from the soil in certain situations with a resultant increase in phosphate availability (Jacobs *et al.*, 1970*a*). Woolson *et al.* (1971*b*) conjectured that the arsenic responses they observed were due to increased activity of the plant systems by small amounts of arsenic. Other pesticides have also been reported to stimulate plant growth at sublethal doses.

3.5.7. Arsenic accumulators

The presence of plants on elevated-arsenic areas and the levels encountered in their tissues have been discussed in Section 3.1.2. Many toxic arsenic sites have minimal plant cover and limited plant diversity, but the observation of plant occurrences on sites is not proof of evolution of tolerance. Wild (1974*a*,*b*) reported the presence of many species on arsenical mine dumps in Rhodesia, but no comparisons with control populations have been made to indicate whether a tolerance difference between the various populations did indeed exist.

In several cases, plant survival has been proven to be due to evolution of tolerance to arsenic (Rocovich and West, 1975; Porter and Peterson, 1975). All *Andropogon scoparius* plants from a mine site in the USA possessed tolerance to arsenate concentrations in nutrient solutions of 1 μg arsenic/ml—which control plants lacked (Rocovich and West, 1975). Only those plants found on soils with over 15 000 μg arsenic/g were shown to tolerate solution values of 25 μg arsenic/ml. Thus the degree of tolerance is a reflection of the arsenic concentration in the spoil material. In the UK, tolerance in arsenic-toxic mine spoil *Agrostis* plants was specific to arsenate (Porter and Peterson, 1977*a*) which was shown to be the major water-soluble arsenic ion in the spoil (Porter and Peterson, 1977*b*). As this is also the predominant form of arsenic in most soils (Woolson, 1973) it is likely that where arsenic tolerance has evolved it is predominantly to this ionic species.

Isoenzyme complements have been used to identify genotypic variation in mine populations of *Agrostis* plants (Benson *et al.*, 1979). Analysis of

esterase isoenzymes from different organs of *A. tenuis* clones showed that the pattern was dependent on the plant organ, although esterase zymograms from any one tissue from the clonal plants were identical. It was apparent that many different genotypes existed on each mine site, which is in agreement with the concept of different mine spoil populations of plants based on morphology and growth habit. Some identical zymograms were found, however, on several different sites. The plants have therefore remained genetically diverse despite the strong selection pressures, which presumably indicates a separate evolutionary origin for at least some of the plants. Perhaps this diversity is not surprising since the chemical and physical conditions of the spoil are heterogeneous even over a small area.

Arsenic tolerance was examined in several of the clones displaying the same esterase patterns (Benson *et al.*, 1979). The tolerance indices of clones from one plant were the same, but different index values were found between clones from three plants exhibiting essentially the same esterase pattern. Thus the esterase patterns, although reflecting different genotypes, are not directly related to arsenic tolerance. Again, the degree of tolerance reflects the arsenic concentrations in the spoil material.

No conclusive evidence on a mechanism of arsenic tolerance has been found. It is possible that the mycorrhizal association reported by Benson *et al.* (1980) can ameliorate arsenite toxicity by increasing phosphate supply or inhibiting arsenate uptake into the plant. Further work is required to elucidate these problems.

4. ANTIMONY

4.1. Antimony in the Environment

4.1.1. Baseline studies

The concentrations of antimony in several major rock types and soils are shown in Table 8.10. Although data is scarce, Mitchell and Burridge (1979) report that the antimony concentrations in soils are higher than the crustal mean of 0·2 quoted by Mason (1966). Soils of urban and industrial areas usually contain more antimony. Elevated antimony concentrations are generally associated with high arsenic concentrations in sulphide ores and several reports have appeared on the use of antimony concentrations in soils as pathfinders for gold (Hawkes and Webb, 1962).

Information on the antimony concentrations in plants is also incomplete, despite the apparently moderate toxicity of antimony to all organisms (Bowen, 1966). Several values of antimony levels in plants are listed in

TABLE 8.10
ANTIMONY CONCENTRATION IN ROCKS AND SOILS

Materials	Concentration (ppm (dw))	Reference
Sandstones	0·05	Bowen (1966)
Igneous rocks	0·2	Bowen (1966)
Limestones	0·2	Bowen (1966)
Shales	1·5	Bowen (1966)
Soils	0·5	Brooks (1972)
Soils	2–10	Bowen (1966)
USA soils	2·3–9·5	Onishi (1970)
Canada soils	1–3	Onishi (1970)
Nigeria soils	1–5	Onishi (1970)
Bulgaria soils	0·8–2·2	Naidenov and Travesi (1977)
Holland soils	0·6–2·1	Chattopadhyay and Jervis (1974)

Table 8.11. Orchard leaves and grass contain quite high concentrations compared with citrus leaves, but whether these values reflect differences between species or reflect the different antimony concentrations in the soils was not reported.

4.1.2. Industrial contamination

The major sources of antimony pollution arise from mining and smelting of metals and metalloids and the burning of coal. Slight contamination by this element may arise from the application of sewage sludge to soil.

