
Levels, Distribution and Chemical Forms of Trace Elements in Food Plants [and Discussion]

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Levels, distribution and chemical forms of trace elements in food plants

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The content of trace elements in plants can vary widely, depending upon the composition of the soil in which they grow, other environmental factors, and the species or cultivar of the plant. A high growth rate of the plant may cause internal 'dilution' of trace elements. Complex formation with soil organic colloids and compounds, cell wall material and ligands in and inside the cell membranes are of critical importance in uptake, though most evidence shows that it is the free metal ion in the external solution that is absorbed; the detailed mechanisms are still unknown. Other processes such as excretion of organic compounds, reductants and hydrogen ions from the root greatly alter availability of trace metals, and iron has to be reduced to the ferrous form before uptake. The mean composition of plant shoots is affected by age and season; element mobility in the xylem and phloem determines translocation, and hence concentrations in individual parts of the plant. The rate of retranslocation can be strongly affected by the abundance of the element. Symptoms of deficiency or excess are well documented, but are often not dependable.

The essentiality of the trace metals depends upon their function as part of enzymes, and these are briefly reviewed, with stress on processes in plants. Only a small fraction of the total amount of an element is bound in the enzyme; of the remainder, some is present as the free metal ion (Mn) or as complexes of small molecular mass (Cu, Zn, Ni, Fe), the rest being bound to cell wall material.

Certain species or genotypes have resistance against high levels of some elements in the soil. Several mechanisms may be involved, one being very strong binding to root cell walls. There are also large genetic differences in susceptibility to trace element deficiencies.

1. INTRODUCTION

The scope of this paper is far-ranging, and the coverage is therefore largely by generalization or example, and not by a detailed discussion. Some trace elements are essential for plants (Cu, Zn, Fe, Mn, Mo, B, Cl), some are 'functional' or beneficial (Si, Na, Co) (see Hewitt 1979) and some have no known function or essentiality in plants, though they are essential to animals (Se, As, Cr, I). A few may be essential or useful to a small number of organisms, e.g. V appears essential for some algae. Most work has been done on elements that are essential to plants, and these will be used as the main examples here.

2. ELEMENT UPTAKE

(a) Relation of growth and uptake

The mean concentration of any element in a plant is a result of the simultaneous processes of growth and uptake. This is most simply expressed (Nye & Tinker 1977), by the formula

$$X = (S/R_w) (M_R/M),$$

where X is the fraction of nutrient to dry matter, which is governed by the specific nutrient-absorbing power of the root (S), the relative rate of growth of the plant (R_w) and the root/plant

mass ratio (M_R/M). S is in no sense a constant, but depends upon the physiological state of the plant, and upon the ability of the growth medium to supply nutrient at the required rate (see Nye & Tinker 1977).

Cases often occur in which the nutrient concentration may be less in a rapidly growing than in a slowly growing plant, when that element is not rate-limiting for growth, and this is often referred to as 'dilution' (Loneragan 1975). The relation between X and R_w (equation (1)) explains this very simply, if other factors remain constant. In fact, the plant possesses a system of complex internal controls that tend to keep X constant, and S or the root/plant ratio are likely to change when R_w or X changes, so that 'dilution' effects may then not appear. In general, however, when growth rate is sharply increased, for example by adding nitrogen, the concentration of trace elements is likely to diminish (see Olsen 1972). In the field the situation is so complicated that such effects are rarely consistent; Rinne *et al.* (1974) found that when nitrogen rates to grass were greatly increased, zinc and copper concentrations increased whereas manganese and iron levels decreased, and Whinham (1979) could detect no consistent effects of nitrogen supply on the trace element content of grass.

For elements essential to plant growth, there is a functional relation between X and growth rate, in which growth increases rapidly with X until the latter reaches a so-called 'critical level'; if X rises still further, growth is not affected at first, then declines as toxic levels are reached. This relation is idealized, and the 'critical level' may vary quite widely with environmental conditions, cultivar or species of plant, and with the level of supply of other nutrients. The elemental composition may thus vary over a wide range without affecting plant health (table 1). For non-essential elements, there is no initial increase in growth, and the only effect is the possible development of plant toxicity. With tolerant strains, and even more with accumulator species (for example *Astragalus* for Se and *Becium homblei* for Cu), the horizontal part of the curve is extended to extremely high levels. The elemental composition is often used as a criterion of the health of a crop, and quite complex leaf analysis procedures exist to determine crop nutrient deficiency.

In table 1, it is clear that manganese has a particularly wide concentration range within the same species, and this element also showed the largest variation between 24 species when they were grown under identical conditions (Loneragan 1975).

