Acta Bot. Neerl. **41**(3), September 1992, p. 229–248

REVIEW

Metal tolerance in plants

W. H. O. ERNST, J. A. C. VERKLEIJ and H. SCHAT

Department of Ecology and Ecotoxicology, Faculty of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

CONTENTS

INTRODUCTION

Nearly 60 years ago, Prat (1934) initiated the research of heavy metal resistance in plants when he was analysing the growth performance of two populations of *Silene dioica* (*Melandrium sylvestre*), one from a copper mine and one from a non-mine soil. He was able to demonstrate a heritable copper resistance in the mine population, relative to the non-mine population, which he explained as a result of evolution by natural selection. Nearly 20 years later Bradshaw (1952) and Baumeister (1954) started further research on ecological and physiological differentiation between plants from metal-enriched and non-contaminated habitats. The species chosen for study were predominantly *Agrostis capillaris* in the Bradshaw group (Jowett 1959; Gregory 1965; McNeilly 1965; Antonovics

Note. Some of the frequently investigated plant species have been taxonomically renamed. *Agrostis capillaris* L. is synonymous with *A. tenuis* Sibth., *Silene vulgaris* (Moench.) Garcke with *S. cucubalus* Wibel and *S. inflata* Smith, *Thlaspi caerulescens* J. et C. Presl. with *T. alpestre* L. and *Noccaea coerulescens* (J. et C. Presl.) F.K. Meyer.

1966) and *Silene vulgaris* in the Baumeister group (Bröker 1962; Ernst 1964; Gries 1965; Rüther 1966). In the late 1950s Duvigneaud (1958), while studying the vegetation on metalliferous soils in Central Africa, added to the above approaches a phytogeographic one and introduced the study of speciation processes in metallophytes.

In the 1950s, the study of evolutionary and physiological aspects of metal resistance was hampered by the absence of convenient techniques for measuring metal concentrations in small plant samples. The techniques available for metal analysis were either time-consuming, such as phase separation (Ernst 1964), or costly and only applicable for laboratory-raised plant material, i.e. radiolabelling (Turner & Gregory 1967; Peterson 1969). Only after applying atomic absorption spectrophotometry on wet-ashed plant material (Reilly 1967) did time and cost-effective metal analyses become possible.

The research in the late 1960s was characterized by the study of (sub)cellular-compartmentation patterns, particularly studies on the binding of metals (zinc) to the cell wall in grasses (Turner & Gregory 1967; Peterson 1969) and metal accumulation (Zn, Cu) in the vacuole (Reilly 1967; Ernst 1969). Also, translocation from root to shoot, allocation within the shoot, age-dependent accumulation in leaves, and activities of metal-stimulated enzymes and metallo-enzymes were studied. Observed differences between resistant and non-resistant plants led to the development of various physiological models for specific metal resistances (Ernst 1975; Mathys 1975a). From the early 1970s onwards, an increasing number of groups became involved in the study of physiological and genetical aspects of metal resistance, nearly all of them using the rewarding approach of a comparison of distinctly tolerant populations or isogenic lines of a single species which differ as far as possible only in the resistance to one or more metals (Strange & Macnair 1991; Schat & Ten Bookum 1992a).

The present review will highlight the progress with respect to the elucidation of the mechanisms of resistance to the heavy metals Cd, Cu, Pb and Zn, and the metalloid As in angiosperms.

MEASUREMENT OF METAL TOLERANCE

Every organism, regardless of whether it lives in a metal-enriched environment or not, has a certain ability to cope with non-essential metals or excessively available essential metals although there are limits in the occurrence of metal tolerances in a lot of higher plants (Bradshaw & McNeilly 1991). Several authors have referred to this ability as 'tolerance' or 'resistance'. In this review, however, we wish to restrict these terms to cases of a heritable increase in the ability to cope with excessive metal, usually naturally or artifically selected under the pressure of a toxic level of metal exposure. Such heritable tolerances occur in a limited number of plant species and have been demonstrated for Zn (Bröker 1962), Pb (Urquhart 1971; Wu & Antonovics 1976), Cu (Macnair 1983; Schat & Ten Bookum 1992a), As (Watkins & Macnair 1991) and other metals. Evolution of tolerant populations may occur as rapidly as within 5 to 10 years (Ernst 1976; Wu 1990), or more slowly, dependent on the selection pressure prevailing (Dueck *et al.* 1984; Dueck 1986; Verkleij *et al.* 1989a; Lolkema 1985).

Tolerances to metals can be demonstrated at different levels of integration. Basically, metal tolerance is a constitutive property, present in every cell, tissue and organ of the plant. It is maintained in cell tissue cultures prepared from tolerant plants (Wu & Antonovics 1978; Qureshi *et al.* 1981). Therefore, tolerance can be tested using different tissues, organs, or the whole plant, although it cannot be excluded that processes taking

place at integration levels higher than the cell may substantially contribute to the tolerance of the whole plant (see below).

Comparative plasmology

Repp (1963) elaborated a test for plasma membrane integrity after exposure to heavy metals. Sections of leaf or stem tissue, between 2 and 4 cell layers thick, are incubated in a series of metal concentrations, either without nutrient addition (Repp 1963; Gries 1966) or in a nutrient solution (Rüther 1967; Ernst 1972a). After 24 h in the case of copper, or 48 h for all other heavy metals, the cells' ability to plasmolyse and deplasmolyse is tested using 1 M glucose. The advantage of this technique is that it allows testing of established dicotyledonous plants in the field and in the laboratory without cloning them. The results are in agreement with those obtained with the rooting technique described below (Ernst 1982).