Areas of gold mineralization, as well as containing high concentrations of arsenic, may contain elevated levels of antimony. Concentrations of

TABLE 8.11
ANTIMONY CONCENTRATION IN PLANTS

Plant	Concentration (ppm (dw))	Reference
Citrus leaves	0·000 04	O'Toole *et al.* (1971)
Pine bark	0·044	Hattula and Johanson (1978)
Kale	0·069	Bowen (1974)
Forage crops	0·1	Ragaini *et al.* (1977)
Raw rice	0·96	Abedinzadeh *et al.* (1978)
NBS Tomato leaves	0·12	Renan *et al.* (1979)
NBS Spinach leaves	0·69	Renan *et al.* (1979)
NBS Pine needles	1·14	Renan *et al.* (1979)
NBS Orchard leaves	2·57	Renan *et al.* (1979)
Grass	3–4	Ragaini *et al.* (1977)

50 ppm antimony derived from stibnite have been reported to extend over several kilometres of sandstone and sands in Rhodesia (Wild, 1974*a*) with localized concentrations ranging up to 2000 or even 5000 ppm. As a result, gold mine spoil materials commonly contain hundreds of ppm antimony and may approach 50 000 ppm in isolated dumps (Wild, 1974*b*; Hill and Nothard, 1973). These are certainly high concentrations compared with mean soil values (Table 8.10).

Contamination of soils and plants by stack dust and smelter fumes from a gold mining and refining site at Yellow Knife, N.W. Territories have been recorded by O'Toole *et al.* (1971). Up to 280 ppm antimony was found in soils near the stack but it decreased with increasing distance from the stack. Plants were also contaminated, with grass containing up to 15·4 ppm exceeding the background values by one to three orders of magnitude. As the area is also contaminated with arsenic, arsenic/antimony ratios can be used to show selective bioaccumulation effects. Mean ratios of 16·8 and 4·2 were calculated for soil and grass respectively.

Antimony contamination of surface soils by fallout has also been reported near a large copper smelter (Crecelius *et al.*, 1974) and a lead smelter (Ragaini *et al.*, 1977) with corresponding increases in the antimony concentration of the plants. Soils containing up to 200 ppm antimony and plants containing up to 111 ppm antimony from the most polluted sites, contrasted with background values of 3–5 ppm for soil and 3–4 ppm for plants.

Lim (1979) reports that coal is slightly enriched with antimony, samples from the USA and UK fall within the range 0·2–9·6 ppm antimony whereas Australian coals can range up to 20 ppm. Antimony concentrations in pfa centre around 4–5 ppm and increase with decreasing particle size (Coles *et al.*, 1979). Crops grown on soils amended with 10% pfa contain high antimony concentrations. For example, onions grown on a pfa-amended soil (pfa antimony concentration 5·3 ppm) were reported by Furr *et al.* (1976*a*), to contain 2·2 ppm antimony compared with 0·8 ppm for plants grown on soil alone (background soil antimony concentration 0·8 ppm). Beans, however, contained the same concentration of antimony (0·4 ppm) when grown on either soil alone or pfa-amended soil. Species effects were obviously important, for in an earlier paper, Furr *et al.* (1975) reported lower concentrations of antimony in clover plants when grown on pfa-amended soil compared with clover grown on a gravelly subsoil.

Antimony concentrations are variable in sewage sludge and may range in the USA from 2·6–44·4 ppm (Furr *et al.*, 1976*b*) and 4·3–15·9 ppm (Nadkarni and Morrison, 1973), although the data is rather scant. In the

UK values range around 15–19 ppm (Wiseman and Bedri, 1975), with a sewage-based fertilizer at 330 ppm (Egan and Spyrou, 1977). Plants collected from sludge-amended soils have been reported to show subtle increases with large applications of sludge (Erdman and Tourtelot, 1976).

4.2. Antimony Cycling

Serious attention has not been given to the global cycling of antimony and only a few fluxes have been quantified. Antimony is present in substantial concentrations in precipitates from hot springs and boreholes and in geothermal waters where it plays an important role in co-precipitation reactions of antimony sulphide (Sabadell and Axtmann, 1975). Concentrations up to 30% have been reported in extreme cases. Concentrations in bamboo leaves of up to 12·6 ppm antimony have been reported for plants growing near a geothermal source (Nakahara *et al.*, 1977). Field data are sparse on antimony contamination in ecosystems from geothermal areas, but Klein *et al.* (1975) calculate that weathering and mobilization contributes 5×10^3 tonnes antimony/year. This is large compared with the inputs from industrial operations and pfa of around $0{\cdot}17 \times 10^3$ tonnes antimony/year.

Seasonal fluctuations of the antimony concentrations in air have been measured, being especially high in winter months (Peirson and Cawse, 1979).

4.3. Antimony in Soils

Very little is known about the details of mobilization and adsorption of antimony in soils. Soluble antimony probably occurs as antimonate although complexes with humates are also possible (Valente, 1978). Antimonates are probably absorbed by the same minerals which bind phosphate and arsenate. Nothing is known about the existence of organic derivatives in the environment as a result of biotransformations. Although microorganisms can liberate arsine and methylated derivatives of arsenic and selenium, there is no information on the occurrence of stibine or methylated antimony compounds in the environment.