(b) *Complex formation*

The greatest complication in the study of trace metals is the ease and frequency with which they form complexes. The overall effect in uptake and translocation involves complex formation with soluble and insoluble ligands in the soil, the root cell wall material and compounds in the xylem, the phloem, the cell and its organelles. The interrelations between these various forms and the free ion is largely the reason why uptake from soil is so poorly understood at a fundamental level. Uptake of the simple ions is therefore discussed first. Manganese has the weakest bonding, and is often present in the soil solution largely as Mn^{2+} ion. Zinc and iron (II) complex more strongly, but copper and iron (III) have the strongest bonding of all the transition elements (Williams, this symposium), and only a very small fraction of copper is present in soil solution as the free ion. For example, the free Cu^{2+} concentration in several soil solutions, as measured with a Cu-specific electrode, was of the order of 1 nM, while the total copper concentration was around 10 μM (Sanders, personal communication).

(c) Uptake from simple solution

The term S (equation (1)) includes all soil and plant factors that determine the average rate of uptake per unit dry mass of root: the fraction of root length that is absorbing, the efficiency and mechanism of the absorbing system, the external concentration of the absorbed ion and the ability of the medium to maintain that concentration at the root surface (Nye & Tinker 1977). The points that I shall discuss here are the first two, and the latter two in so far as the plant

TABLE 1. RANGE OF ELEMENT CONCENTRATIONS IN PLANT TOPS (MICROGRAMS PER GRAM)

	sufficient†	toxic†	in 24 species‡
B	20–100	> 200	—
Cu	5–20	> 20	4–11
Fe	50–250	—	90–600
Mn	20–500	> 500	30–500
Mo	0.5?	—	—
Zn	25–150	> 400	11–70

† Suggested by Jones (1972).

‡ All grown under identical conditions (Loneragan 1975).

itself affects them. Factors relating only to the soil have been dealt with by Dr West in the preceding paper in this symposium.

It is now generally accepted that uptake of most of the major elements occurs over most of the root system, and that it is not prevented even by extensive suberization at the root endodermis (see Clarkson & Hanson 1980). However, some elements are absorbed only by root lying within 1 or 2 cm of the tip. Iron is the only trace element that has so far been investigated (Clarkson & Sanderson 1978), and this was absorbed over only a very limited length of barley roots. Further work on this point for other elements is urgently needed.

There is still debate about whether trace element uptake is passive or active (with or against the electrochemical gradient). The distinction should be made on the ground of differences in concentration between the external solution and the xylem sap, after allowing for the electrical potential difference between them, but the latter measurement is rarely available. It is peculiarly difficult to apply this criterion consistently for most of the trace metals because they complex so readily and therefore may have different charges, but most studies have pointed to active uptake, at least at low concentrations.

Silicon (Jones & Handreck 1967) is particularly interesting, because it is certainly transported as uncharged silicic acid, so there are no complications due to the electrical gradient. The concentration ratio indicates that in some species (most Gramineae) uptake appears to be passive (i.e. external and xylem concentrations are about the same), whereas in rice it is active (i.e. there is an increased concentration in the xylem sap), where the concentration of silica in dry tissue can rise to above 10%. In others there is exclusion (decreased concentrations in the xylem), and the concentration of silica in the dicotyledons tends to be relatively low. This shows that it is difficult to generalize about uptake mechanisms for a particular ion or molecule.

It is assumed that active uptake proceeds by mechanisms analogous to those believed to operate for the major nutrients, i.e. uptake sites or carriers on the cell plasmalemma that are specific for a small group of closely similar ions or for a single ion. There are considerable uncertainties in our understanding of this process at a single membrane for major simple ions such as potassium (see Spanswick *et al.* 1979). These difficulties are much greater for ions possibly of

variable valency, which are (at least in Nature) largely present in the complexed state. We also have to consider a complicated total uptake process that transports ions across the symplasm of the cortex, an endodermal cell layer, and a series of cells in the pericycle, before finally discharging the ions into the free space around the latter cells, or directly into the xylem vessels.

It has often been shown that the usual indicators of metabolic control (Q_{10} , low temperatures, metabolic poisons) suggest an active uptake process (see Loneragan 1975). A metabolic effect

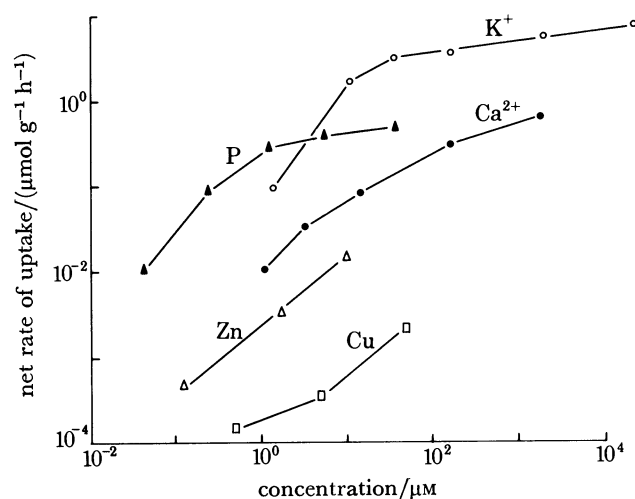


FIGURE 1. Relations between specific uptake rate of elements into roots, on a fresh mass basis, and the external solution concentration. K, P, Ca and Zn data after Loneragan (1979); results for Cu are for ryegrass, and were kindly provided by Dr S. Jarvis of the Grassland Research Institute, Hurley.