Rooting technique

Roots are more directly confronted with heavy metals in the environment than shoots, except in the case of aerial metal deposition (Ernst 1980). Therefore, root growth usually responds more rapidly to metal exposure than shoot growth. This is probably the reason that root growth is the most widely used parameter in metal tolerance tests. A commonly used measure of tolerance is the tolerance index TI (Wilkins 1957, 1978; Jowett 1958):

$$TI = \frac{\text{root growth in metal solution}}{\text{root growth in control solution}}$$

The use of TI certainly allows a perception of the coarse patterns of interspecific and intraspecific variation in metal tolerance (Woolhouse 1983, for a review). The resolution of this index, however, is limited and depends on the metal concentration chosen for testing, as well as the range of variation in tolerance present among the plants to be tested (Macnair 1981, 1983; Schat & Ten Bookum 1992a, for a detailed discussion). Moreover, low metal concentrations may stimulate root growth, especially in tolerant plants, which questions the use of a metal-free control solution. Macnair (1983) developed an alternative single concentration test. He exposed cuttings to a fixed metal concentration, chosen at such a level that presumed non-tolerants consistently failed to root, whereas presumed tolerants showed normal rooting. Although this test does not measure quantitative variation in tolerance, it was successfully applied in a genetical analysis of copper tolerance in *Mimulus guttatus* (Macnair 1983). The use of TI in genetical studies has never yielded clear-cut interpretable results. Apparently, Macnair's test more effectively identifies the non-tolerant homozygote (his test concentration was approximately equal to the lowest effect concentration (EC) where root growth in non-tolerant plants stop [EC_{100}]). Schat & Ten Bookum (1992a) developed a multiple concentration test, which also used the lowest EC_{100} as a tolerance measure. The latter is established by exposing each individual to a test solution in which the metal concentration is raised in time in a stepwise manner until root growth is completely arrested. The advantage of this test over Macnair's test is that it measures quantitative variation in tolerance among tolerant plants. It has been successfully applied to analyse the genetics of copper tolerance in *Silene vulgaris* (Schat & Ten Bookum 1992a). Compared with conventional multiple concentration tests, it has the advantage that it allows to estimate the tolerance level of individual plants without the need for cloning them. A general advantage of multiple concentration tests over single

concentration tests is a higher resolution. Unfortunately, multiple concentration tests for metal tolerance are only scarcely applied (e.g. Davies & Snaydon 1973; Craig 1977; Nicholls & McNeilly 1979; Schat & Ten Bookum 1992a,b). A major problem of testing for metal tolerance is that tolerance measures are sensitive to innate variation in root growth unrelated to tolerance (Macnair 1981). This restriction applies to all the tolerance measures used, but probably not to the same extent. The apparent success of the use of the lowest EC_{100} for root growth as a tolerance measure in genetic studies (Macnair 1983; Schat & Ten Bookum 1992a) seems to be due to a comparatively low sensitivity to root growth factors other than tolerance genes. The lack of success in former genetic studies, in which TI was used, may be explained by disproportional effects of root growth factors unrelated to tolerance on the two component parts of TI (Macnair 1981; Humphreys & Nicholls 1984).

The usefulness of root growth as an effect parameter in metal tolerance tests probably relies on the fact that metal-imposed root growth reduction is due to a direct effect of the metal on the root itself, such as demonstrated by split root experiments (H. Schat, unpublished data) and experiments with isolated root segments. Both cell division and cell elongation are affected. In non-tolerant *Festuca rubra*, exposure to 3 μM Zn results in a more than 100% increase in the length of the cell cycle, compared to less than 20% in a zinc-tolerant one. In non-tolerant plants especially, the G1 phase appears to be sensitive. Zinc also differentially affects the protein content of the meristem and the proximity of root hairs and xylem elements to the root tip in non-tolerant and metal-tolerant populations (Powell *et al.* 1986a,b,c; Davies *et al.* 1991a,b).

Whole plant testing.

Several authors have used the relative growth rate of the whole plants as an effect parameter in metal tolerance tests (e.g. Verkleij & Prast 1989). In view of the fact that tolerant and non-tolerant plants may differ in the rate of translocation of metals from root to shoot or in the capacity to store metals in the root, it is conceivable that they might also differ in the degree of shoot growth inhibition, relative to the degree of root growth inhibition, if they are exposed to a toxic metal concentration. If so, then whole plant testing may be expected to yield results which differ from those obtained with a rooting test. For example, compared at equal levels of root growth inhibition, Cu-tolerant *Silene vulgaris* exhibit a stronger shoot growth reduction than non-tolerant *S. vulgaris* (Fig. 1), possibly due to a more rapid root to shoot translocation of copper in the tolerant plants (Lolkema *et al.* 1984). It is obvious, however, that this does not produce large differences between a rooting test and a whole plant growth test. Systematic comparison between whole plant tests and rooting tests with the same clone or isogenic line have not been published, as far as we know.

COMPARTMENTATION AND TOLERANCE AT THE CELLULAR LEVEL

The cell wall

The walls of the root cells are directly exposed to the metals in the soil solution. Association of metals with the cell wall has been frequently established, either through cation exchange techniques (Ernst 1969, 1972a; Peterson 1969; Pickering & Puia 1969; Turner 1970; Farago & Pitt 1977), or by electron microscopical techniques (Ernst & Weinert 1972; Mullin *et al.* 1985). Most of the cell wall-associated heavy metals are bound to polygalacturonic acids, to which the affinity of metal ions decreases in the order

