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METAL TOLERANT PLANTS

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CONTENTS

A. INTRODUCTION

Metal ions are particularly important for healthy plant life [1—5]. Excesses or deficiencies of metal ions have effects on plant growth and morphology which are well documented [1—5]. The physiology of metal toxicity in plants has been reviewed recently [6,7]. Excessive concentrations of some metals in soils, producing toxic symptoms, may come about in a variety of ways. They may be the result of natural mineralization caused by the presence of undisturbed ore bodies near the surface, known as geochemical anomalies. High concentrations of metals may be the result of the exploitation of mineral resources, e.g. mining activities, ore tailings, tips, smokes and dusts. Lastly high concentrations can result from agricultural and waste disposal practices.

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B. INDICATOR PLANTS

Plants which are diagnostic of particular environmental conditions are known as indicator plants [8,9]. The qualitative term metal indicator species can be used to describe species which occur over mineralization and show a distinct or morphological variant with respect to mineralization, enabling anomalies to be detected [10]. They can also indicate the presence of high concentrations of metals from other than natural sources. Plants which grow on soils with metal concentrations which are normally toxic are metal tolerant. Mechanisms of metal tolerance have been reviewed [11—16].

C. THE USE OF METAL TOLERANT PLANTS AS INDICATORS OF MINERALIZATION

Lists of plants used as indicators in various parts of the world have been presented by Cannon [8,17], Malyuga [18], Antonovics et al. [12], and Brooks [9]. Attempts have been made [9,12] to distinguish between "local" indicators, which are adapted to grow over mineralized ground but will grow elsewhere, and "universal" indicators which will grow only in mineralized locations. The latter can be used as indicators wherever they occur. However there are doubts [9,10] about claims to universal indicators since studies may have been confined to one country and taxonomy may be confused. Many plants have metal tolerant ecotypes adapted to heavy metal environments, for examples see ref. 8—22.

The history of plants as indicators of mineralisation is long: *Lychnis alpina* was used to locate copper in medieval Scandinavia [23]. *Campanula rotundiflora* was associated with smelting sites and lead mineralization in the early nineteenth century [24], and in 1857 Henwood [25] noticed the association between thrift (*Armeria maritima*) and copper. As early as 1588 *Minuartia verna* was recognized as a metal indicating species [26].

In more recent times indicator plants have been used to discover copper in Zambia (*Becium homblei*) [12] and uranium in the Colorado Plateau area, USA, [27] using *Astragalus* species. *Astragalus* takes up selenium, which acts as a "pathfinder" element for uranium. In 1964, Cannon, again using *Astragalus*, found uranium ores which had not been located using drilling methods [28]. Whereas the early work in the field used the presence of indicator plants for prospecting, later work used the analyses of the plants, and many such studies are reported [9], for example, for prospecting for manganese in Tennessee [29] and for nickel in Oregon [30].

Studies have been carried out in Australia, where *Polycarpaea synandra* has been used to locate lead-zinc anomalies [31], and in the Dugald River area of Queensland, *Polycarpaea glabra* was also found to be indicative of lead-zinc mineralization [32].

This last area has been subject to the more recent development of remote sensing [33], where it has been used to assess remote sensing techniques

[34,35]. It is possible that remote sensing techniques could assist in mineral exploration by revealing ore bodies in inaccessible parts of the world. The integrated use of multi-spectral photography and thermal imagery from aircraft and satellites allows the detection of distinctive vegetation or plant communities over ore bodies. These anomalies can be detected because of the difference in reflectivities of the species of the plant communities and possibly of the soils and bedrock in such areas. At present there are no sensing techniques capable of penetrating the surface covers of vegetation, soil, or fossil weathering horizons. Consequently, the value of the technique depends on the interpretation of the data. Such an interpretation was carried out in the Dugald River area [34,35] where there was a little-disturbed major lead-zinc ore body, the Dugald River lode, and where the geochemical and geobotanical field investigations had already been carried out [32] (Fig. 1). The area dominated by the species *Polycarpaea glabra* and *Eriachne mucronata*, which demarks the lode can be seen running in a north-south direction.

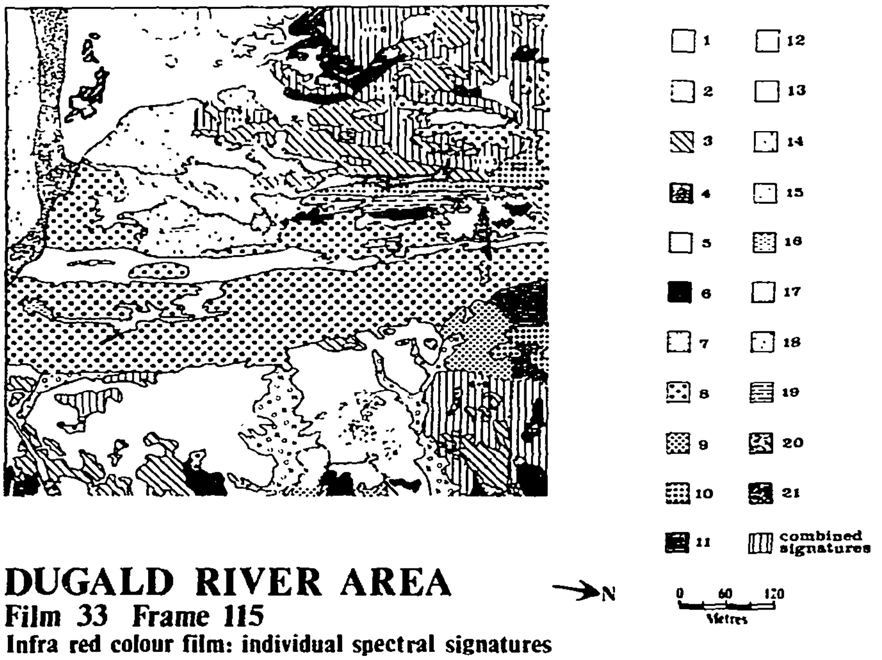

Fig. 1. Dugald River lode area (scale 1 : 15 000), spectral units recognised by interpretation of infrared colour photography. Unshaded area shows Dugald River lode with plant communities of *Polycarpaea glabra* and *Eriachne mucronata* with *Bulbostylis barabata* and *Fimbristylis* sp. as subsidiary species. For composition of other shaded units see ref. 35. (Reprinted with permission from ref. 35.)

D. THE COLONIZATION OF MAN-MADE HABITATS BY METAL TOLERANT PLANTS

As well as growth on undisturbed sites, metal tolerant plants also colonize man-made habitats [36–40]. The main factors which determine plant distribution on contaminated sites have been reviewed by Antonovics et al. [12], and those which govern the inhibition of plant growth on industrial wastes have also been discussed [36,40,41].