4.4. Antimony in Plants

The accumulation and metabolism of antimony has been neglected in plants and little is known of its toxicity. Indeed the element was omitted from the recent review by Gough and Shacklette (1976) of elements of environmental importance. Considerable research is required before its significance in the environment can be assessed.

4.5. Accumulation of Metalloids by Lower Plants

Throughout this review the emphasis has been on higher plants but it is both appropriate and timely to mention that there are observations of the occurrence of *Equisetum*, mosses, liverworts, lichens and fungi on habitats rich in certain elements, including arsenic and antimony (Shacklette, 1965; Cannon *et al.*, 1968; Pyatt, 1973, 1975; Benson *et al.*, 1980), and fungal sporophores in particular have been found to accumulate arsenic when collected from polluted areas (Peterson *et al.*, 1979*a*). Concentrations of up to 1720 ppm arsenic have been found in *Thelophora terrestris* growing on a derelict arsenic mine and smelter site in the UK. Byrne *et al.* (1980) have also found evidence of arsenic-accumulating fungi, with up to 182 ppm arsenic in *Laccaria amethystina* being the highest value reported.

Edible mushrooms, when grown on normal soil, have been reported to accumulate up to 7·8 ppm selenium which is a concentration factor of 150 (Stijve and Besson, 1976). Toadstools belonging to the genus *Tubiporus*, collected in Europe, contained up to 20 ppm selenium (Stijve and Besson, 1976) and *Amanita*, collected in New Zealand, contained up to 16·8 ppm selenium (Watkinson, 1964). In this latter case the surrounding herbage contained only 0·047 ppm selenium, which indicates that the toadstool is a moderate accumulator. Appreciable selenium concentrations have also been found in some wild fungal species in Yugoslavia with a maximum value of 47·7 ppm in *Boletus edulis* (Byrne *et al.*, 1980). Other species, however, do not accumulate this element to any great extent. As more data are compiled and metal accumulation differences between species become clearly established, it may be possible to use trace metal profiles as additional taxonomic markers for species identifications. It would seem that accumulation of trace elements by higher fungi is a promising new area for further research. However, as sample sizes are small and material not readily available, progress may not be rapid unless sensitive analytical techniques are employed.

5. CONCLUSIONS

Studies on the metalloids in soil, plant and animal systems have evolved from recognition of their adverse effects in earlier years, to one of essentiality; at least for selenium and arsenic in animal nutrition.

In recent years there has been a considerable advance in methods of analysis and instrumentation and these have been applied to the transition metals in particular. New spectroscopic methods including electron spin

resonance, Mossbauer and nuclear magnetic resonance have been effectively employed to study metallo–enzyme systems. The applications of these and other techniques should enable many of the long-standing questions concerning the chemical nature of the metalloids in soils and their uptake, concentration and metabolism by plants to be answered. But the problem is a complicated one.

The properties and behaviour of individual species, particularly crops and forages, ecotypes, physiotypes and cultivars are extremely important in determining the quantities of elements which plants obtain from soils. Some of these differences may have important consequences, resulting in deficiencies or toxicities of these elements to plants and to animals grazing them. The reasons for their different behaviour are not understood and additional efforts to evaluate likely mechanisms would be a profitable line of research. Methods of alleviating the problems associated with the metalloids become apparent as research continues. From a practical point of view the growth of tolerant cultivars may reduce or avoid toxicity problems encountered with the revegetation of industrially polluted soils. Alternatively, the use of cultivars capable of accumulating an element from low concentrations in soils could have agricultural implications and avoid the problems associated with trace element supplementation. A strategic programme of cultivar/soil interactions should be initiated and only then can recommendations for their possible field use be reliably assessed.

Our knowledge is of course still far from complete, especially for plants; as is clearly shown by the lack of data in many sections in this review. The pathways whereby these elements are released from the geological environment, absorbed by plants and translocated to their sites of deposition are not well understood, although some progress has been made in this area, especially with arsenic and selenium. Despite the reported toxicity of antimony and its high concentration—at least in some soils and plants—it is surprising that so few studies have been concerned with this element. The scope for further work is great.

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Index

Acer pseudoplatanus, 118, 120, 121
Acer rubrum, 66
Acer saccharinum, 194
Acid-dissociation constant, 12
Aconitum excelsum, 220
Activation techniques, 26
Activity coefficient, 15
ADP, 256
Adsorption, 36
Agaricus, 243
Agropyron spp., 232
Agrostis, 200, 232, 321
Agrostis gigantea, 196, 202
Agrostis stolonifera, 132, 203
Agrostis tenuis, 117, 121, 201, 202, 283, 301, 322
Airborne metal burdens, 34
Albizia amara, 200
Alcaligenes eutrophus, 172
Algal responses to nickel, 183–5
Alkali-metal coordination chemistry, 41
Allium cepa, 67
Allium macropetalum, 259
Aluminium, 195, 214–18
  coals, in, 214
  earth's crust, in, 214, 215
  ore, 214
  plants, in, 217
  soils, in, 215
  tolerance, 215, 217
  toxicity, 215, 217
Aluminium—*contd.*
  toxicity measurement, 216
  toxicity susceptibility, 216
Alyssum bertolonii, 199, 200
Alyssum murale, 200
Alyssum serpyllifolia, 200
Alyssum serpyllifolium subsp. *lusitanicum*, 199
Amanita, 326
Amanita muscaria, 259, 260
Amino acids, 237, 295
AMP, 256
Anabaena circinalis, 258
Anacystis, 183
Analytical techniques, 22–7
Andropogon filifolius, 255
Andropogon scoparius, 321
Anion-exchange resin, 21
Anisopappus davyi, 233
Anode-stripping voltametry, 26
Antagonism, 187
Anthoxanthum odoratum, 175
Antimony, 279, 322–6
  baseline studies, 322
  cycling, 325
  environment, in, 322–5
  industrial contamination, 323–5
  plants, in, 280–1, 323, 325
  rocks and soils, in, 323
  soils, in, 280–1, 325
Aqueous phases, sampling, 19–20
Aqueous solution, 9–15, 22