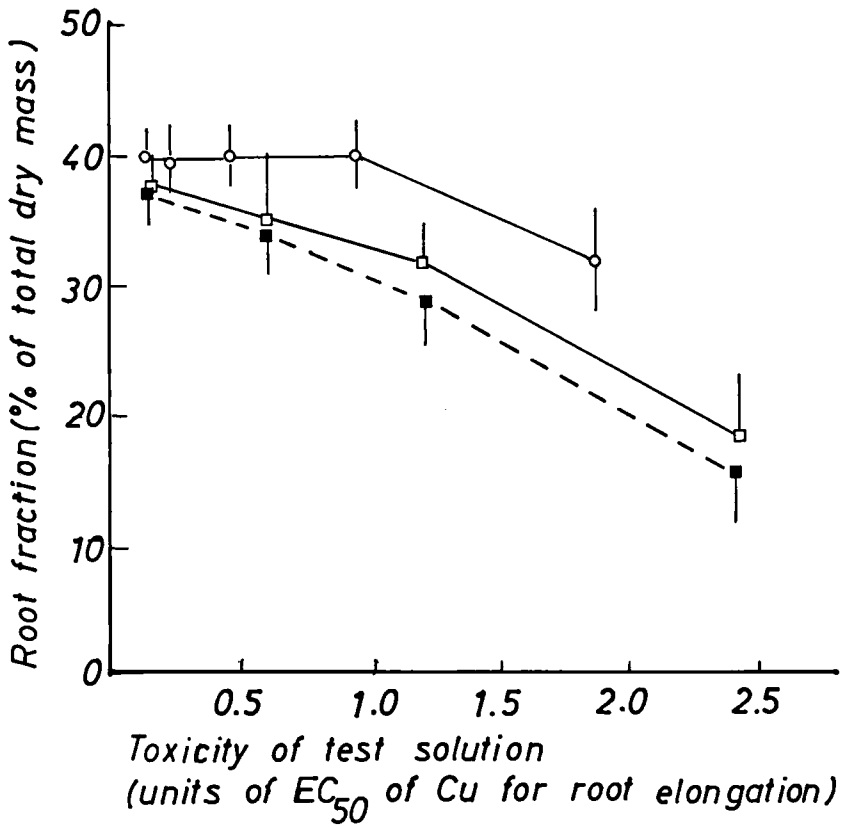

Fig. 1. Root fractions (percentage of total dry mass) of Cu-tolerant and non-tolerant *Silene vulgaris* after 7 days of growth in copper-toxic solution, compared at equal levels of root elongation inhibition (H. Schat, unpublished). ■ non-Cu-tolerant (Amsterdam), □ moderately Cu-tolerant (Marsberg), ○ highly Cu-tolerant (Imsbach).

Pb > Cr > Cu > Ca > Zn (Ernst 1972a,b; Jellinek & Sangal 1972; Muzzarelli 1973; Farago & Pitt 1977). Therefore, lead can be used to desorb other metals, such as Cu and Zn from cell walls in uptake studies (Harrison *et al.* 1979; De Vos 1991).

The suggestion of an involvement of the cell wall in zinc tolerance has been made by Peterson (1969), Turner (1970) and Wyn Jones *et al.* (1971). Turner & Marshall (1971, 1972) observed a positive correlation between Zn tolerance and the cell wall's Zn-binding capacity in *Agrostis capillaris*. They suggested that Zn tolerance involves an altered carbohydrate composition of the cell wall. This could not be confirmed by Farago & Pitt (1977) who investigated populations of the Australian plant species *Polycarpea glabra* from areas of high and low zinc concentration. In general, there is also a tolerance-unrelated interpopulation variation in the cation exchange capacity of cell wall material (Ernst 1972b). The amount of metals bound to the cell wall, even when crystal-like metal bodies are present in it (Ernst & Weinert 1972), is usually less than 10% of the total cellular amount in metallophytes (Ernst 1969, 1972a,b, 1974).

The idea of an involvement of the cell wall in metal tolerance has been frequently disputed (Turner & Marshall 1971; Ernst 1976; Thurman & Collins 1983; Verkleij & Schat 1990). The main problem is that it is not easy to imagine how an increase in binding

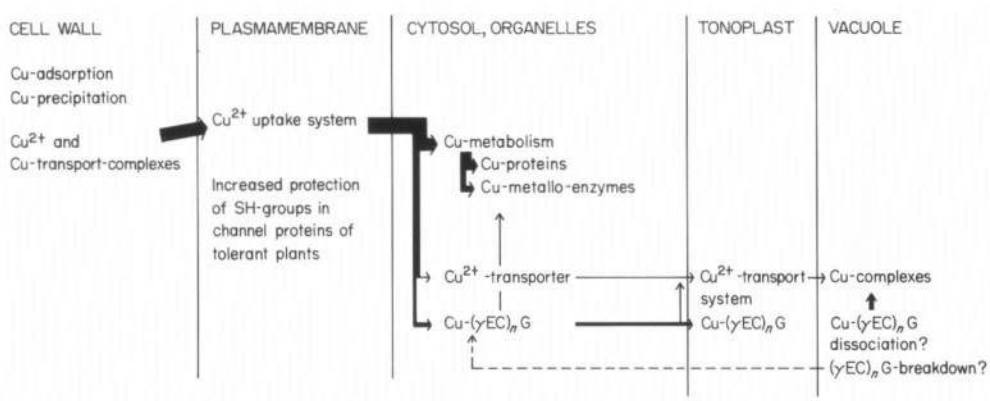

Fig. 2. Model of the copper tolerance mechanism.

capacity of the cell wall can reduce the free-metal activity near the plasmalemma surface, if the soil solution, the cell wall solution and the cell wall matrix are at equilibrium. Co-ion exclusion has been suggested as a possible mechanism, but this cannot explain metal-specific tolerance (Thurman & Collins 1983). A specific role of the cell wall in metal tolerance may be related to processes within the cell wall. There are some indications that cell-wall-bound enzymes such as the acid phosphatase of metal tolerant populations form less stable complexes with the habitat-specific metals than their counterparts from uncontaminated soil (Wainwright & Woolhouse 1975; Cox & Thurman 1978). It has been claimed that the cell wall does play a significant role in lead tolerance, which occurs in plants from mine waste and from roadside soils (Urquhart 1971; Wu & Antonovics 1976). Poulter *et al.* (1985) observed a loss of Pb tolerance in protoplasts prepared from Pb-tolerant cell cultures of *Anthoxantum orodatum*. On the other hand, protoplasts and cells were equally tolerant to Cu and to Zn, which was taken as evidence for a specific role of the cell wall in Pb tolerance. These authors, however, did not make the necessary precautions to prevent a drop in the free-lead concentration in the incubation medium upon addition of the cells due to the binding of Pb to the cell wall. In view of the amount of cells added per ml of incubation medium, it seems possible that the apparent higher tolerance of cells represents an experimental artefact. Differences between Pb-tolerant and non-tolerant clones may be caused by the indole-3yl acetic acid-induced cell elongation, which is strongly affected by lead, stimulating IAA-oxidase activity (Mukherji & Maitra 1977; Lane *et al.* 1978).