There has been mining activity in the United Kingdom since the Bronze Age [26]. The most important areas have been Devon, Cornwall, Somerset, central and north Wales, the Pennines, the Lake District and the southern uplands of Scotland. In the past, mining in Britain concentrated on the extraction of high grade ores from fissure-type deposits and in certain areas of the country. Unexploited mineralisation of this type still exists [42]. However, the once numerous mines are virtually all abandoned for economic reasons. In many cases these metalliferous sites are colonized with indicator plants which have ecotypes exhibiting tolerance to metals [42,43].

Minuartia verna and *Thlaspi alpestri* occur widely in mineralised areas in England and Wales [43]. *Armeria maritima*, *Silene maritima* and *Cochlearia officinalis* are coastal species with some inland occurrences which are related to mineralized soils. *Lychnis alpina* is associated with mineralization in Cumbria, and Derbyshire *Voila lutea* is found in mining areas.

E. FACTORS AFFECTING COLONIZATION OF METALLIFEROUS SOILS [12,38]

The analysis of the metal content of a soil or spoil is generally carried out by atomic absorption spectroscopy after digestion of a sieved sample in 8 mol dm^{-3} nitric acid. Some results of such analyses are given in Table 1 [45]. Sometimes the "available" metal is determined after extraction with EDTA

TABLE 1

Mean metal content of soils (ppm dry soil) (data from ref. 45)

Metal	Total [a]		Available [b]	
	Uncontam. [c]	Contam. [d]	Uncontam. [c]	Contam. [d]
Pb	37	3300	15	1816
Zn	94	2923	4.4	330
Cu	13	28	8.3	12.7
Cd	1.5	23	0.23	4.7

[a] Nitric acid extractable.
[b] EDTA extractable.
[c] Uncontaminated soils from Devon.
[d] Contaminated soils from Halkyn, Wales.

or acetic acid or water [36,45] Gemmel [36] suggests that water extraction gives the most realistic indication of the soluble metal which might be available to the plant roots. Some metals may have different concentrations at different depths, and some may not move readily within the soil. For example, cadmium pollution which results from aerial fallout remains in the top 0—5 cm of soil [46—48] and seems not to move downward. In such cases the depth or root of the plants is very important. Other factors affecting the vertical distribution of metals in soils are leaching, accumulation in decomposing vegetative litter and the existance of ore bodies at different depths [18].

The major essential macronutrients required by plants from the soil are nitrogen, phosphorus, potassium, calcium, magnesium and sulphur. Most wastes are deficient in one or more of the macronutrients N, P or K, and are usually very rich in sulphur. These factors may act directly upon the plants, or may act indirectly by alleviating or enhancing the toxicity of the metal present in the soil. Thus the plants may be tolerant to another factor as well as to the metal. For example, it was shown [32] that in an Australian copper soil, the distribution of the plants was determined not only by the metal content, but also by that of phosphorus. The soil in this metalliferous area has higher phosphorus content than the surrounding areas, in contrast to mine wastes which are generally low in phosphorus.

Waste and mine soils are often deficient in organic matter and humus. Not only does organic material provide a source of nitrogen and other nutrients both for plants and for soil organisms, it has a high water-retaining power. Organic matter also provides ligands which chelate with toxic metal ions, and these can be important in the movement of toxic metals through soils [49—51]. Metal ions and their complexes may be adsorbed by, for example, clays or hydrous oxides [7,52,53]. Adsorbed metal ions are in equilibrium with chelated soluble metals in the "labile pool" which are in turn in equilibrium with those being absorbed by plants from the soil solution [54]. Both diffusion and mass flow of the soil solution are important in the movement of ions to the roots [55—57]. Since both occur simultaneously, a mathematical treatment including both factors has been developed by Passioura [58] and by Nye and Marriott [59].

Chelating agents in soil can have two opposing effects on toxic metal ions [50,60]: (a) they can promote solubilization and movement of metal ions, thus increasing their toxicity; (b) they can sequester the toxic metal ion and make it less available to the plant. Humic and fulvic acids are well studied, but ill-defined, organic constituents of soils, natural waters and sediments. There are several reviews of the chemistry of these compounds in the literature [61—65].

In a series of papers, Bloomfield and co-workers [66—70] found that some insoluble metal oxides were rendered soluble by anaerobically and by aerobically decomposing plant material. The organic constituents of the decomposing plant matter, which resembles natural humose material, mobilizes Mo, V, U, Cu, Mn, Co, Ni, Zn and Cd, partly in association with colloidal humified organic matter and partly in the form of soluble anionic complexes. The nature and constitution of these complexes is unknown. Recently humic and fulvic acids and their metal complexes have been studied by IR spectroscopy [71], ESR spectroscopy [72—75], NMR spectroscopy [76,77] and electron microscopy [78].

The complexing capacity of natural waters has been discussed [79,80]. In a consideration of the binding capacities of the polyelectrolyte chelating agents in natural waters, it has been shown that the conditional binding constants are a function of the total ligand concentration. Langford et al. [80] have pointed out that the conclusions concerning the relative binding capacities and structure of polyelectrolyte ligands derived from different soils may not be reliable if the mathematical treatments applicable to small model systems have been used.

Both the chelation and the surface adsorption of metal ions and organic materials are strongly affected by pH [81]. This can be particularly important in the toxic soils in old mining areas: copper mine soil has a pH value in the range 3—5; lead mine areas may be acid or alkaline and zinc mine soil is around neutral.

Calcium is an extremely important element in the consideration of toxic soils. Addition of calcium to metalliferous soils is generally considered to decrease the acidity and thus to reduce the availability of the metals. Thus the availability of boron, iron, zinc, nickel, cobalt and manganese is decreased when the soils are limed. In contrast, that of molybdenum is increased. When copper is present at toxic levels, it is also rendered more available by a lowering of the pH. At low levels of copper, there is no relationship between copper availability and soil pH. This is explained by the marked selectivity of soil cation exchange sites for copper, which become saturated at high concentrations of copper, which then occupies also nonselective exchange sites, from which the copper is exchanged at low pH values.

F. RECLAMATION OF CONTAMINATED WASTELAND

Metal tolerant species have been used in attempts to reclaim and recolonize metal contaminated wastelands [36—41,82—93]. Not only do the plants have to withstand high concentrations of metals but also low nutrient status. The addition of fertilisers and organic materials has been found to be beneficial [36,89]. Goodman [41] has reviewed the problems in the rehabilitation of industrial wastelands. The most important environmental inhibitors to the growth of plants are: instability, spontaneous combustion, steep slopes, floods and droughts, high level of toxic elements, cementation and compaction, temperature extremes, low nutrient status, excessive stonyness, uneven surfaces, no soil micro-organisms.

In mine spoil habitats it appears that metal tolerant species have evolved very rapidly [39], for example, tolerant plants have been found in areas polluted for fewer than 20 years [86]. Tolerant individuals can be selected from non-tolerant populations in one step [87].