does not, however, necessarily imply specific uptake sites or ATPases, or indeed active uptake in our sense at all, because the metabolic process could be necessary for the maintenance of a particular membrane conformation or the electrical potential across it. Selenium is interesting, because the selenate ion is accumulated actively, probably by the same mechanism as sulphate, whereas selenite is accumulated passively (Peterson *et al.* 1981). On the other hand, both arsenate and arsenite appear to be actively absorbed (Asher & Reay 1979).

The distinction between active and passive uptake is often confused by the strong physico-chemical adsorption of ions, particularly zinc and copper, on the root cell walls (Lindsay 1972; Loneragan 1975). This adsorption of metal on cell wall material is independent of temperature, rapid, and depends on the concentration of the ion in solution (Wainwright & Woolhouse 1975). The chemical bonding of the adsorbed or complexed metal is still uncertain. The lack of relation of the adsorption to the N content of the cell wall material, and the small release of metal after treatment with proteases, argues against metal-protein complexes, but Wainwright & Woolhouse (1975) do not regard this as conclusive, despite the fact that cellulases can release large amounts of the bound zinc from cell walls (Wyn Jones *et al.* 1971).

The relation between external solution concentration and uptake rate is crucial to understanding the nutrition of plants with any element. Loneragan (1975) has discussed this in some detail for the main trace elements Cu, Zn, Mn and Fe. In general, uptake responds approximately linearly up to at least 100 μM concentration of the metal, supplied as the uncomplexed salt, which is very high compared with the concentration of the free metal ions in the soil solution (Lindsay 1979). It seems that the efficiency of uptake of trace metals may be low

compared with that of the major elements when evaluated in terms of the uptake rate per unit amount of root from a given external concentration (figure 1). The use of excised roots or other plant tissues in short-term experiments is unreliable in this context, because of the large effect of ions adsorbed on cell wall surfaces. Also, much early work on the uptake of trace elements by plants is of little relevance, because it used very large concentrations of the trace elements or

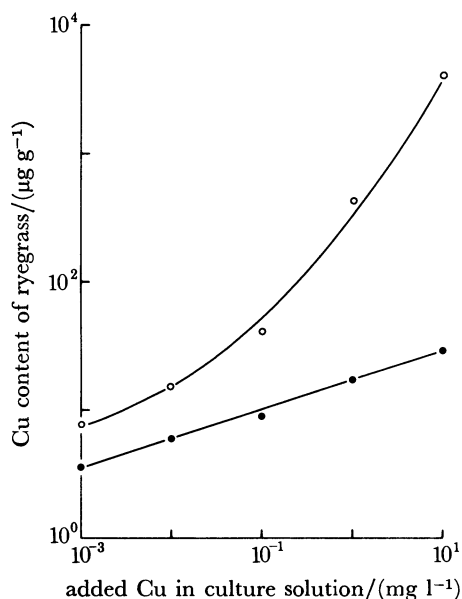


FIGURE 2. Uptake of copper into shoots and roots of ryegrass grown in flowing solution culture (after Jarvis 1980): ○, roots; ●, shoots.

imperfectly stirred solutions so that the actual concentration of solution in contact with the root was uncertain. Since the development of flowing solution systems (Asher *et al.* 1965), much more valid experimentation has become possible.

This technique has allowed careful studies of uptake and distribution of at least two trace elements. Carroll & Loneragan (1968) studied zinc uptake rates in relation to growth and nutrient concentration. Jarvis (1980) used a similar installation to measure copper uptake into ryegrass and into clover (figure 2). The specific uptake rate was similar for the two species. The copper contents in the roots were always much larger, and more affected by solution concentration, than those in the shoots, owing to the strong root cell wall adsorption of copper, shown by Jarvis (1978) to range from 30% to over 75% of the total root copper. In both species the fraction of total uptake retained in the roots was around 70%.