The plasma membrane

Membrane structure. Exposure of plants to increased concentrations of Cu^{2+} and Hg^{2+} causes leakage of potassium from the cells, as originally shown for *Chlorella* (McBrien & Hassall 1965; De Filippis 1979). Copper-induced potassium loss has also been observed in root cells of higher plants, i.e. *Agrostis capillaris* (Wainwright & Woolhouse 1977; Woolhouse 1983), *Silene vulgaris* (De Vos *et al.* 1989), and *Mimulus guttatus* (Strange & Macnair 1991). In Cu-tolerant ecotypes or clones, the Cu concentrations required to induce membrane leakage (Fig. 2) are much higher than in Zn-tolerant or non-tolerant ones (Wainwright & Woolhouse 1977; De Vos *et al.* 1991; Strange & Macnair 1991). In view of the instantaneous occurrence of leakage, i.e. within a few minutes, and the remarkable similarity between the highest no-effect-concentration (NEC) for K-leakage (Fig. 2)

and for root growth, both in tolerant and non-tolerant plants (De Vos *et al.* 1991; Strange & Macnair 1991), it has been postulated that membrane damage is the primary effect of toxic Cu (De Vos *et al.* 1989) and that Cu tolerance involves alterations of plasma membrane structures (De Vos *et al.* 1989; Strange & Macnair 1991).

Experiments with the sulphhydryl reagent *N*-ethylmaleimide (NEM) and the free radical-producing compound cumene hydroperoxide (CHP) have shown that Cu tolerance in *Silene vulgaris* is not due to a general resistance to sulphhydryl reagents or free-radical formation (De Vos *et al.* 1989). In view of the high sensitivity of H^+ efflux and plasma membrane polarization to Cu (Kennedy & Gonsalves 1987) we suggest that Cu-tolerant plants are able to prevent Cu ions to react with sulphhydryl groups of proteins, for example those of potassium channels (Hille 1984). This seems to hold true for all Cu-tolerant plants, independent of their taxonomic rank, from the bluegreen alga *Anabaena doliolum* (Rai *et al.* 1991) to angiosperms, such as *Silene vulgaris* (De Vos 1991). As soon as the highest no-effect concentrations for root growth are exceeded, then K-leakage and lipid peroxidation with concomittant changes in lipid composition proceed in the same way in tolerant and non-tolerant plants (De Vos *et al.* 1991; De Vos 1991; Slivinskaya 1991). Perhaps patch-clamp methods (Maathuis & Prins 1991) may be useful to validate the hypothesis of structural alterations of the plasma membrane in Cu-tolerant plants.

In contrast to copper, zinc protects membrane lipids and proteins against oxidation (Bettger & O'Dell 1981; Cakmak & Horst 1988) and does not damage isolated membranes, even at high concentration. Therefore, it is not surprising that Zn does not cause leakage of membranes in non-tolerant plants.

Uptake kinetics. It has often been suggested that metal tolerances could rely on a decreased uptake of the metal in question, at least in part. This suggestion is mainly based on the observation that metal contents of the tissue of tolerant plants, especially those of the shoots, may be lower than those of non-tolerant plants grown at the same level of metal exposure, although this is certainly not a rule (Ernst 1972b; Wu *et al.* 1975; Baker 1978; Lolkema *et al.* 1984; Verkleij & Bast-Cramer 1985; Verkleij & Prast 1989; De Vos 1991; Schat & Kalff 1992). Tissue contents, however, may not only reflect differences in uptake rates, but also differences in translocation or in growth rate. Moreover, comparisons of tissue contents have often been performed at exposure levels that are toxic to the non-tolerant plants, but not or rarely to the tolerant ones. Under these conditions, differential tissue contents may be a mere consequence of differential tolerance, rather than a cause, especially after a long period of exposure. Comparative studies on the kinetics of metal uptake at non-toxic exposure levels have hardly been performed. Strange & Macnair (1991) compared the kinetics of copper uptake in root segments of copper-tolerant and non-tolerant *Mimulus guttatus*. Their best-fit model indicated a very similar K_m and V_{max}, but a higher Cu uptake in non-tolerant roots, due to a linear term that was considered to represent passive diffusion as a result of copper-imposed membrane damage. The models tested by these authors, however, do not account for biphasic Cu uptake (Nissen 1973), such as observed in leaves and roots of *Elodea nuttallii* (Marquenie-van der Werff & Ernst 1979) and roots of *Hordeum distichum* (Veltrup 1976, 1977), with a phase transition between 6·5 and 7·5 μM, i.e. half the concentration range chosen by Strange & Macnair (1991). Further comparative studies of metal-uptake kinetics in tolerant and non-tolerant roots are not available to date. In general, the mechanisms of trans-plasma membrane metal transport in higher plants are poorly understood. Trans-membrane transport of metal ions is often assumed to be protein-mediated (Gutknecht 1983). Specific carrier

systems for essential heavy metals have not been found thus far. Interactions between heavy metals have been frequently registered (Veltrup 1977, 1978, 1979).

A clear example of tolerance by means of a reduced uptake is arsenic tolerance (Meharg & Macnair 1990, 1991a,b; Watkins & Macnair 1991). Both phosphate and arsenate are taken up by the systems in angiosperms (Asher & Reay 1979). Arsenate-tolerant plants of *Agrostis capillaris, Deschampsia cespitosa*, and *Holcus lanatus* exhibit a reduction in arsenate uptake, due to a decrease in the V_{max} of the high- and the low-affinity uptake system and by an increase in the K_m of the high-affinity system for phosphate uptake. However, the reduced uptake cannot explain how As-tolerant plants, often containing very high As concentrations (Porter & Peterson 1975; De Koe 1991), can prevent the well-known interaction of arsenate with phosphate in glycolysis.