G. MEASUREMENT OF TOLERANCE

The index of metal tolerance is usually measured by the root elongation method [94—98]. Young plants or tillers are grown both in calcium nitrate solution (1 g dm^{-3}) and in calcium nitrate solutions containing known concentrations of the metal under test. The growth in the toxic solution is expressed as a percentage of that in the calcium nitrate solution. Using such methods, tolerance of various species has been demonstrated, and this is usually correlated with the available metals in the plant habitat. In 1958 Jowett [97] demonstrated that populations of *Agrostis tenuis* tolerant to one metal were not tolerant to another unless it was also present in the soils where the plant occurred. It was later demonstrated [38] that tolerance of *Agrostis* is specific for copper, zinc and lead separately.

TABLE 2

Possible mechanisms of metal tolerance in plants

- A. External mechanisms
  - (1) Metal is not available to plant root
    - (a) metal is present in water insoluble form
    - (b) metal is present in soluble, but chelated form not available to plant root
    - (c) concentration of freely diffusable metal ions is small
- B. Internal mechanisms
  - (1) Metal is available to plant root but is not taken up
    - (a) alteration of cell wall membrane of roots giving decreased permiability to toxic metal ion
    - (b) alteration of surface enzymes of roots
    - (c) excretion of substance by root, rendering toxic metal insoluble or unavailable
  - (2) Metal is taken up but rendered harmless to metabolism within the plant
    - (a) metal bound in insoluble precipitate or complex
    - (b) metal bound in soluble, innoccuous, complex of high thermodynamic stability or low kinetic lability
    - (c) metal removed by spatial separation, e.g. in cell walls or vacuole
  - (3) Metal is taken up but excreted
    - (a) by loss of collecting organ e.g. shedding of leaf
    - (b) by guttation
    - (c) by leaching of soluble metal by rain
  - (4) Metal ion is taken up but metabolism is altered to accommodate increased concentration of metal ions
    - (a) increase of enzymes inhibited by metal
    - (b) inhibited enzyme systems by-passed
    - (c) alternative metabolic pathway not requiring products of inhibited enzyme system
    - (d) metal required for metabolism

H. POSTULATED MECHANISMS OF TOLERANCE [11—16]

Table 2 gives some of the possible mechanisms of tolerance of plants to metals. Wainwright and Woolhouse [13] have pointed out that much of the evidence for the mechanisms is flimsy. Several difficulties arise in the investigation of metal tolerance. Studies may be made of tolerant and non-tolerant populations of a particular plant from metalliferous and non-metalliferous habitats. Not only is the metal content of the respective soils different, but other factors vary also, e.g. nutrient status and water availability. In a search for possible metal storage compounds, metal organic complexes have been isolated from plant sources. Many metal ions form kinetically labile complexes and the breaking down of the plant cells will lead to the release of many organic potential complexing agents. The complexes isolated may be those with the greatest thermodynamic stability in the extraction mixture and not those which exist in the living and intact plant.

I. UPTAKE OF IONS BY PLANTS

The current theories concerning the uptake of ions by plant roots and transport to the aerial parts of the plant have been discussed by Bowling [99] and Epstein [100].

Plant cells [99—101] (Fig. 2) are encased in a wall consisting of cellulose and other polysaccharides. As the plant cell ages, the wall becomes impregnated with other substances, notably lignin. The walls are cemented together by a substance mainly composed of pectates, in which Ca^{2+} acts as a structure former. Pectic acid (Fig. 3.), poly(α-galacturonic acid), is made up from β(1—4) linked α-galacturonic acid monomers. Pectin has many of the carboxyl groups esterified as methyl ethers [102]. The cell walls are produced by the living part of the cell.

Ions and water are taken into the plant by the root [99]. The outer layer of the root, the epidermis, is provided with root hairs, which usually have a pectic coating, allowing them to adhere to soil particles. A transverse section of a root is shown in Fig. 4. Water and inorganic salts can pass easily through the outer portion of the root, the cortex, where the cellulose walls and intercellular spaces are freely available to aqueous solutions. The centre portion of the root, the stele, contains the vascular system: the phloem which conducts organic substances and the xylem which conducts water and mineral salts. The stelar portion of the root is surrounded by a distinct layer of cells, forming a boundary which separates the stele from the cortex. This layer is the endodermis, which contains a strip of suberised (waxy) material, the Casparian strip. Water and dissolved salts cannot pass the Casparian strip and it thus forms a barrier between the cortex and the stele. In order to reach the

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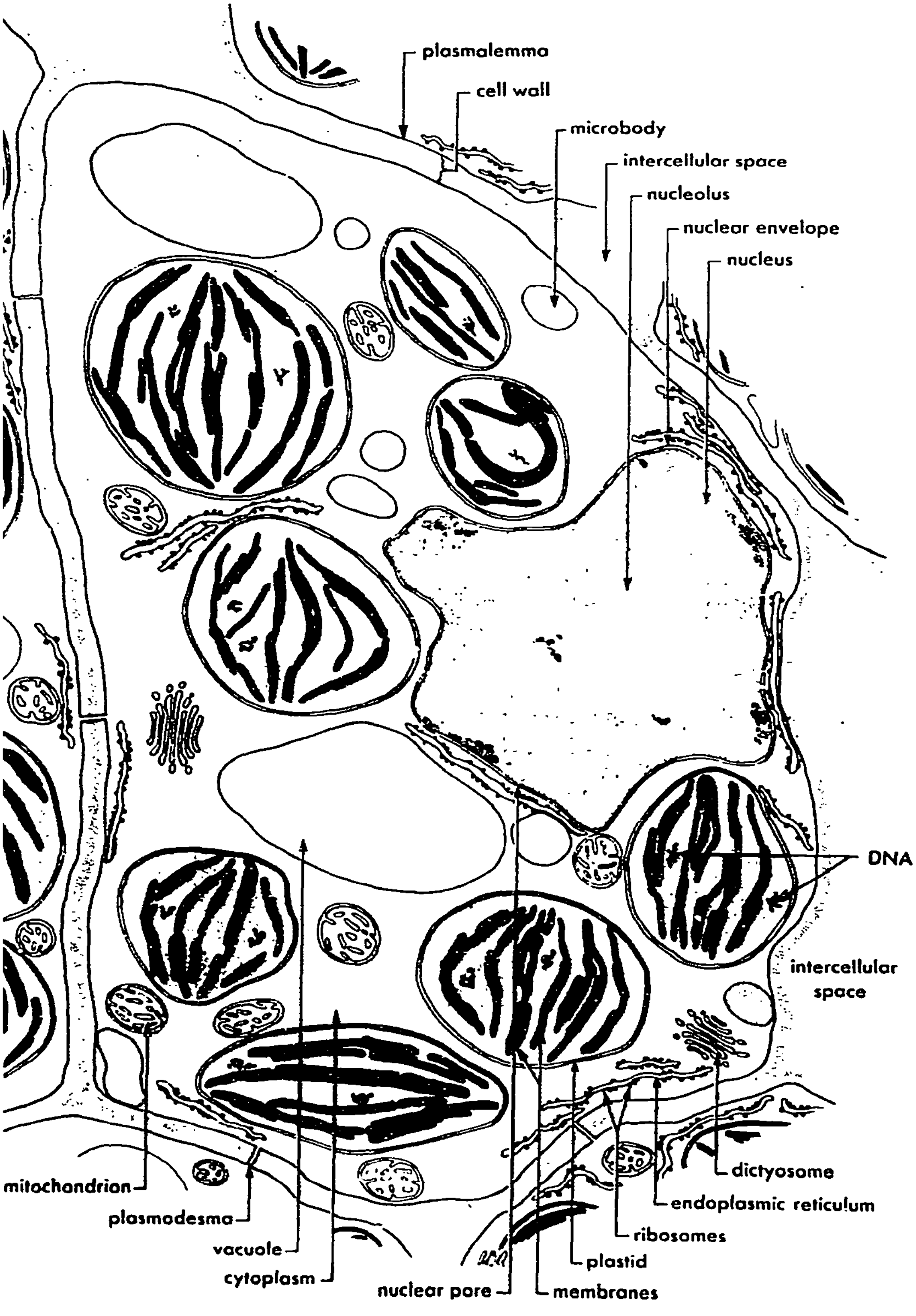
Fig. 2. Leaf cell from bean seedling (from ref. 101(a), p. 31).