Arachis hypogae, 182
Arsenic, 3, 279, 299–322
  accumulators, 321
  agricultural effects, 309–12
  background studies, 299
  baseline studies, 299
  coal, in, 302
  compounds, 304
  crops, in, 313
  cycles, 303–4
  environment, in, 299–303
  essentiality, 320
  industrial contamination, 300
  metabolism, 320
  microbiological transformations, 305–6
  occurrence, 300
  phytotoxicity, 303, 309–12
  plant accumulation, 313
  plants, in, 280–1, 313–22
  soil chemistry, 306–9
  soils, in, 280–1, 304–9
  tolerance, 322
  uptake and translocation, 318–20
Arsenicals, 309, 311, 315
Artemesia canadensis, 284
Artemesia terras alba, 237
Aspergillus niger, 246
Association constant, 12
Aster, 298
Aster novae-angliae, 285
Astragalus, 280, 284, 291, 292, 295–8
Astragalus bisulcatus, 293, 295
Astragalus crotolariae, 295
Astragalus pattersoni, 255, 260
Astragalus pectinatus, 297
Astragalus preussi, 255, 259, 260
Astragalus racemosus, 285, 295
Atmophile elements, 279
Atomic absorption spectrometry, 23–4, 30
Atomisation procedures, 23
ATP, 60, 61, 256, 293
ATPase, 69, 70
Atriplex, 217, 298
Avena byzantina, 173
Avena sativa, 62, 66, 69, 82, 87
Azotobacter, 258

Barium, 218–19
  coal, in, 218
  earth's crust, in, 218
  granite, shales, limestones and sandstones, in, 218
  plants, in, 219
  soils, in, 219
  toxicity, 219
Barleria aromatica, 200
Beilschmedia tawa, 244
Bertolletia excelsa, 219
Beryllium, 220–2, 261, 262
  coals, in, 220
  industry and commerce, in, 221
  phytotoxicity, 222
  plants, in, 220
  soils, in, 220
Beta vulgaris, 62, 180
Beta vulgaris var. *cicla*, 81–3, 87, 101
Betula, 251
Betula papyrifera, 193
Biogeochemical prospecting, 236
Bio-inorganic chemistry, 6, 41
Block sampling, 17
Boletus edulis, 243, 326
Boron, 3
Bouteloua gracilis, 297
Brassica napus, 82
Brassica oleracea botrytis, 88
Brassica oleracea L., 101
Brassica oleracea var. *capitata*, 98
Brassica rapa L., 96
Bronze, 249

Cacodylic acid (CA), 306, 308–10, 312, 319
Cadmium, 63, 77–109, 118, 183, 203, 213
  absorption by plants, 77, 80–98
  absorption characteristics, 83, 97
  accumulation by lettuce, 87
  accumulation by plants, 83
  accumulation characteristics, 103
  assessment of detrimental effects to plant growth, 104
  chemical extraction procedures, 102
  detection limits, 25