Laboratory studies of metal uptake (Cathala & Salsac 1975; Hassan & Tang Han Hai 1976; Cataldo *et al.* 1983) usually consider the uptake of free heavy-metal ions. It is well known that population-specific excretion of metal-binding compounds can modify the availability of these metals (Hall *et al.* 1979; Butler *et al.* 1980). In metal-enriched soils, except for pioneer stages of vegetation development, part of the heavy metals will be present as soluble organic metal complexes (Ernst 1974; Van der Werff 1981). Complexed metal ions are taken up to a lesser degree than free-metal ions (Ernst 1968; Coombes *et al.* 1977; Van der Werff 1981; Laurie *et al.* 1991), which explains the higher floristic diversity and greater variation in metal tolerance of plant species on heavy metal soils with profile development. Also, the presence of mycorrhizal (VAM) fungi might affect the uptake and toxicity of metals in host plants, possibly through biocomplexation (Dueck *et al.* 1986; Ietswaart *et al.* 1992). On copper soils, however, vesicular–arbuscular mycorrhizal fungi are virtually absent (Griffioen & Ernst 1990), because of the fungicidal properties of this metal.

The cytosol

Enzymes. Cytosolic enzymes of tolerant plants, possibly in contrast to apoplastic ones (Woolhouse & Walker 1981), are generally as metal-sensitive as those of non-tolerant plants (Ernst 1975, 1976; Mathys 1975b; Cox *et al.* 1976, 1978; Smirnoff & Stewart 1987). Therefore, tolerance must include a mechanism to keep the activity of potentially harmful metal species in the cytosol within certain limits. At the same time, the intracellular distribution of essential heavy metals must guarantee the supply to metalloproteins (Lolkema & Vooijs 1986) so that undesirable metal substitution cannot occur (Van Assche & Clijsters 1990; Clijsters *et al.* 1991). This could be affected by the production of metal-complexing compounds which may at the same time promote the transport to other cellular compartments.

Two groups of compounds have been suggested to act as cytosolic metal buffers or metal carriers during cytosol transport, i.e. metal-binding peptides (Reilly *et al.* 1970; Rauser & Curvetto 1980) and, for zinc and nickel, organic acids (Ernst 1975, 1976; Mathys 1975a; Sasse 1976). In the following section we will discuss whether metal-specific tolerances can be explained by increased production of these compounds.

Metallopeptides (phytochelatins). Although genes coding for metallothioneins (MT) are present in higher plants (De Miranda *et al.* 1990; Evans *et al.* 1990; Tommey *et al.* 1991), most of the metallothionein-like substances (Robinson & Jackson 1986), isolated during

the early 1980s (Rauser & Curvetto 1980; Bartolf *et al.* 1980; Weigel & Jäger 1980; Lolkema *et al.* 1984; Wagner 1984; Robinson & Thurman 1986) are probably poly(γ-glutamylcysteinyl)glycines (poly(γ-EC)$_n$G's), also called cadystin (Murasugi *et al.* 1981), phytochelatins (PC; Grill *et al.* 1985a,b) or metal-binding polypeptides (Jackson *et al.* 1990) and peptides (Me-BC; Rauser 1991). In this review they will be named (γ-EC)$_n$G's. They are induced by various heavy metals and even by the non-metals selenium and arsenate in all the plant species investigated so far (Grill *et al.* 1987; Delhaize *et al.* 1989; Salt *et al.* 1989; Gupta & Goldsbrough 1991). The tendency of metals to induce (γ-EC)$_n$G's in cell-suspension cultures of *Rauvolfia serpentina* decreases in the order Hg > > Cd,As,Fe > Cu,Ni > Sb,Au > Sn,Se,Bi > Pb,Zn (Grill *et al.* 1987). This order, however, is based on the total metal concentration in the Linsmaier and Skoog medium. For free ionic metals the order may be different. The affinity of metals for (γ-EC)$_n$G's depends on the value of *n*, but in a metal-specific way. In the case of Cd, for example, it increases more or less proportionally with *n*, whereas in the case of Cu, it is largely independent of *n* (Matsumoto *et al.* 1990). Therefore, depending on the metal concerned, tolerance might be obviously dependent on an increase in the chain length of the (γ-EC)$_n$G's produced. Another factor with a conceivable bearing on tolerance is the incorporation of labile sulphur into the metal-(γ-EC)$_n$G complexes (Steffens 1990).

There are many indications that phytochelatins are not involved in metal tolerance. First, tolerant plants or cells often do not produce more (γ-EC)$_n$G's than non-tolerant ones (Verkleij *et al.* 1989a,b, 1990; De Vos *et al.* 1992; Schat & Kalff 1992). Secondly, in the case of exposure to high copper levels, maximum (γ-EC)$_n$G production occurs only at concentrations where growth is not even possible, both in tolerant and non-tolerant plants of *Silene vulgaris* (De Vos *et al.* 1992). Moreover, distinctly copper-tolerant and non-tolerant populations and isogenic lines of the latter species produce equal amounts of (γ-EC)$_n$G's if they are grown at concentrations which cause an equal degree of root growth inhibition (Schat & Kalff 1992). This suggests that differential (γ-EC)$_n$G production should be taken as a consequence, rather than a cause of differential tolerance. It would imply that (γ-EC)$_n$G levels can be used as a tolerance-independent biomarker of metal stress (Ernst & Verkleij 1991). Another argument against a role for (γ-EC)$_n$G's in metal tolerance is that (γ-EC)$_n$G production cannot account for metal-specific tolerances, because it can be induced by any metal, both in tolerant and non-tolerant plants. Tolerance-correlated and metal-specific increases in (γ-EC)$_n$G inducibility have never been found to date. Thus, if cadmium tolerance would rely on increased production of (γ-EC)$_n$G's, such as claimed for tomato cell lines (Steffens *et al.* 1986), then Cd-tolerant plants should also be tolerant to Cu, for example, which is obviously not the rule (Schat & Ten Bookum 1992b).

According to Vögeli-Lange & Wagner (1990), (γ-EC)$_n$G's should be considered as a carrier for metal transport into the vacuole rather than as a purely cytoplasmic buffer system, which means that their impact will depend on their turnover rate rather than their concentration in the cell. This could imply that the role of (γ-EC)$_n$G's in tolerance, whenever it would exist, could be masked by a tolerance-correlated increase in the rate of their breakdown, which presumably takes place in the vacuole. It is not easy to imagine however, how such a possible variation in turnover can be reconciled with the above mentioned tolerance-independent relationship between (γ-EC)$_n$G levels and the degree of root growth inhibition such as found in *Silene vulgaris* (Schat & Kalff 1992). The recent description of CdS crystallite coated with (γ-EC)$_n$G's in Cd-treated tomato plants (Reese *et al.* 1992) makes it more difficult to consider Cd-(γ-EC)$_n$G as metal transporter.