plasmalemma
cell wall
microbody
intercellular space
nucleolus
nuclear envelope
nucleus
DNA
intercellular space
dictyosome
endoplasmic reticulum
ribosomes
plastid
membranes
nuclear pore
cytoplasm
vacuole
plasmodesma
mitochondrion

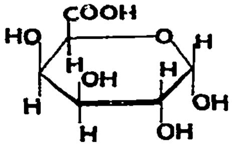


Fig. 3. α-galacturonic acid.

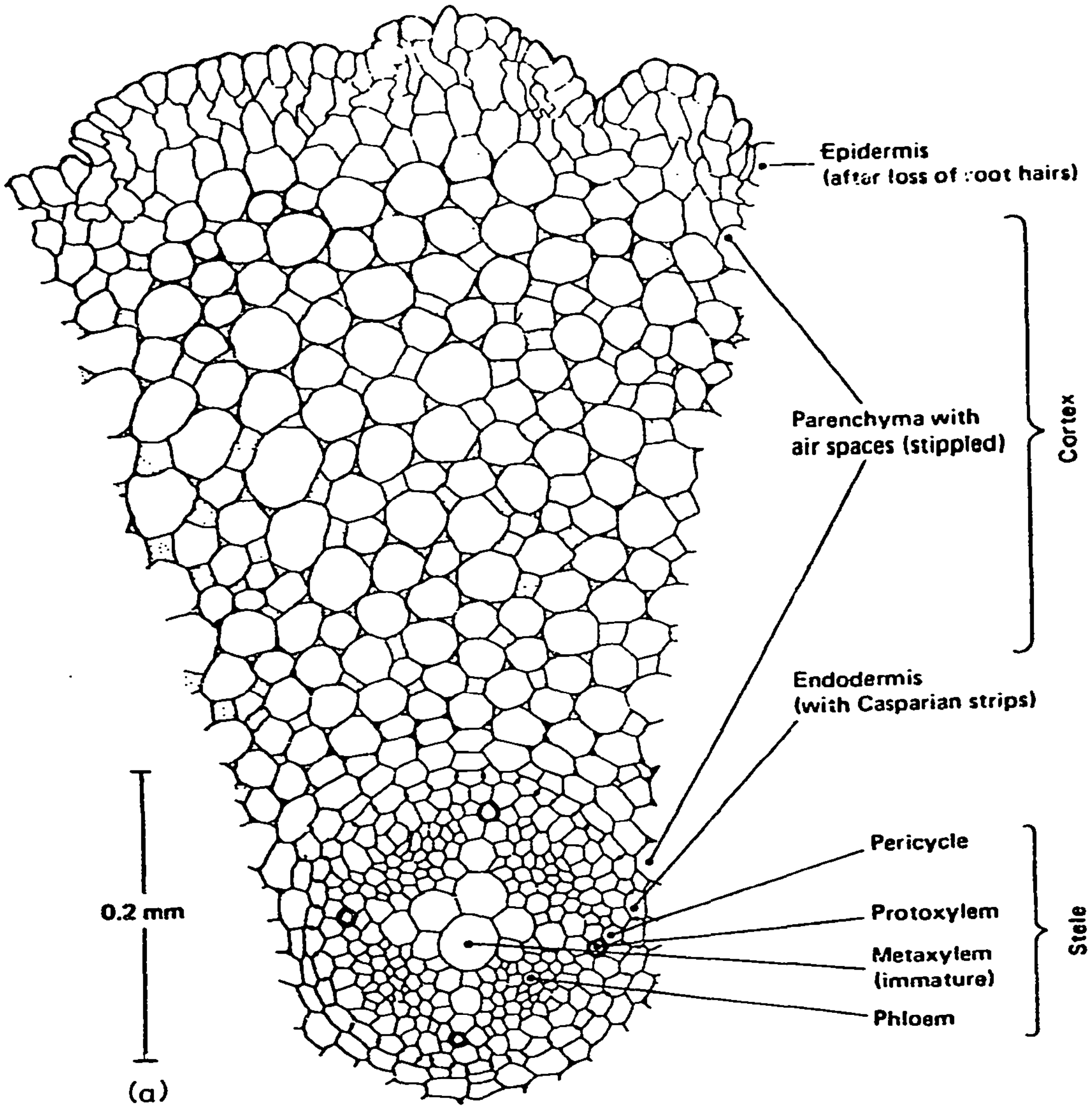


Fig. 4 (a).

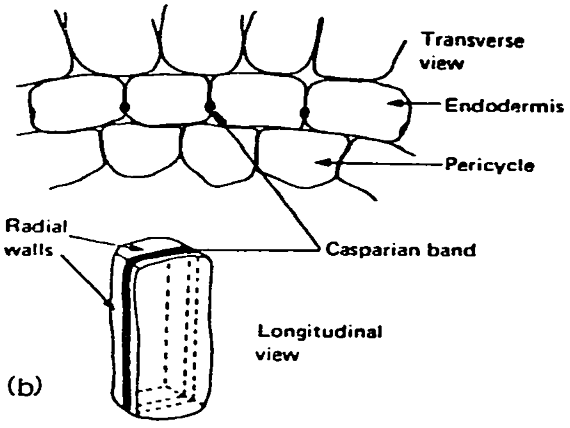


Fig. 4. (a) Transverse section of root of *Ranunculus repens.* (b) Location of Casparian band. (From ref. 99, p. 4.)

xylem, the water and dissolved salts have to pass through the living portions of the cells (protoplasts) by passing through a membrane (plasmalemma) which probably provides the control for the entry of water and dissolved salts into the plant.