(d) Uptake from chelating solutions

There has been debate on whether the free ion is translocated through the plasma membrane, possibly immediately after its liberation from a complex, or whether the complex as a whole is taken up. The wide variation in chemical composition and molecular mass of the natural complexing agents in the soil makes it difficult to believe that uptake sites could be specific for many forms of the complexed metal. During iron uptake from labelled ferric chelates (Tiffin 1970), in some cases no complexing agent appears in the xylem exudate, indicating that the free ferrous ion only is taken up after reduction at the root surface (see Moore 1972). If a

complexing agent for ferrous iron is added, uptake is sharply decreased (see Tiffin 1977). Dragun *et al.* (1976) measured uptake of copper and other elements into maize, from a solution culture with and without a chelating agent. For similar copper concentrations in solution, the chelating compound decreased copper concentration in the shoot to less than half, and in the root to about one-tenth. They concluded that it was the activity of Cu^{2+} that determined uptake, and that Cu^{2+} concentrations greater than $10^{-5.78}$ M caused toxicity, and less than 10^{-13} M caused deficiency. The complications that result from the competition for a limited amount of complexing agents by different metal ions have been considered by Halvorsen & Lindsay (1977), who concluded that Zn^{2+} ion only was absorbed by roots, and that $10^{-10.6}$ M Zn^{2+} was adequate for plant growth, whereas chelated Zn was insufficient even at 10^5 times this concentration.

Graham (1979) has made a particularly interesting study of copper uptake, because he measured the concentration of ionic Cu^{2+} in both external solution and the xylem with a specific ion electrode, and also the electrical potential difference between the external solution and the xylem fluid. From this he concluded that the Cu^{2+} ion in the xylem and in the external solution were near equilibrium, and hence that uptake of the ion could be passive. In contrast, negatively charged organic complexes would have to move against both an electrical and a chemical potential gradient. The value of complex formation in the soil solution is thus not that the chelates are absorbed, but that the total concentration in solution is increased, and thus increases the possible flux of the metal towards the root surface, where the free ion concentration is maintained by complex dissociation.

(e) Special mechanisms of the plant that affect uptake

It is probably rare for the simple root to absorb directly from the unchanged soil solution, and some of the complicating factors are noted here.

1. It has long been established that healthy roots excrete compounds such as citric and malic acids, and amino acids (see Tinker 1980), and these increase the concentration of the chelated metal in the soil (Elgawhary *et al.* 1970). However, the importance of this mechanism in practice remains to be determined.

2. Iron can only be absorbed in the divalent state (Chaney *et al.* 1972; Brown 1978), and reduction at the root or plasmalemma surface appears to be an essential first step. This capacity of roots to produce reductant is greatly increased when the plant suffers from incipient iron deficiency, at least for dicotyledonous species (Marschner *et al.* 1978). Hydrogen ion excretion by root tips greatly increases the solubility of Fe^{III} . These and other processes immediately around the root allow the plant to affect the rate of iron uptake greatly, so that there are especially clear and well recognized differences between species and cultivars in iron uptake efficiency (see Brown *et al.* 1972; Olsen & Brown 1980).

3. Bacterial breakdown of root exudates (see above) may well produce more complexing agents than would normally be present, and it has been suggested that bacterial siderophores are exceptionally efficient chelators of iron (see Emery 1980). The uptake of manganese can be diminished by bacterial oxidation of Mn^{2+} to MnO_2 at the root surface (Bromfield 1978), the oxide being much less available than the divalent ion. Zinc is absorbed and translocated by the fungal hyphae of vesicular-arbuscular mycorrhiza and they can increase the rate of supply of zinc from the soil to the host plant (see Tinker 1979). There is now clear evidence that copper

is also absorbed by mycorrhiza more rapidly than by uninfected roots (A. Gildon, personal communication), and can alleviate copper deficiency. Vesicular–arbuscular mycorrhizas are involved in the well known copper–phosphorus and zinc–phosphorus interactions (Lambert *et al.* 1979).

(f) *Differences in content in relation to age, season and site*

Almost all nutrient element concentrations in plant dry matter decrease with time (see Antonovics *et al.* 1971), as the amount of structural tissue in the plant increases, if all else is constant. For example, in wheat the mean copper concentration in the shoot declined from 12 to 4 µg/g during 120 days (Loneragan *et al.* 1980). However, when crops are grown in the field, many complicating factors can arise. Thus, in pasture samples from dairy farmers in Scotland, manganese and copper concentrations increased steadily from May to September, whereas zinc concentrations changed irregularly (Thompson & Arren 1979). There is a remarkably consistent and large increase in foliar lead levels in pasture species during winter (see Foy *et al.* 1978), probably from atmospheric deposition.

It is clear that despite the control mechanisms of the plant, which are discussed above, the composition of the same plant part from different crops must vary widely. Welch & Cary (1975) analysed samples of wheat grain from 21 sources, and found that the ranges were 80–349 µg/kg for nickel, 3–43 µg/kg for chromium and 6–20 µg/kg for vanadium.