Cadmium—*contd.*
diagnostic criteria, 98–102
diagnostic procedures, 103
diet, in, 77
domestic water, in, 78
earth's crust, in, 78–9
effect of substrate concentration, 82–7
fruits, in, 90
grains, in, 94
health effects, 77
most recently matured leaf at early bloom stage, in, 96
natural levels in plants, 89–96
natural occurrence, 78–80
phytotoxicity, 98–102
plant species and cultivars, in, 96–8
plants, in, 103
poisoning, 77
saturation extracts, in, 101
soil factors, 87–9
soils, in, 79–80, 86, 89, 103
solid wastes, in, 80
tolerance studies, 98
toxic levels, 103
various plant parts, in, 96
vegetables, in, 85–6, 92
Calamagrostis arundinacea, 220
Calcium, 221
Calcium nitrate, 202
Calluna vulgaris, 175
Camellia sinensis, 217
Canavalia ensiformis, 172
Capsicum annuum, 62
Carbon dioxide, 65, 196–7
Cassiterite, 249
Castillega, 298
Castillega angustifolia, 259
Cation-exchange, 56
Caulerpa, 243
Caulerpa prolifera, 244
Ceratophyllum demersum, 65
Ceratophyllum sp., 178
Cetraria cucullata, 187
Cetraria nivalis, 187
Chalcopyrite, 111
Chelates, 149–51, 153–4, 157
Chelating agents, 37
Chelation, 32
Chemical analysis, 15–30, 98
Chemical properties, 6, 112
Chlamydomonas, 67
Chlamydomonas eugametos, 183, 184, 189
Chlorella, 132
Chlorella fusca, 184
Chlorella saccharophila, 178, 185
Chlorella vulgaris, 183, 184, 189
Chlorophyceae, 183
Chromium, 42, 118, 197, 222–9
accumulator plants, in, 226
biochemistry, 222
biogeochemical survey, 223
biological effects, 222
chemical form in plants, 226–7
coal, in, 228
compounds, 226
distribution, 222
earth's crust, in, 222
essential element, 222, 227
food plants, in, 225
fruit and seeds, in, 225
lichens and mosses, in, 225
ore, 222
pfa, in, 229
physiology, 222
phytotoxic effects, 227
serpentine soils, in, 223
shrubs and trees, in, 224
soils, in, 222, 224
translocation, 226
transport in roots, 225
uptake in roots, 225
wastes, in, 227
Chromosomal aberrations, 70
Chrysanthemum, 126
Chrysanthemum morifolium, 123, 126
Chrysothamnus viscidiflorus, 259
Citrus, 174
Cladina stellaris, 187
Cladonia rangiformis, 59
Clavulina, 243
Clavulina cinerea, 244
Clitopilus prunulus, 243
Clostridium butyricum, 258
Clostridium stricklandii, 296

Clostridium thermoacetium, 296
Cobalt, 197, 202, 203, 229–34
  coal, in, 230
  deficiency, 231
  earth's crust, in, 229
  endemics, 233–4
  health effects, 234
  plant accumulation, 233
  plants, in, 230–2
  ruminants, for, 230
  ruminants, in, 231
  soils, in, 231, 233
  toxicity, 234
  ultrabasic rocks, in, 233
  uses, 229
  valence states, 230
Colorimetric analyses, 23
Combretum molle, 200
Complexing agents, 183
Complexometric analysis, 23, 39
Computational speciation, 13–15, 28
Conopsis, 297
Conservation equations, 10
Copper, 31, 41, 88, 111–43, 187, 192, 195, 197, 200–3, 261, 262
  availability to plants, 112–14
  biological role, 112
  chemical properties, 112
  complexes, 113, 115, 119
  deficiency, 112, 113, 118, 124–9
  deficiency diagnosis and correction, 127–9
  earth's crust, in, 111
  effect on root extension, 131
  excess, 114, 129–33
  excess counteraction, 133
  excess symptoms, 130–1
  extractability, 113
  interactions, 116, 118
  loss from plants, 122
  metabolic processes, in, 131–3
  ore deposits, 111
  organo complexes, 112
  plant metabolism, in, 123–4
  plants, in, 112
  root uptake, 114–19
  soils, in, 113–14, 118, 124–33
  toxicity, 130, 191
Copper—*contd.*
  transport within the plant, 119–21
  trees, in, 118
  uptake by whole plants, 117–18
  uptake mechanisms, 112
Copper sulphate, 115, 116
Copper sulphides, 111
Coprosma australis, 256
Coulombic attraction, 41
Coulombic forces, 40
Crotalaria cobalticola, 233
Cucumis sativus, 182
Curcurbita pepo L. var. *medullosa* Alef., 101
Cynodon dactylon, 203

Dalbergia melanoxylon, 200
Daucus carota, 182
Daucus carota sativa, 83
Defoliants, 309
Deschampsia cespitosa, 153, 172, 196, 202, 203, 301
Deschampsia flexuosa, 181, 193, 216
Desiccants, 302
Desorption, 38
Dicoma macrocephala, 200
Diffusion
  coefficients, 151
  Fick's first law of, 150
Digestion, 22
Disodium methanearsonic acid (DSMA), 305–6, 309, 312, 314, 315, 318–20
Dissolved gases, 13, 19
Dithizone, 23
DNA, 159, 216, 221
Dryas octopetala, 241
DTPA, 115, 153
DTPA–TEA test, 101

EDDHA, 115, 128
Edge-effects, 17
EDTA, 11, 115, 128, 131, 150, 172, 221, 225, 231, 288
Elaeagnus umbellata, 65
Elodea canadensis, 178
Eminia antennulifera, 200

Emission techniques, 24–5
Enzyme(s), 112
  function, 158–9
  kinetics, 36
Epilobium angustifolium, 255
Equilibrium constant, 9, 33
Equisetum, 176, 236, 326
Erica cinerea, 175
Erica tetralix, 175
Erigonum ovalifolium, 243
Eritrichium chamissonis, 241
Escherichia coli, 296
Essential elements, 171–3, 222, 227, 238, 257, 296
Euglena gracilis, 159
Euglena mutabilis, 184
Exhaust emissions, 61
Extractability, 32

Fagus sylvatica, 122
Fertilizers, 115, 290, 291, 316, 325
Festuca, 236
Fick's first law of diffusion, 150
Filtration, 18
Flame-resonance spectrometry, 25
Food chain, 71, 78
Formation constants, 10
Fragaria spp., 89
Fungi, 196–7
Fungicides, 129