Outside the Casparian strip salts move by diffusion, although passage through a membrane probably requires energy, and is thus termed active transport. Bowling et al. [103] showed by application of the Nernst equation that the polar ion flux was powered by active transport of anions, the cations following to maintain neutrality. There is a potential difference across the root [104] in which the trans-root potential behaves like the trans-membrane potential of the individual cell. It has been suggested that this is evidence that the Casparian strip is an effective barrier to water movement. Two electrical compartments are thus produced; one outer to the endodermis, one inner [105,106].

Early experiments [107] using coloured salts showed that their diffusion into the root stopped at the endodermis. More recently, electron microscope examination of barley roots fed with uranyl acetate showed uranium up to the Casparian strip, but not beyond [108].

Ions on their way to the xylem must cross at least one membrane. There are two general pathways:

(i) Ions cross cortex by means of cell walls, then cross membranes of the endodermis by active transport.

(ii) Ions cross the cell membranes of the epidermis, then cross the cortex by means of cytoplasmic drift through the small common areas between cells (the plasmodesmata) (Fig. 2)). The cytoplasm is continuous and extends from cell to cell through the plasmodesmata. This continuum is called the symplasm.

There is good evidence for ion transport by both mechanisms. It has been shown [109] using a soluble lead compound, that it is most likely that water is transported via the cell walls, and that ions are transported by the symplasm. More evidence on this point is required.

The xylem sap has been examined and analysed for a number of plants by decapitation and collection of the exudate. Iron is not taken into the plant as ferric chloride, but is taken up as the EDTA complex. It has been suggested [110] that Ca^{2+} forms a complex for transport across the root, and that in contrast zinc and manganese are present as aquo ions [111,112].

There has been much less success in the correlation of active cation transport and membrane bound ATPase activity in plants than in animal cells. In particular there has not yet been found a relationship between uptake of metal ions by roots and ATPase activity. As plant cells are less specialised they contain more types of ATPase and activities can be masked [113]. Specific inhibitors are also lacking. It may be that specific cation transporting ATPase do not occur [114]. However there does appear to be a connection between ATPase activity and salt uptake [115].

J. TOLERANCE MECHANISMS FOR INDIVIDUAL ELEMENTS

(i) Aluminium

The toxicities of the metals aluminium, manganese and iron and tolerance to them, particularly for crop plants, have recently been considered in detail [6].

The availability of aluminium in soils is governed by the soil pH [116]. Plants from acid soils are generally tolerant of aluminium [117,118]. The effect of aluminium on the reduction of root growth is similar to that produced by the heavy metals Pb, Zn and Cu and this has been suggested to be due to the inhibition of mitotic cell division [119,120].

The roots of some Al tolerant plants: wheat, barley, rice, peas and corn grown in nutrient solutions are able to increase the pH of their solutions [6,121]. This effect has also been demonstrated when the root is in soil [112]. This ability to change the pH has been linked with proton release as more cations than anions are absorbed [123,124]. However, Foy et al. [6] have pointed out that the pH changes appear to be more clearly related to the nutritional status of the root, and are possibly the result of growth under aluminium stress [125].

Some plants achieve aluminium tolerance by excluding the metal [126], while some take Al into the roots but it is not transported to the aerial parts [127]. Henning [125] found that aluminium was absorbed by wheat roots, and located the position of uptake by using chelating agents which gave fluorescent compound with Al. Considerable amounts were retained in the cortical cells of the root, and it was suggested that aluminium tolerance in this wheat cultivar is brought about by exclusion by the plasmalemmas of the root cells, due to a difference in the molecular make-up of the membranes compared with non-tolerant varieties. It has also been suggested that the plasmalemmas may contain a complete new protein network which reduces permeability to aluminium [128]. Difference in cell wall pectins between tolerant and non-tolerant plants has also been suggested as a tolerance mechanism. Particularly in the extent of methylation of the galacturonic acids [129]. However, in aluminium-treated pea roots, no interaction was found between Al and galacturonic acids.

Russian work on Al-tolerant peas has also suggested aluminium accumulation in combination with root cell wall carbohydrates [130]; other effects have been an increase in the SH groups in root homogenates of Al-tolerant pea cultivate grown in Al containing nutrient solutions, and a decrease in the total concentration of free radicals [131,132].

Aluminium has been shown to be precipitated in the cortex, endodermis and later roots of Al sensitive sugar beets [133,134]. The accumulation of aluminium in roots in association with phosphorus has been shown in other species [135—139]. Aluminium may also act as a sink for phosphorus by extracting this element from other cells [140]. As well as the precipitation of inorganic aluminium phosphate [133,134,141] Al tolerant varieties have also been shown to have high activities of root phosphatases [142]. Woolhouse [143] in a study of the grass, *Agrostis tenuis*, which is capable of rapid evolution of races adapted to heavy metal containing soils [39,86], investigated the effect of Al^{3+} on the surface phosphatases of three ecotypes: calcarius, acidic and lead spoil. It was found that the inhibition of the enzyme activity is less in the case of the acid race.

Tolerant plants of *Medicago sativa* have been found to contain more calcium and less aluminium in the shoots than non-tolerant plants [144], and it was concluded that calcium reduced aluminium transport to the shoots. Davies and Snaydon [145] found that *Anthoxanthum odoratum* from unlimed plots in the Park Grass experiment, Rothamsted, contain more calcium and phosphorus within the shoots than the plants from the limed plots. They further showed that the populations from the acid soils were more tolerant of high aluminium concentrations in culture solutions than were those from the calcarius soils. [146]. Thus populations from acid soils may tolerate Al because of their additional tolerance of low concentrations of Ca and P.

In some aluminium tolerant plants the metal is transported to and accumulated in the aerial parts of the plant; examples are tea [147] and mangrove [148]. In the tea plant Al is found in the epidermal cells of old leaves [147, 149] where flavonoid type complexes have been suggested [149]. Root exudates of tea plants contain malic acid which may solubilize Al, P and Fe in the soil [150].

(ii) Manganese

Mn(II) is available to plants and is taken up rapidly by most species and appears to be very mobile within the plant. Mn(III) is not available and the manganese tolerance of rice is associated with the oxidising power of its roots [151]. In addition rice plants are able to accumulate Mn in the leaves, where up to 7000 ppm have been reported [152]. Many other plants have been shown to take Mn into their aerial parts; for example, concentrations in excess of 2600 ppm have been recorded in carrot tops [153], and tomatoes have been shown to take up more than 5000 ppm [154]. Manganese is thought to exist in the simple aquated Mn^{2+} ion [155], although association between Mn and amino acid and carbohydrate fractions in sunflower exudate has been reported [156]. A complex in which the manganese is bound to a protein has been isolated from peanut seed [157].