(g) *Ion interactions*

The most common effect is that of antagonism, which is most readily understood when the two ions are chemically very similar; for example, copper and zinc, phosphate and arsenate, and selenate and sulphate compete. Interactions of iron, copper, zinc and molybdenum with phosphorus are common. It is often concluded that applying an antagonist will reduce ion uptake but the results in practice are often not so simple. Thus phosphate added to As-toxic soil does not consistently reduce As uptake, possibly because the phosphate displaces arsenate from the sorption sites on the soil colloids, and hence, increases the concentration in solution in the soil (Thomas 1980).

The most striking and frequently reported interaction with micronutrients is that between iron and phosphorus. Thus the application of phosphorus greatly reduced iron concentration in potato stems (Boawn & Leggett 1964). The mechanism for this interaction is not yet clear. Phosphate may ‘dilute’ zinc if it causes the plants to grow larger, and it certainly alters its distribution in favour of the root, presumably by reducing the mobility of the element in the plant.

3. DISTRIBUTION

(a) *Translocation mechanisms and element mobility*

All absorbed elements, whether essential or not, find their way to some degree into the xylem sap, and are translocated in the transpiration stream. According to Tiffin (1972, 1977) almost all copper, iron(III) and nickel in the xylem is negatively charged, zinc is mainly negatively charged, but manganese behaves mainly as the Mn²⁺ ion. Most iron moves as citrate, but there also appear to be other amino acids and carboxylic acids that strongly chelate copper and zinc (Tiffin 1977). Uptake by individual cells from the xylem stream is therefore again from an

almost entirely complexed metal source. Chromium moves as the chromate anion, boron probably as borate, borate-sugar or borate-hydroxyacid complexes, and selenium as selenate (Peterson *et al.* 1981).

The phloem system is a much more complex transport system, but not all ions have access to this system of vessels; e.g. calcium, sodium and iron are present in relatively very low concentrations (Tammes & van Die 1964). It seems highly unlikely that phloem contents move uni-

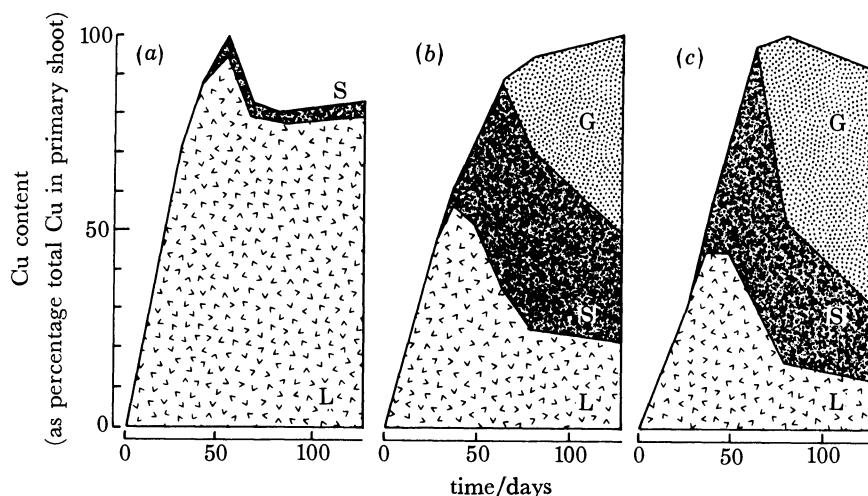


FIGURE 3. Distribution of copper in leaves (L) stem plus chaff (S) and grain (G) of wheat at different times, and with three different rates of soil copper supply: (a) low supply, total uptake 340 ng; (b) adequate supply, total uptake 2570 ng; (c) luxury supply, total uptake 8836 ng.

formly in bulk, as those of the xylem do, so the concentrations determined in the phloem sap give only the most general information about which ions are phloem-mobile. In extracted phloem sap of *Ricinus* plants, zinc, nickel and cobalt were bound to negatively charged complexes with relative molecular masses in the range 1000–5000, though manganese was mainly as the free ion (Van Goor & Wiersma 1976; Wiersma & Van Goor 1979). Information on the chemical composition of these complexes is extremely scanty, but those authors suggest that polynucleotides may be the major complexing compounds.

Nickel, as a non-essential element, is a little unusual in the ease with which it is translocated within the plant and incorporated in seed. It may act as an analogue of Cu or Zn inside plants, since it competes with these elements in uptake. Cataldo *et al.* (1978) concluded that various nickel complexes of relative molecular mass less than 10 000 were found in the root, and that these were transported in the xylem stream. Most of the nickel in all organs was in the soluble fraction, and was readily retransported to the seed. Most complexes were anionic, but cationic complexes have also been found, especially in nickel accumulators (Timperley *et al.* 1973).