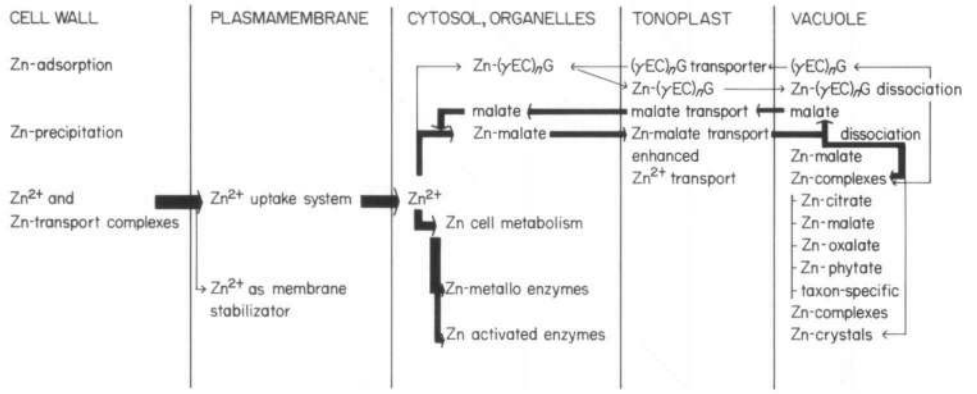

Fig. 3. Model of the zinc tolerance mechanism (modified after Ernst 1975 and Mathys 1975a).

Organic acids. Organic acids have been proposed to play a role as metal-binding compounds, e.g. malic and citric acid for Zn (Ernst *et al.* 1975; Mathys 1977; Godbold *et al.* 1984; Harmens *et al.* 1989), and malonic, citric and malic acid in the case of Ni tolerance (Sasse 1976; Pelosi *et al.* 1976; Lee *et al.* 1988). This hypothesis is based on the observation that Zn-tolerant and Ni-tolerant plants often exhibit increased concentrations of these compounds. It is uncertain, however, whether this is due to increased cytosolic or vacuolar concentrations, or both (see below).

Vacuoles as storage compartments

Vacuoles of metal-tolerant plants, but also those of non-tolerant plants after exposure to a high metal concentration (Mullins *et al.* 1985), often contain high concentrations of heavy metals, especially of zinc and nickel (Ernst 1969, 1972a, 1974, 1980; Sasse 1976; Brookes *et al.* 1981) and to a lesser degree copper and lead (Ernst 1974; Mullins *et al.* 1985) and cadmium (Ernst 1980; Heuillet *et al.* 1986; Rauser & Ackerley 1987; Krotz *et al.* 1989; Vögeli-Lange & Wagner 1990).

Ernst (1975) and Mathys (1975a) postulated the zinc-malate-shuttle hypothesis (Fig. 3) for Zn transport over the tonoplast. Malic acid would bind Zn in the cytosol, thereby detoxifying it, and the Zn-malate complex would be transported over the tonoplast and dissociated in the vacuole, after which malate would be retransported into the cytosol. Vacuolar Zn would remain bound to stronger chelators, such as citrate, oxalate or anthocyanidines, when present.

According to Vögeli-Lange & Wagner (1990), Cd is transported over the tonoplast as a Cd-$(\gamma\text{-EC})_n$G complex. In the vacuole the complex will dissociate, dependent on the concentration of and the affinity to Cd of other chelators, as well as on the chain length of the Cd-$(\gamma\text{-EC})_n$G's in question. In the absence of other chelators with a high affinity to Cd, only low molecular Cd-$(\gamma\text{-EC})_n$G's will rapidly dissociate at a normal vacuole pH of about 5 (Reese & Wagner 1987; Matsumoto *et al.* 1990). At high Cd exposure levels, accumulation of undissociated Cd-$(\gamma\text{-EC})_n$G's in the vacuole may be unfavourable, as this would drain too much of the cytoplasmic pool of glutathione (Scheller *et al.* 1987), or other low molecular sulphur compounds. Therefore, re-transport of $(\gamma\text{-EC})_n$G, or of the composing amino acids seems to be a prerequisite for an effective compartmentation of Cd. Nothing is known, however, about these processes.

Recent application of X-ray cryo-microanalytical techniques have provided some information on the final speciation of heavy metals in vacuoles. At high levels of Cd exposure, globular deposits containing Cd, K and P, first detected in root cells of *Agrostis gigantea* (Rauser & Ackerley 1987), were found in mature cells, but sheet-like deposits with Cd and S in immature cells (Van Steveninck *et al.* 1990a,b). In contrast to Cd, Zn-treated cells contain vacuolar deposits containing Zn phytate (Zn, K and P or Zn, Mg, K and P) (Van Steveninck *et al.* 1987, 1990a,b, 1992). Crystal formation has also been observed after incubating epidermal cells of *Silene vulgaris* for 48 h in 0·2 M solutions of zinc sulphate or zinc chloride, but was not found after incubation in other metal sulphate solutions (Gries 1965). The high concentrations of water-soluble metals in plant extracts indicate that crystals are an exception rather than a rule with respect to the form of storage of heavy metals in vacuoles.