(iii) Iron

Tolerance to iron occurs with tolerance to waterlogging [158]. In addition it has been found that the roots of plant species which are tolerant to waterlogged conditions oxidize iron and have reduced transport to the tops [159]; examples of such species are reed canary grass and rice. No ability to oxidise iron in the soil is shown by alfalfa, which is also sensitive to wet soil conditions, and it is suggested [159] that the oxidation of iron in the wet soil around the root constitutes the tolerance mechanism. The work of Tiffin and co-workers [160] has shown that the uptake of iron in soybean involves the reduction of Fe^{3+} to Fe^{2+}, the redox site probably being at the plasmalemma. Since translocation of iron from the roots to the shoots involves ferric citrate [162], re-oxidation must take place within the cytoplasm [161, 163,164]. Zinc and iron are mutually antagonistic in terms of uptake [165, 166]. Similarly, an excess of Fe in soil can induce manganese deficiency, and iron-tolerant navy beans have been found to be less susceptible to Mn-deficiency symptoms than non-tolerant plants, indicating that the tolerant plants were able to exclude iron [166].

The nutrient status of a plant also influences its tolerance of high iron levels in the soil. Plants which are deficient in Ca, Mg, P, Mn and K do not exclude iron. For example, rice plants deficient in these elements accumulate iron in their shoots and are susceptible to iron toxicity which may be alleviated by an adequate supply of these nutrients [167]. There is also interaction between iron and other metal ions: Fe^{3+} in the form of the EDTA complex reduces the toxicity of many metals to mustard grown in culture solution as compared with Fe^{3+} added in the form of the chloride.

(iv) Lead

Lead in the environment has been the subject of several reviews (160—171). The use of biogeochemical techniques, including the analysis of vegetation in the search for contamination and for lead anomalies has been discussed by Warren [172]. pH has a great effect on the availability of lead to plants [173], low humus content and high pH values favouring the uptake of lead. In general lead is taken into the roots and little is translocated to the aerial parts particularly in fertile soils [174]. However, on humus deficient mine spoils plants are found to have high lead levels in the leaves [90,175].

As with the uptake and translocation of iron, that of lead also depends on nutrient status. There are indications of the importance of sulphate status in lead uptake [176]. Phosphate forms insoluble lead compounds in soil [177], but if the phosphate level is low, e.g. in lead mine soils, more lead will be available to the plant. If the phosphate status is normal or high, plants do not usually accumulate lead [174]. Seasonal variations in lead concentrations of up to 10 fold have also been found in vegetation from lead mining areas [178,179].

Lead was found to be incorporated into the nuclei of moss leaf cells as a non-diffusable complex [180]. Studies of the distribution of lead within plant tissues have shown in corn, lead is associated with the cell walls in crystal form [181]. In the leaves of *Pisum* lead has been shown to be bound to chloroplast membranes [182]. Lane and Martin [183] showed that in radish, lead moves across the cortex of the root and collects at the endodermis. The endodermis thus provides a partial barrier to lead movement between the root and the shoot, accounting for the accumulation of lead in the roots [184] when compared with the shoots [176]. Thus in radish the Casparian strip is a barrier to the diffusion of lead from the cortex into the stele, in agreement with the report [181] that in barley roots most of the lead taken up can be extracted with water, and only a small proportion migrates into the vascular tissues and the aerial parts of the plant. In beans much more lead is translocated throughout the plant, however [185]. Water plants have also been shown to take up lead [186], and electron micrographs demonstrated that lead passed through the thin cuticle into the thick outer epidermal cell wall. Such aquatic plants do not have root systems in the same way as other plants, and take up ions directly from the external bathing medium. Lead was shown to be associated with pectates both in the cell wall and plasmalemma.

In both a theoretical [187] and an experimental [188] study, Ramamoorthy and Leppard, have examined the release of particle bound cations, in particular lead, to the roots. These suggest that pectin rhizoplane fibrils produce large contact areas for ion exchange by the penetration of the surface of soil particles. Each fibril exploits a zone around it and can thus "feed" on the solid soil by a "contact ion exchange process". The effect of lead on soil respiration has recently been discussed [189].

High lead levels in leaves and trees can also result from pollution by aerial fallout. Such lead can largely be removed by washing and consists of superficial dust coating [190,191]. Lead content of tree rings has been used as a chronological tracer of atmospheric pollution [192,193] where lead content can be correlated with the use of leaded petrol, and this type of measurement gives an indication of the distribution of lead from motor vehicles.

(v) Nickel and chromium

High concentrations of nickel and chromium occur in serpentine soils in various parts of the world, and in spoil heaps from chromite mining. Serpentine soils have a characteristic lack of fertility, which has been considered to be a lack of calcium (194—196) or to the high levels of nickel and chromium, (196—202). The addition of calcium to serpentine soils has been shown to decrease the acidity, which in turn decreases the absorption of nickel [196]. Conversely the addition of ammonium nitrate reduced the pH of such soils and increased the availability of toxic metals [203]. The addition of calcium to serpentine soils and vegetation does not decrease the total amount of nickel take up by plants, which are larger and thus contain lower concentrations of nickel [203]. Grasses from Rhodesian serpentine soils show the ability to take up calcium from toxic soils, yet yield less than grasses from control soils [204]. Stunted growth on nickel rich soils has been reported [203—206].

Very high concentrations of metals have been found in indigenous plants from nickel-chromium soils [18,205—211]. Some of the plants reported in these papers accumulate very high levels of nickel in their tissues: *Hybanthus floribundus* contains up to 1.3% nickel on a dry mass basis [208,209,212]; *Psychotria douarrei* has been reported as having Ni in the plant ash up to 44% [211]. A large proportion of the nickel contained in *Hybanthus* [212, 213], *Psychotria douarrei* and *Allysum bertolonii* [214] is soluble in water. Nickel in an aqueous extract of *Allysum bertolonii* has been shown to be associated with malic and malonic acids [214]. The major portion of nickel, in *Hybanthus floribundus* is accumulated in the leaves [212] where it is associated with pectic carbohydrates [215]. *Dicoma nicollifera* [216,217] which is generally confined to serpentine soils with high concentrations of nickel, translocates less nickel to the shoot than other species of *Dicoma* [210].

Shewry and Peterson [202] have shown that concentrations of nickel and chromium in plants are species specific for a range of species from various serpentine sites, including chromite spoil heaps. Positive correlations between chromium and nickel levels were obtained, since nickel concentrations reflected those in the soil, whereas chromium concentrations in roots were well below the soil values. This was explained by the non-availability of chromium, which was shown to be strongly bound in soils and not solubilized by a variety of extractants. It was concluded that the metal level in the plants gives the best measure of availability.