Gas exchange, 65
Gleichenia linearis, 251
Gleocapsa, 183
Glycine max. L., 63, 65
Glycine soja, 82, 88, 89
Gold, 235–8
  analytical methods, 236
  coal, in, 235
  deposits, 235
  mineralization, 236
  phytotoxicity, 237
  plant accumulation, 237
  plants, in, 237, 238
  sea water, in, 235
  soils, in, 236
Grindelia, 298

Haematococcus capensis, 183, 189
Haplopapus, 297
Hard-acids, 7
Hard water, 13
Heavy metal, definition, 5
HEEDTA, 115
Helianthus annuus, 62, 65
Herbicides, 302
Hordeum, 115, 117, 118, 122
Hordeum vulgare, 62, 82, 98
Humic acids, 113
Hybanthus austrocaledonicus, 182, 200
Hybanthus caledonicus, 182, 200
Hybanthus floribundus, 181, 199, 200
Hydrogenomonas, 172
Hydrolysis reaction, 11–13
Hylocomium splendens, 187
Hypnum cupressiforme, 228, 259

IAA-oxidase activity, 131–2
Ilex aquifolium, 217
Indigofera setifera, 201
Inductively coupled plasma (ICP), 25
Insecticides, 302
Instrumental methods, 22
Ion-binding sites, 36
Ion-exchange chromatography, 21
Ion-exchange resin, 21, 30
Ion-microprobe mass spectrometry, 26
Ion-selective electrodes, 30
Iron, 67, 88, 238–40

Juncus gerardii, 121
Juniperus communis, 244

Kinetic effects, 40

Laccaria amethystina, 326
Lactuca sativa L., 64, 67, 82, 83, 87, 88, 97, 98
Lactuca sativa var. *longifolia*, 101
Lagochilus intermedius, 236

Lead, 17, 42, 55–76, 117, 200, 202, 203, 213, 251
  aerosol effects, 59–61
  burden of plants, 61
  detection limits, 24, 25
  distribution in plants, 58–9
  earth's crust, in, 55
  effects on *in vitro* plant systems, 68–9
  environmental factors, 70
  impaction on plant leaves, 57
  influence on plant growth, 58
  plants, in, 57–69
  rooting medium, in, 61–7
  soils, in, 57–8
  specific *in vivo* effects, 64–7
  toxicity, 62, 67
  uptake by plants, 55–6, 64
  whole plant effects, 61–4
Lead acetate, 67
Lead chloride, 60, 62, 66, 68
Lecythis ollaria, 298
Ledum decumbens, 241
Ledum palustre, 253
Lemna, 123
Lemna gibba, 126
Lemna minor, 126
Lemna triscula, 178
Lemna valdiviana, 189
Lepidium sativum, 98
Leptospermum scoparium, 223
Lewis acid, 3, 7, 9, 41, 42
Lichens, 186–7, 225
Ligands, 8–12, 32, 33, 39, 41
Linaria triphylla, 248
Linum usitatissimum, 173
Liquid scrubber, 18
Litter decomposition, 193, 197
Lolium perenne, 246, 283
Lolium spp., 85
Lonicera, 243
Lumbricus terrestris, 194
Lychnis alpina, 181, 200
Lycoperdaceae, 243
Lycopersicon esculentum, 62, 89, 182
Lycopodium circinatum, 244
Lycopodium spp., 217
Lycospersicon esculentum Mil., 101
MAA, 319, 320
Magnesium, 197, 221
MAMA, 315
Manganese, 67, 88
  deficiency, 31
  soils, in, 238–40
Mass-flow budgets, 28
Mass spectrometry, 25
Medicago sativa, 67, 69
Melilotus alba, 284
Melilotus indica, 248
Melilotus officinalis, 229, 284
Mentha aquatica, 236
Mercury, 17, 213
Merismopedia, 183
Metabolic rate, 39
Metal(s)
  availability, 31, 37
  binding, 33
  classification, 3–9
  definition, 3
  displacement, 33
  exchange, 36
  functional classifications, 3–5
  hydroxide, 12, 13
  ion binding, 36, 40
  ion chemistry in aqueous solution, 9–15
  ion mobility, 30
  ions
    classification, 7
    primary capture, 35–7
    root capture, 36–7
    uptake by biological systems, 30–42
  tolerance in plants, 201
Metalloids, 279–342
  accumulation by lower plants, 326
Metallothioneins, 163
Micronutrient, 5
Microspora, 185
Mimulus guttatus, 202
Mining, 55, 80, 196, 201, 228, 236, 300
Monosodium methanearsonic acid (MSMA), 306, 309, 310, 312, 315, 319, 320
Morinda reticulata, 298

Moss bags, 18
Mosses, 186–7, 225
Munroa squarrosa, 297
Mycorrhizal formation, 193
Myosotis monroi, 233