It has often been postulated that tolerance may be due to an increased ability to transport metals into the vacuole. The latter could conceivably rely on (a) a higher concentration in the cytosol of substances forming metal complexes which can be transported over the tonoplast, such as $(\gamma\text{-EC})_n$G's and, possibly, malic acid, (b) a higher affinity or capacity of the metal-transporting system in the tonoplast itself, and (c) a higher metal storage capacity in the vacuole. It may be of importance that zinc-tolerant plants, including *Silene vulgaris* also exhibit increased malate concentrations if they are grown in the absence of excessive zinc (Ernst 1975, 1976; Ernst *et al.* 1975; Mathys 1975a,b; Brookes *et al.* 1981; Godbold *et al.* 1984). Also Ni-tolerant plants exhibit increased concentrations of malate, malonate or citrate (Pelosi *et al.* 1976; Sasse 1976; Brooks *et al.* 1981). As outlined earlier, however, it remains to be shown whether the cytosolic concentrations of these compounds are also increased and whether these compounds really mediate metal transport over the tonoplast. They might as well serve as vacuolar metal sequestrants. Brookes *et al.* (1981), using compartmental flux analysis, found that both zinc-tolerant and non-tolerant clones of *Deschampsia cespitosa* were able to pump zinc into the vacuole, but that the mechanism broke down in non-tolerant clones at higher exposure levels. This does not necessarily mean, however, that tolerance would rely on a higher transport capacity. The breakdown of the transport in non-tolerant clones may also be taken as a consequence of metal strain, rather than as a primary cause of strain. Ernst (1974) observed that leaf mortality occurs always at the same fixed internal zinc concentration in the Zn-tolerant and Zn-hyperaccumulating plant *Cardaminopsis halleri*, suggesting that mortality is a consequence of a saturation of the vacuolar storage capacity. This does not mean, of course, that tolerance would be due to an increase of the vacuolar storage capacity. In general, it is difficult to reconcile the idea of tolerance by means of an increased production of organic acids with the metal-specific nature of tolerances. For example, Zn-tolerant *Agrostis capillaris* and *Silene vulgaris*, which both exhibit increased malate levels (Ernst 1975, 1976), are only slightly Ni tolerant (Gregory & Bradshaw 1965; Schat & Ten Bookum 1992b), whereas Ni-tolerant *Alyssum bertolonii*, which is very rich in malate (Pelosi *et al.* 1976), is non-tolerant to zinc (J.A.C. Verkleij & P. Pancaro, unpublished data). This strongly suggests that these tolerances involve specific changes in the transport systems in the tonoplast itself, rather than a mere increase in the concentration of chelating organic acids.

In summary, although it is likely that vacuolar compartmentation plays a role in the tolerance to metals, at least in the case of Zn and Ni, the precise mechanisms by means of which tolerant plants could accomplish a more effective vacuolar compartmentation

are completely unknown. Studies on isolated vacuoles and tonoplasts of tolerant and non-tolerant plants may provide more insight.

COMPARTMENTATION AT THE WHOLE PLANT LEVEL

Although tolerance is apparent in any organ, tissue and cell of a tolerant plant, it is conceivable that compartmentation processes taking place at the level of tissues and organs can contribute to tolerance at the whole plant level. Several possibilities will be discussed below. Differences between tolerant and non-tolerant plants in the distribution of metals over root and shoot have been frequently reported (e.g. Ernst 1972b; Wu *et al.* 1975; Baker 1978; Coughtrey & Martin 1978; Lolkema *et al.* 1984; Verkleij & Prast 1989). Increased retention in the roots in tolerant plants is certainly not a rule (Baker 1981), but it could be of adaptive significance if shoots are more sensitive than roots, which is uncertain. Regardless of whether it is adaptive or not, retention in the root might represent a mere consequence of the tolerance mechanism operating in the root cells (e.g. increased vacuolar storage). It has never been proven that tolerance involves genetic changes other than those involved in the tolerance mechanism present in every cell.

Transport of heavy metals from root to shoot via the xylem is mediated at least partially by organic complexes (Höfner 1970 for Mn and Fe; Van Goor & Wiersma 1976 for Mn and Zn; Graham 1981 for Cu, but not Mn; White *et al.* 1981a,b for Cd, Ni and Zn; Mench *et al.* 1988 for Cu, Pb and Zn). Analyses of xylem exudates of non-tolerant and metal-tolerant plants of *Silene vulgaris* have not shown qualitative differences of metal complexes (H. Harmens, pers. comm.), but quantitative differences between free and complexed metal ions may affect the plant-internal transport.

Allocation pattern of metals and leaf age

A potential advantage of allocating metals into leaves is that it creates the possibility of removing metals from the plant via natural leaf shedding. The oldest leaves of metal-exposed plants generally exhibit the highest metal concentrations (Ernst 1982, 1984, 1990). In contrast to Ca, which accumulates gradually with ageing, Zn accumulates especially during the last week prior to shedding (Fig. 4), suggesting that plants make use of leaf fall as means of reducing their metal burden. Due to the low mobility of Pb in the environment and its high accumulation in the roots, the concentration of Pb in senescent leaves remains about constant throughout the year. It is unknown whether tolerant plants exhibit special adaptations at this point (see above).

Reproductive organs

Due to the toxicity of metal-enriched soils, non-tolerant plants do not survive to the reproductive phase in such environments, neither naturally nor experimentally. Therefore it is impossible to compare metal accumulation in reproductive organs at high levels of metal response through the life cycle of tolerant and non-tolerant plants. Plants on heavy metal-enriched soils do not exhibit a decreased seed production. In metal-tolerant plants growing in the natural environment, e.g. *Armeria muelleri, Thlaspi coerulescens, Silene vulgaris* (Ernst 1982; Ernst *et al.* 1990), or in metal-enriched nutrient solution, e.g. *Mimulus guttatus* and *Silene diocia* (Searcy & Mulcahy 1985a,b), metals are not excluded from the reproductive parts. Although the metal concentrations in all reproductive plant parts are lower than in vegetative ones, pollen selection, due to metal accumulation in the pistils has been demonstrated in the laboratory (Searcy & Mulcahy 1985a,b,c). Also, a