An example of the complexities of retranslocation of copper from leaves to seed is given in the study by Loneragan *et al.* (1980) (figure 3). Copper is highly mobile in the plant when present in adequate supply, but immobile when present at low levels, explaining the apparent anomaly of deficiency symptoms appearing just near the growing point, for an element that is mobile in the phloem. They also confirmed earlier work showing a strong association between copper and nitrogen content of leaves and movement out of them. It suggests that much of the copper is bound to nitrogen, and is only translocated when nitrogen moves.

(b) Distribution between plant parts

This follows largely from the mobility and function of the trace elements. Immobile or less mobile ions tend to accumulate in the older leaves simply because the largest total amount of transpiration occurs through them. There may be a tendency for elements to be deposited at the leaf margins where transpiration is maximal; thus boron can accumulate there to a concentration 4 or 5 times that in whole leaves (Jones 1970). Immobile elements can accumulate to very high concentrations in the leaves, to levels well above those essential for growth.

The micronutrients in seeds are particularly important, because of the frequency with which the latter serve as food, and because they form the early supply to the developing plant. Molybdenum may be present in amounts sufficient for at least one whole growth cycle. Duffus & Rosie (1976), in a very careful study of trace elements in barley grain, found very rapid rates of accumulation of iron and zinc at certain times, whereas manganese and copper accumulated more steadily. The fraction of the total copper in the plant translocated into grain depends upon the copper supply, and can be well over half (Loneragan *et al.* 1980). Most effort appears to have been devoted to potentially toxic metals. A careful comparison of cadmium levels in leaves and in the edible parts of a range of plants (Bingham *et al.* 1975) showed that the latter were almost always lower, by factors of up to 20. Cadmium was almost excluded from seeds. Translocation of Fe out of the seed is enhanced if the seedling is growing in an iron-deficient medium (Tiffin 1972).

(c) Appearance of deficiency or toxicity symptoms

Visual symptoms of trace element nutrition disturbance may be clear and unique, but they are more often affected by element interactions or environmental conditions, so it is best to obtain confirmation by other means. Symptoms are usually most localized for immobile elements, such that growing points and new leaves show B and Fe deficiency first.

4. FUNCTION AND FORM IN PLANTS

(a) Function

Price *et al.* (1972), Nicholas (1975), Hewitt (1979) and Clarkson & Hanson (1980) have reviewed this topic. I list here briefly the main functions that are particularly relevant to plant processes.

Metallic proteins, of small molecular mass, with Fe-S clusters have been isolated from many tissues. These ferredoxins are necessary for a range of different processes, including photosynthesis, nitrogen fixation and electron transport generally. Their redox potentials tend to be low; e.g. soluble ferredoxin from chloroplasts has a potential of -0.45 V at pH 7.5, and mediates the transfer of electrons from photosystem 1 to NADP⁺ (Boardman 1975).

Cytochrome, which contains the haem group, functions in electron transport in photosynthesis. Other systems, e.g. nitrate reductase (see Hewitt 1979), which converts nitrate to nitrite, and sulphite reductase, which converts sulphite to sulphide, also employ cytochrome. Price *et al.* (1972) argued strongly that iron is involved in RNA metabolism of chloroplasts.

Copper is contained in several essential enzymes, particularly those reducing oxygen to water, such as ascorbate oxidase and laccase. A copper metalloprotein, plastocyanin, is involved in the electron transport chain in photosynthesis.

Many enzymes, especially dehydrogenases, contain zinc. Zinc rather resembles Mg and Mn in its function, in that it does not exhibit valency change, but influences conformation, and

binding between enzyme and substrate. In the cell, zinc is concentrated in the cytoplasmic ribosomes, which may be connected with the fact that an early sign of zinc deficiency is a sharp decrease in RNA synthesis, which thus inhibits protein formation. Zinc is also involved in the synthesis of indoleacetic acid, and hence its deficiency may affect stem elongation. Carbonic anhydrase merits mention, because of its possible involvement in transfer of CO_2 at necessary rates into chloroplasts. Both zinc and copper are present in superoxide dismutase.

Manganese forms part of phosphotransferases and arginase and is also essential for the evolution of O_2 in photosynthesis and other processes. Its most general function appears to be to bridge ATP with the enzyme complex.

Molybdenum is required, in particular, in nitrogenase, which catalyses nitrogen fixation by *Rhizobium* bacteria, and in nitrate reductase in higher plants. The former of these enzymes also contains iron. There has been uncertainty about the presence of other Mo-containing enzymes, but it appears that the element has no other essential functions (Hewitt & Gundy 1970). Since the most important function of Mo in plants is nitrate reduction, a deficiency of Mo causes symptoms similar to those of N deficiency.

Boron and chlorine have not been identified as components of any specific enzymes. Chloride is clearly involved in the oxygen-liberating step in photosynthesis, in which it probably can be replaced by bromide. The functions of boron are by no means clear, but they show up most clearly in tissue differentiation, especially when growth is rapid. Boron deficiency causes a general disturbance in carbohydrate metabolism and transport, and usually the associated symptoms include damage to the growing point. Strangely, boron is not essential for some fungi and algae (see Price *et al.* 1972).