Two exceptions to the general rule that tolerance is normally specific to the metals found at the site of origin have been reported for nickel: populations of *Agrostis tenuis* from zinc contaminated areas showed co-tolerance to nickel without prior exposure to nickel [38] and a clone of *Agrostis gigantea* was shown to be tolerant to nickel [218]. This nickel tolerant clone was, however from an area with normal Ni levels in the soil [219,220] and it is suggested that tolerance to nickel might be more widespread than tolerance to copper [218].

Nickel is possibly an essential element for plants, since urease from jack beans has been found to be a nickel metalloenzyme [221].

(vi) Copper

Much information about copper tolerant plants has come from the study of the metal rich regions of Africa south of the Sahara. The ecology of the copper flower *Becium homblei* [223] from this region has been reported. The biochemistry and copper levels in this plant have been studied [224—227] and it has been shown that the level of copper in the leaves is high, unlike those of the grasses which grow in the same locality, where high levels of copper are accumulated in the roots and only a limited amount is transported to the aerial parts [227]. A comparison of green and chlorotic plants showed a significant correlation between the copper and nitrogen contents of both, and Reilly [228] has shown that the total range of amino acids is qualitatively the same and suggests therefore that the explanation of copper tolerance of *Becium homblei* should be sought elsewhere than in soluble amino acid complexes. There are biological links between copper tolerance and proteins, however, in copper resistant yeast and copper tolerant algae [229,230]. Farago et al. have shown that *Armeria maritima* from a copper containing bog in Wales is highly tolerant of copper [22], and the metal accumulates in the roots and is translocated to the leaves. In the roots 85% remains in the outer portion and on sequential extraction the major portion of the copper appears in the same fractions as pectates. The roots of copper tolerant *Armeria maritima* contain very high levels of the amino acid proline [231], which is present not only in the plants growing in the bog, but also in plants grown from seed collected from bog plants, and grown with and without additional copper. The high proline concentrations were not observed with plants from a maritime, non-copper site, or in the roots of plants grown from non-tolerant seed with or without additional copper. Thus high proline content does not appear to be a response to toxic levels of copper, but an inherited characteristic, the function of which is not clear at present.

In a study of grasses from metal rich African soils, Reilly and Reilly [232] confirmed high levels of copper in the roots in contrast to herbacious plants which accumulate it in their leaves, and suggested a difference in the mechanism of copper tolerance in monocots and dicots. The grass, *Stereochlaena cameronii* takes up higher levels of copper, zinc and lead when growing on arable soil than on poor soil. There appears to be no direct correlation between the total sulphur and the heavy metal content of this grass, in agreement with the indication that sulphur is unimportant in the metal tolerance of *Agrostis tenuis* [11,233] and *Becium homblei* (228). A connection between copper tolerance and sulphur has been postulated in a yeast [234]. In *Stereochlaena cameronii* heavy metal accumulation is not associated with an increase in nitrogen content [232] in contrast to *Becium homblei* [228].

In a study of the Californian monkey flower, *Mimulus guttatus*, Allen and

Sheppard [235] found that the roots of non-tolerant plants were damaged by copper, both in soil and in aqueous solutions, leading to a selection for copper tolerance on copper soil at the germination and seedling stage. Plants from a copper area were shown to be tolerant also to zinc, lead and nickel. The importance of the cell wall in accumulation of copper in clones of the grass *Agrostis tenuis* has been discussed by Turner [233]. An even distribution of copper throughout bean leaves has been observed [236] using X-ray microprobe analysis.

Recent studies of enzyme systems of zinc- and copper-tolerant clones of *Agrostis tenuis* [13,16], have shown that in copper tolerant and sensitive clones the phosphatase enzymes associated with the cell surfaces had different copper sensitivities, a difference which was not reflected in zinc tolerant and non-tolerant clones. Thus Woolhouse [13,16] suggests that in the case of copper, the tolerance mechanism in the grass *A. tenuis* resides in the modification of the enzyme systems of the cell surfaces, and that phosphate transport systems of the plasma membranes are modified in the copper tolerant clones.

Toxic levels of copper has been shown to interfere with enzyme mechanisms in lettuce seedlings [237].

Thus with copper it appears that in the case of grasses the metal is either excluded from the roots or taken to the root but not translocated to the shoots, whereas in the case of herbaceous plants, copper may be transported to the leaves where very high levels may be found, for example in the African species of *Crotalaria* [238].

(vii) Zinc and cadmium

Much work on the mechanisms of zinc tolerance has been carried out using tolerant clones of the grass *Agrostis tenuis*. Peterson [239] has studied the distribution of ^{65}Zn in zinc tolerant and non-tolerant *A. tenuis* and has shown that the main difference between these two populations is that the former contains a higher percentage of the radio-isotope in the pectate fraction of the root cell wall. Similarly Turner discussed the importance of metal accumulation in *Agrostis tenuis* [233] by the cell wall. It was further shown by Turner and Marhsall [240] using ^{65}Zn that in cell-free systems extracted from the roots of clones of *A. tenuis*, the cell wall fraction was the major site for zinc accumulation especially in zinc tolerant clones, where more zinc accumulated in this site than in non-zinc-tolerant clones. Significant correlations were found between zinc accumulation in the cell wall and the index of tolerance of the populations, and its was suggested [241] that the greater the tolerance of the population, the greater is the capacity for accumulation in the cell wall site.

The presence of toxic concentrations of accumulated zinc does not cause changes to the plasma membrane [16] as do high levels of copper. No differential sensitivity of the enzymes of the cell surface to zinc was demonstrated, unlike the case of copper. However, in a study of zinc tolerant and non-tolerant *Anthoxanthum odoratum*, the phosphatase enzymes did possess differential sensitivity to zinc [242].

In a study of zinc tolerant *Silene cucubalus*, Mathys [243] found that the enzymes of the zinc tolerant plants had higher activity at zinc levels which would have been toxic to the enzymes of the non-tolerant plants, and it was suggested that the zinc tolerant plants needed high levels of zinc.

In a series of papers on zinc tolerant *Silene maritima*, Baker [244—246] showed that zinc transport differed in mine populations from those in coastal and arctic-alpine populations. All populations accumulated zinc in the root relative to the shoots. In the mine populations, however, zinc was excluded from the shoots. Baker concluded that zinc tolerance in *Silene maritima* can be accounted for by the following mechanisms: an overall reduction in uptake; lack of transport from root to shoot; or accumulation in the root alone or together with shoot exclusion. Since little correlation was found between uptake characteristics and indices of tolerance, it was concluded that mean shoot accumulation levels and root/shoot accumulation ratios would provide more satisfactory criteria of tolerance than root elongation methods in the case of *Silene maritima* [244]. Total zinc uptake was little affected by increases in the calcium levels with the tolerant plants, but was decreased in non tolerant plants [245]. At high Ca^{2+} concentrations zinc uptake was stimulated in the tolerant plants. There is evidence to suggest that Ca^{2+} is involved in cell permeability [247] and is thus very important in metal tolerance mechanisms [16,248]. Large differences in P uptake occurred between the zinc tolerant and non-tolerant populations of *S. maritima* [246]. Zinc-phosphorus interactions have been reported previously [249] and zinc deficiency is associated with high concentrations of phosphate from phosphate fertiliser and it was concluded that zinc is essential for phosphate metabolism [250]. In both *Silene maritima* [245] and *Thlaspi alpestri* [250] increase in P concentration in the culture solution resulted in a decrease in zinc content and a rise in the P content in shoots of tolerant plants whereas an increase in the Zn concentrations in the culture media produced the opposite effect.