NADH oxidation, 68
Navicula pelliculosa, 184
Neptunia amplexicaulis, 295, 298
Neurospora crassa, 163
Neutron activation analysis, 22, 26–7
Nickel, 88, 118, 171–211
  accumulator species on serpentine soils, 199–200
  algal responses to, 183–5
  effects on micro-organisms, 196–7
  essential element, 171–3
  excessive, 173
  fruits and vegetables, in, 194
  impacts from smelter emissions, 189–96
  lichens and mosses, in, 186–7
  plants, in, 171–9, 182–3
  serpentine soils, and, 187–201
  soil–plant relationships, 179–82
  soils, in, 173, 191, 195
  synergism and antagonisms, 187–9
  tolerance in higher plants, 201
  toxicity, 173–4, 184, 187, 191, 195, 197–201
  translocation, 182
  vegetation, in, 191
Nicotiana tabacum, 220, 246
Nitrogen
  fixation, 193, 231, 258
  metabolism, 172
Nostoc, 183
Nuphar advena, 178
Nutrient cycling, 193
Nutrition, trace element, 31
Nutritional content, 5
Nymphaea, 176
Nymphaea odorata, 178
Nyssa sylvatica, 232, 234

Oleifera sp., 64
Optical emission spectroscopy, 24
Organic matter, 199
Oryza sativa, 62, 77, 81, 96, 101
Oscillatoria, 185
Oxidation states, 42
Oxidative phosphorylation, 68
Ozone phytotoxicity, 189

Palladium, 240–2
  occurrence, 240–1
  plants, in, 241
  sludges, in, 241
  soils, in, 241
  uses, 240–1
Papaver escholtszia, 248
Particulates, 59–61, 71
  sampling, 18
Pearsonia metallifera, 234
Penicillium waksmannii, 197
Periodic Table, 3, 5, 213, 238
Peroxidase activity, 60
Pesticides, 55, 309
pH effects, 11–13, 19, 35, 68, 81–2, 96, 182–3, 199, 215–16, 221, 229, 239, 253, 288, 305, 308
Phacelia, 238
Phacelia sericea, 237
Phaeodactylum tricornutum, 184, 185
Phalaris canariensis, 62
Phaseolus aureus, 226, 293
Phaseolus spp., 85
Phaseolus vulgaris, 62, 67, 122, 159, 160, 162, 173
Phleum pratense, 177
Phosphatases, 197
Phosphorus, 20, 155, 316
Photosynthesis, 60, 65, 66, 70
Phragmites communis, 172, 196, 203
Physical properties, 3
Picea abies, 122
Pimelea suteri, 233
Pinus banksiana, 194
Pinus ponderosa, 228
Pinus radiata, 171
Pinus sibirica, 253
Pinus taeda, 65
Piper–Steenbjerg yield curves, 128
Pisum abyssinicum, 248

Pisum sativum, 62, 66, 96
Plantanus occidentalis L., 64, 65
Platinum, 240–2
  occurrence, 240–1
  plants, in, 241
  sludges, in, 241
  soils, in, 241
  uses, 240–1
Pohlia nutans, 187, 193
Polarography, 30
Polygonum amphibium, 236
Polytrichum commune, 177, 194
Potamogeton pectinatas, 58
Potamogeton sp., 178, 179
Potassium, 20
Potederia condata, 178
Preconcentration, 21–3
Proton activation, 27
Prunus laurocerasus, 237
Pseudomonas, 252
Pseudomonas maltophilia, 246
Pseudotsuga menziesii, 299
Pterocarpus rotundifolius, 200

Quantitative analysis, 16, 22–4, 27–8

Radio-analytical methods, 26–7
Radiogenic nuclides, 21
Raphanus sativus, 64, 83, 88, 102
Raphanus sativus radicula L., 64
Redox
  behaviour, 41, 42
  chemistry, 42
  potential, 13
  reactions, 13
Refining, 194
Rhizosphere, 32, 38
Rhytidiadelphus squarrosus, 57
Ricinus communis, 182
Risk potential, 213
RNA, 159, 160
Root capture of metal ions, 36–7
Rosa kokanica, 243

Saccharomyces cerevisiae, 249
Salix spp., 253
Salvinia natans, 189
Sample
  collection, 16–20
  handling, 24
  preparation, 21–2
Sampling
  aqueous phases, 19–20
  atmospheric metal burdens, 17–18
  particulates, 18
  plant tissues, 20
  solid phases, 20
  statistics, 16
  theory, 17
Sarcobatus, 255
Scenedesmus acuminata, 183, 184, 189
Scenedesmus acutiformis, 184, 185
Scenedesmus acutiformis var. *alternans*, 184
Scenedesmus obliquus, 257
Scirpus cespitosus, 177
Sclerolinia fructicola, 246
Sebertia acuminata, 200
Secale cereale, 84
Seed germination, 193
Selectivity
  charge, by, 41–2
  generation, 39–41
  Lewis acidity, by, 42
  redox behaviour, by, 42
  size and stereochemistry, by, 41
Selenium, 3, 255, 279, 281–99
  accumulation in plants, 290–9
  accumulators, 297
  atmospheric levels, 285
  background concentrations, 281–3
  baseline studies, 283
  compounds, 294
  cycling, 286–7
  environment, in, 281–6
  essentiality of, 296
  forage levels, 290
  industrial contamination, 284–6
  localization, 295
  metabolism, 292
  microbial transformations, 289
  phosphate rocks, in, 285
  plants, in, 280–1, 288
  soil chemistry, 287–9