(b) Chemical form

Information on this is remarkably scanty, and where investigations have been made, they have often not been quantified in relation to the total concentration within the plant tissue. Trace metals are essentially present in three forms: metalloprotein (enzymes); complexed with cell wall celluloses, pectins, proteins and lignins; soluble metal in xylem and phloem sap and vacuoles, as free ions or as complexes of small molecular mass.

For all their importance, the enzymes contain little of the total metal present (Hewitt 1982). Bremner & Knight (1970) found that only 2% of the zinc in ryegrass may have been present in enzymes, and DeKock *et al.* (1960) found only 0.1% of the iron in plant leaves in the haem group, most being present as phytoferritin. Recent work indicates that less than 10% of the molybdenum is present in nitrate reductase, even when the plant suffers from molybdenum deficiency (E. J. Hewitt, personal communication). These low values explain why different healthy crops or plants can have such different total concentrations. However, despite these small amounts in the enzymes, the greatest accumulations may occur at sites where the enzymes function. Possibly high local concentrations are necessary to ensure that the enzyme is saturated with the appropriate metal ion. Thus Timperly *et al.* (1973) found iron and copper to be largely in the chloroplasts, and Neish (1939) found 60% of leaf copper in the chloroplasts (table 2).

Some results of various authors have been listed in table 3, and may be compared with information on the xylem and phloem contents in §3*a*. Very roughly, one may generalize that manganese is almost always present as the free ion, or as complexes of very low molecular mass, with a positive charge. Nickel is mainly soluble, but is in complexes with a variable charge. Zinc and copper are strongly bound in complexes with a negative charge, which form rather

more than half of the total metal present in the plant. The chemical form of the metals in the insoluble residue is often not investigated, but Peterson (1969) determined that zinc bound to protein was about 40% of this fraction, and that bound to pectin about 58% in the shoot, but larger in the root. The chemical composition of the solution complexes is so far unknown.

TABLE 2. MEAN ELEMENT DISTRIBUTIONS (PERCENTAGES) IN LEAVES OF FOUR TREES

(After Timperley *et al.* (1973).)

	chloroplasts	mitochondria	ribosomes	supernatant
Ni	9	2	2	87
Cu	38	6	5	51
Zn	30	6	4	60
Fe	77	12	3	8

TABLE 3. CHEMICAL FORMS OF TRACE ELEMENTS IN PLANTS

ion	species	plant part	M_r	charge	fraction of total (%)	reference
Cu	ryegrass	shoot	cell wall		12-24	1
Cu	clover	shoot	cell wall		7-35	1
Cu	sunflower	root	soluble	-	?	3
Cu	ryegrass	shoot	1500	-	60	7
Cu	trees	leaf	soluble	-2	50	5
Mn	sunflower	root	free Mn	+	?	3
Mn	lucerne	leaf	300	+	100	4
Mn	ryegrass	shoot	free Mn	+	60	7
Zn	trees	leaf	soluble	-2	60	5
Zn	ryegrass	shoot	≤ 1500	-	60	7
Zn	<i>Agrostis</i>	shoot	> 700	-	60	8
Ni	soybeans	root	< 10000	(+/-)	'most'	2
Ni	trees	leaf	soluble	(0/+)	60-80	5
Fe	trees	leaf	citrate	-	8	5
Cr	<i>Leptospermum</i>	leaf	trioxalatochromate	-	15	6

References: 1, Jarvis (1980); 2, Cataldo *et al.* (1978); 3, Graham (1979); 4, Grady *et al.* (1978); 5, Timperley *et al.* (1973); 6, Lyon *et al.* (1969); 7, Bremner & Knight (1970); 8, Turner (1969b).

In a study of the chemical composition of ryegrass, Bremner & Knight (1970) were able to remove 98% of the zinc by ethanol and water extractions, followed by cellulose digestion. Only 11% of the zinc was considered to be associated with protein initially.

The existing measurements of trace metals in complexes are useful, but much more specific information about the chemical composition of the ligands, and the fraction of the element held in them, is necessary.

4. GENETIC EFFECTS

(a) Deficiency levels

Wide variations in the susceptibility of strains and cultivars to deficiency occur (Brown *et al.* 1972). The mechanisms of tolerance to deficiency are known with certainty only for iron, where it is in the reduction and uptake step (see §2e), and various cultivars of, for example, soybeans are well known as iron-efficient or iron-inefficient. It is natural that iron-efficiency should have received most attention, because a deficiency of this element is not easy to prevent by soil

treatments. The value of such efficient cultivars for other trace element deficiencies, where the condition is easily and cheaply cured, is more debatable.