Polycarpaea species from Queensland in Australia, have been shown to grow over copper and zinc anomalies [32]. The copper and zinc content of herbarium specimens of Australian species of *Polycarpaea* have been studied by Brooks and Radford [251]; the zinc content of *Polycarpaea* was very variable and did not exceed 730 ppm; the mean value was 90 ppm.

Crotalaria novae hollandiae is a zinc accumulator from Queensland [34] and has been shown to take up to 8975 ppm of zinc in the leaves [212]. This zinc has been found mainly as the water soluble aquo-zinc ion [252], the zinc accumulating mainly in the cell wall and the phloem. It seems unlikely that zinc is travelling in the phloem in high concetrations, but the phloem membrane may restrict entry to zinc ions; thus the metal becomes immobile at the membrane. This mechanism becomes operational only when very large amounts of metal are accumulated, as with *Crotaria novae hollandiae* and zinc, or *Hybanthus floribundus* and nickel [253].

With *Polycarpaea glabra* populations from high zinc areas in Australia, the metal is found in the pectate fraction [252] in the same way as in *Agrostis tenuis* [239]. In both Australian species *C. novae hollandiae* and *P. glabra*, the pectates separated from the plants did not show large differences in zinc absorptive capacities in plants from high and low zinc areas. The pectates also showed greater affinities for Cu^{2+} ions than for Zn^{2+} ions. It was concluded that in these plants, although zinc was stored in ion-exchange sites in the carbohydrates of the cell walls, these sites are not specific for zinc, and thus are not the mechanism of zinc-specific tolerance.

Mathys suggests that malic and oxalic acids chelate zinc in tolerant clones of *Agrostis tenuis* [254] which might possibly prevent transport of metals in the leaf.

Ernst [255] has found 73.5 $\mu g\ ml^{-1}$ of zinc, 66.0 $\mu g\ ml^{-1}$ of copper and 105 $\mu g\ ml^{-1}$ of iron in the cell sap of the African species *Indigofera dyeri*. This represented only part of the total metal content and Ernst suggested that the cell walls must also be involved in accumulation. The transport of metals from the cell sap, the vacuole system, to the metabolic sites is restricted by the tonoplast. Ernst [255] further suggests that one significant difference between monocotyledons and dicotyledons is that the former can replace their roots continuously without harm to the whole plant. This specialisation may explain the dominance of monocots (grasses and sedges) on metalliferous soils.

Cadmium is a relatively rare element but of widespread occurrance in association with zinc, zinc-lead and zinc-copper ores. Several grass species have recently been found to show cadmium tolerance. Very high tolerance indices were shown by *Agrostis tenuis* growing on old zinc-lead mines in Europe and near an industrial site characterized by aerial pollution consisting of Zn, Pb and Cd. *Festuca ovina* from an old mine area was also cadmium tolerant [256]. The grass *Holcus lanatus* from a site in England contaminated by aerial fallout also shows cadmium tolerance [257].

Aerial pollution by cadmium in the vicinity of smelting works and the uptake of cadmium by vegetation in such areas has been reviewed [258].

The contamination of plants around smelters, in particular trees, is mainly by aerial, particulate fallout [30,41–44,48]. It has been suggested that the fallout exists mainly in the form of insoluble particles which are metabolically inactive [47,48,259–264].

The accumulation of cadmium in surface soils, particularly where these contain large proportions of vegetative litter and humus, seems well established, as does the immobility of cadmium in soil, and its consequent falling off in concentration down the profile [47,48,260]. Thus it has been suggested that cadmium is not taken up via the roots of deeply rooted plants such as trees [47,259]. High levels of cadmium have been found in tree leaves in the vicinity of smelting operations, although mostly without chlorotic symptoms [47]. As much as 4500 ppm of zinc and 70 ppm of cadmium were found in the foliage of trees. Unwashed leaves contained up to six times more metal than those which had been washed before analysis. Buchauer [260] suggests, in agreement with Little and Martin [47,265] that the zinc and cadmium are in biologically unavailable forms and that it is possible that particles can enter directly through the stomatal apertures.

A wide variety of plants have been found to be sensitive to cadmium, e.g. [266—269]. Leaves treated with 5 ppm Cd^{2+} show increased net photosynthesis and transpiration [269], and significant Cd^{2+}/SO_2 interactions have been found. Although Cd^{2+} concentrations of around 5 ppm reduce stomatal opening, higher concentrations of 20 ppm almost completely inhibit stomatal function [259]. Thus it seems possible that the deposition of particulates containing cadmium, part of which is soluble in rain water, would render stomatal pores non-functioning, and thus provide entry points for further particles to penetrate inside the leaf. Since oxide particles in smelter dusts are usually less than 1 μm in diameter [260,270] and stomatal pores range from 5 to 30 μm in diameter, this appears to be a possible mechanism. However, recent work by Godzik et al. [271] has demonstrated that the stomata are of little significance as a route of entry for dust particles. Dust particles were shown to adhere to the wax structures of the epidermal walls without changes in surface structure.

In an investigation of the cadmium uptake by oats and lettuce, as influenced by other elements from culture solution, John [272] showed that the uptake and translocation of cadmium to the tops were reduced the most by an increase of K or an increase of pH. When additional Ca, Zn, P or Al was supplied, Cd levels in the tissues were also reduced. Varying the concentration of applied Cd affected the tissue concentrations of P, Fe, Mn, Al and Ca. An antagonistic effect on root zinc concentration by cadmium has been reported [273,274]. However enhanced Cd supply increased Zn concentrations to the tops of some species [273,275]. There appear thus to be very complex interrelationships between applied Cd and other elements.

K. CONCLUSIONS

A large number of parameters is involved in the study of the mechanisms of metal tolerance in plants, and at present no clear overall picture has evolved, although some particular mechanisms seem to be established for some metals and certain plants under specified conditions. In general, storage mechanisms appear to involve the cell wall carbohydrates, but the mechanisms of uptake, transport, and in particular specificity, are not yet understood. A great deal of research needs to be carried out in this area, which has great importance in agriculture and more generally in the understanding of our natural environment.

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