Selenium—*contd.*
soils, in, 280–1
toxicity, 297
uptake and transport in plants, 291–2
Selenocysteine, 293
Serpentine soils, 197–201, 223
Setaria italica L., 64
Sewage sludge, 228, 241, 249, 252, 286, 324
Silene acaulis, 181
Silene cobalticola, 233
Silene maritima, 181, 203
Silver, 203, 242–7, 261
environment, in, 245
occurrence, 242
oligodynamic action on bacteria, 245
plants, in, 243–6
soils, in, 243
toxicity, 245
Smelting, 55, 80, 189, 194, 195, 197, 201, 247, 300
Soft-acids, 7
Soil chelate stability–pH diagrams, 149
Soil-metal content, 29, 31, 34
Soil–metal plant system, 31–4
Soils, serpentine, 197–201, 223
Solanum tuberosum, 83, 88
Solid phases, sampling, 20
Solidago canadensis, 194
Solubilisation, 32
Solvent extraction, 21
Sorghum vulgare, 82
Spark-source mass spectrometry, 25
Speciation
analytical, 28–30
computational, 13–15, 28
Sphagnum, 187
Sphagnum acutifolium, 244
Spinacia oleracea, 98
Stability, 9–13
complexes, 183
constants, 9–11, 13–15, 33, 182
Stanleya, 284, 297
Stanleya pinnata, 255, 284, 295, 297
Stereocaulon paschale, 186, 193
Stoichiometric stability constants, 14
Streptomyces arenaca, 244
Sulphur, 261
Sulphur dioxide, 189, 191, 195
Superphosphate, 285
Surfactants, 21
Sutera fodina, 223
Symplocos tinctoria, 217
Synergism, 187, 189

Tagetes sp., 64
Tailings, 196, 228
Thallium, 247–9, 261, 262
coal, in, 247
earth's crust, in, 247
occurrence, 247
soil, in, 248
stable isotopes, 247
stimulatory affects on micro-organisms, 248–9
toxicity, 247–9
Thelophora terrestris, 326
Thermodynamic effects, 40
Thioglycollate chelating resin, 21
Tillandsia usneoides, 217, 219
Tin, 249–52
coal, in, 249
occurrence, 249
organo compounds, 250
plants, in, 250–2
shales, igneous rocks and sandstones, in, 249
trees, in, 250
valence states, 250
Tolerance Index, 202
Toxicity pattern, 6
Trace metals
biological systems, in, 1–54
capture via aerial parts, 35
classification by Periodic Table, 5
definition, 5
plants, and, 1
synergistic and antagonistic effects of, 88
Trifolium pratense, 177, 283
Trifolium repens, 246, 283

Trifolium subterraneum, 124, 125, 127, 157
Trimethylarsine (TMA), 305, 306
Triticale, 129
Triticum spp., 82, 87, 96, 98
Triticum vulgare, 62
Tubiporus, 326
Tungsten, 252–3
  coal, in, 252
  igneous rocks and limestones, in, 252
  ores, 252
  plants, in, 253
  sewage sludges, in, 252
  soils, in, 252
Turraea nilotica, 200
Typha latifolia, 177, 196

Ulothrix, 185
Umbilicaria deusta, 186, 193
Uptake processes, 37–9
Uranium, 253–6, 261, 262
  igneous rocks, 254
  ores, 255
  plants, in, 254–6
  radiotoxic hazard, 256
  soils, in, 254
  valency states, 253
Utricularia sp., 178

Vaccinium angustifolium, 181, 193
Vaccinium uliginosum, 255
Vanadium, 256–61
  biological availability, 258
  coals, in, 257
  earth's crust, in, 256
  essentiality, 257
  fresh water, in, 258
  immobility, 257
  occurrence, 256
  oxidation states, 256
  plants, in, 258–61
  soils, in, 258
Verbesina, 260
Vicia sativa, 62
Vicia sylvatica, 220

Xenophyophora, 218
X-ray
  emission, 22
  fluorescence, 26
Xylorhiza, 297

Zea mays, 63, 65, 66, 69, 82, 84, 85, 96, 97, 102, 237
Zebrina pendula, 67, 69
Zinc, 41, 67, 88, 117, 118, 145–69, 183, 202
  action on physiological processes, 162
  availability in soil, 164
  chelates, 149–51, 153, 157
  complexes, 159
  concentration in lithosphere, 146
  deficiency, 145, 147, 153, 156, 159–61, 163
  detection limits, 25
  effect on membrane permeability, 163
  effect on phloem translocation, 162
  enzyme function, and, 158–9
  interaction with macronutrients and hydrogen ions, 154
  interaction with micronutrients, 154–5
  interaction with P, 155–7
  intermediary metabolism, in, 159
  metabolism, 163
  movement to root surface, 150–2
  soil chemistry, 145
  soils, in, 145–9
  solubility in soil, 146
  toxicity, 161–4
  toxicity symptom, 161
  toxicity tolerance, 163
  translocation, 157–8
  uptake in plants, 152–4
  xylem fluid, in, 157
Zygnema, 185