(b) *Toxicity*

The mechanisms of tolerance have received much discussion (see Antononovics *et al.* 1971; Wainwright & Woolhouse 1975; Foy *et al.* 1978). The simplest suggestion is differential binding in plant cell walls, and there is no doubt that this occurs for zinc and copper, with the adsorption of up to 5 or 6 times more zinc in resistant lines of grass (Turner 1969*a*; Peterson 1969). However, this mechanism is only useful in certain circumstances. When plants are grown in solution culture, tolerant and intolerant lines often seem to accumulate about the same concentration of metal in the shoots (Wainwright & Woolhouse 1975; Johnson & Proctor 1977). It therefore seems that accumulation in the roots is little protection when there is an uninterrupted supply of the metal at the root surface, but that in soil where the rate of supply of metal is limited by ion mobility, the cell walls act as an alternative sink and reduce the amount that can be truly absorbed by the roots. Secondly, it is suggested that the element is absorbed but confined to some part of the plant or cell where it has little effect. This is so for some metal accumulators, in which the element may reach very high levels in some parts, and concentration in cell walls or vacuoles may confer some use in tolerance generally (Ernst 1969). Finally, enzymes and membranes of tolerant genotypes may be structurally different, and able to function in presence of higher metal concentrations. Wainwright & Woolhouse (1975) have argued for these explanations, and they found different activities in the cell wall acid phosphatases of *Agrostis*, in the presence of the same solution concentrations of copper ion. It appears likely that a variety of mechanisms contribute towards the total metal tolerance of a genotype, which is in agreement with the polygenic nature of tolerance.

A major question concerning adaptation to element deficiency or toxicity, and the different properties of cultivars, is whether the accumulation of the element in the shoots or other harvested parts is greatly changed by this. In general the concentration of metal in the shoots of tolerant lines will be less than in intolerant ones grown in the same conditions. However, if the tolerant line is grown on highly contaminated soils, it may have a higher total metal concentration in the shoot than the intolerant line could ever produce.

The concentration of an element is normally well buffered in a plant, in that an increase in external concentration does not lead to a correspondingly great increase in rate of uptake to the shoot (figure 1). In addition, the plant availability of heavy metals in old mine tailings and similar contaminated materials is often very low. Hence the internal concentrations of metal in plants in such environments do not always increase very greatly: for example, a thirtyfold increase in soil lead and a sixfold increase in cadmium did not affect the content of the metals in cabbage leaves, and a tenfold increase in soil zinc only doubled the zinc level (Thornton & Webb 1975; Thornton 1977).

The effects of toxicity in plants must vary between elements, but root extension usually slows rapidly. Later chlorosis and stunting of growth follows. Zinc functions appear to be antagonized by nickel, cadmium and copper, while copper, nickel and possibly zinc induce iron deficiency by interfering with its transport from the root, but the physiology and biochemistry of toxic effects in plants (Foy *et al.* 1978) is very confusing, and much work is of dubious relevance.

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Discussion

A. MACPHERSON (*The West of Scotland Agricultural College, Auchincruive, Ayr, U.K.*). There are two points that I wish to raise with Dr Tinker.

1. He has shown how both Cu and Fe are involved in the photosynthetic process. Would he be able to quantify the concentrations of these elements that would prove limiting in terms of crop production?

2. He has also shown that it is the free ion that is absorbed by the plant and that chelated complexes have to break down to release the ion for absorption to take place. Is there in that event likely to be any benefit from the application of chelated trace elements, as opposed to that of the inorganic salts?

P. B. TINKER.

1. I do not think that we can quantify the limiting concentrations inside the chloroplast yet, and the total concentrations in the soil may vary within very wide limits. The concentrations within the plant tissues can vary within the limits given in table 1; below the lower value one may expect some loss of yield.

2. Yes, there are many cases where it is of advantage to apply the chelated metal rather than the simple salt to soil or solution culture. This ensures that a sufficient concentration of metal is kept in solution, and hence is free to move to the root surface, where the chelate may then dissociate and provide a buffered, though low, concentration of the free ion.

T. S. WEST (*Macaulay Institute for Soil Research, Aberdeen, U.K.*). Dr Tinker mentioned that in studying the relation between the total organic matter content of the soil and the concentration of free copper ions, values of the order of 1 nM Cu²⁺ had been found by the use of an ion-selective electrode. I feel that this is pushing the sensitivity of the technique well beyond the limits of its capability (*i.e.* potentioposing of electrodes at such low dilutions is almost impossible).

P. B. TINKER. Dr West has raised an important point, because of the great importance of being able to measure these free ion concentrations. I agree that the ion electrode is probably beyond its limit for an unbuffered solution of 1 nM, but in Dr Sanders's work there was a thousand times as much complexed copper present in the solution, which buffers the free ion concentration. The measurements are reproducible.