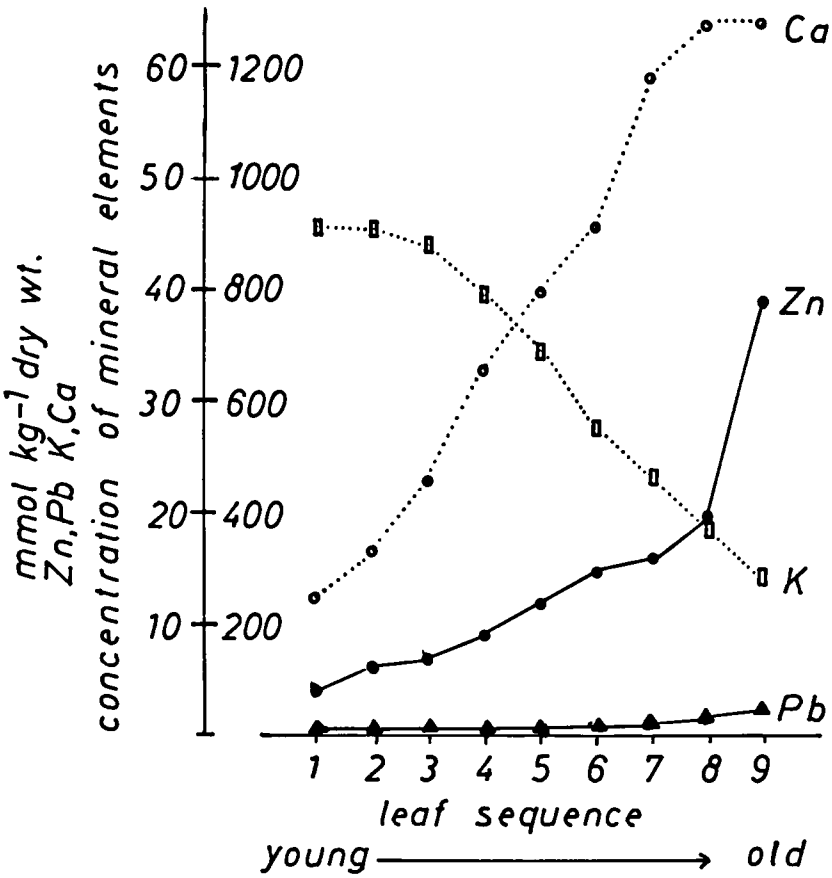

Fig. 4. Concentration of some mineral elements in relation to leaf initiation in Zn-tolerant plants of *Plantago lanceolata*. Data from Ernst (1984) and unpublished.

reduction in the percentage of viable pollen in metal-tolerant plants after metal treatment has been observed (Searcy & Mulcahy 1985b). It is unknown, however, whether these processes play a role in the natural environment.

In metal-tolerant plants, seeds have a lower metal concentration than any other plant part (Ernst 1974, 1982). The concentration in the testa is often two to four times higher than in the embryo, which suggests that the placenta represents a barrier to metal translocation. The low metal concentration of the embryo may be advantageous since it creates extra storage capacity during germination and early seedling growth.

THE COSTS OF METAL TOLERANCE

Selection against metal tolerance on unpolluted soils has been clearly demonstrated (McNeilly 1968; Cook *et al.* 1972; Hickey & McNeilly 1975), which implies that metal tolerance has a cost. In the case of tolerances to essential metals, the costs of tolerances may be (partly) explained by an apparent increased need for the metals in question (e.g. Baumeister 1954; Schat & Ten Bookum 1992a,b). The fact that tolerant plants often

exhibit maximum growth at elevated, normally toxic levels of the metal to which they are tolerant, has been explained as a consequence of the operation of the tolerance mechanism itself. The reduced uptake, or increased cellular sequestration of the metal would reduce the activity of metallo-enzymes or metal-stimulated enzymes. If compared at a low external zinc concentration, Zn-tolerant *Silene vulgaris* exhibits a 50% lower carboanhydrase activity than non-tolerant plants (Mathys 1975a,b), and, possibly as a consequence, a lower photosynthetic activity too (Baumeister 1954). Only at an increased zinc concentration, tolerant plants reach normal activity levels. Such a shift in the optimum zinc concentration has also been observed for nitrate reductase activity (Ernst *et al.* 1975; Mathys 1975a,b). Tolerance-correlated increases in the metal demand for maximum growth have been observed for Zn (Mathys 1975a,b), Ni (Sasse 1976), Cu (Lolkema *et al.* 1984; Schat & Ten Bookum 1992a,b), and although only in short-term experiments, some non-essential metals, too (Baker & Walker 1989, for a survey).

Even when grown at the optimum metal concentration, tolerant plants or clones usually grow slower than non-tolerant ones (Ernst 1983; Wilson 1988). This difference in maximum growth has been tentatively explained as the energy cost of the tolerance mechanism (Ernst 1983). It might as well represent, however, a consequence of adaptation to environmental conditions other than the elevated metal availability itself, e.g. the nutrient status of the soil. A more certain interpretation of these phenomena requires a genetic analysis.

REFERENCES

Antonovics, J. (1966): *The genetics and evolution of differences between closely adjacent plant populations with special references to heavy metal tolerance.* PhD. Thesis, University of Wales.

Asher, C.J. & Reay, P.F. (1979): Arsenic uptake by barley seedlings. *Austr. J. Plant Physiol.* **6:** 459–466.

Baker, A.J.M. (1978): Ecophysiological aspects of zinc tolerance in *Silene maritima* With. *New Phytol.* **80:** 635–642.

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

Baker, A.J.M. & Walker, P.L. (1989): Physiological responses to heavy metals and the quantification of tolerance and toxicity. *Chem. Speciation Bioavailability* **1:** 7–17.

Bartolf, M., Breman, E. & Price, C.A. (1980): Partial characterization of a cadmium binding protein from the roots of a cadmium-treated tomato. *Plant Physiol.* **66:** 438–441.

Baumeister, W. (1954): Über den Einfluss des Zinks bei *Silene inflata* Smith I. *Ber. Deutsch. Bot. Ges.* **67:** 205–213.

Bettger, W.J. & O'Dell, B.L. (1987): A critical physiological role of zinc in the structure and function of biomembranes. *Life Sci.* **28:** 1425–1438.

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

Bradshaw, A.D., McNeilly, T. (1991): Stress tolerance in plants—the evolutionary framework. In: Rozema J. and Verkleij J.A.C. (eds): *Ecological Responses to Environmental Stresses*, 2–5. Kluwer Academic Publishers, Dordrecht.

Bröker, W. (1962): *Genetisch-physiologische Untersuchungen in* Silene inflata Sm. Inaugural-Dissertation Universität Münster.

Brookes, A., Collins, J.C. & Thurman, D.A. (1981): The mechanism of zinc tolerance in grasses. *J. Plant Nutr.* **3:** 695–705.

Brooks, R.R., Shaw, S. & Asensi Marfil, A. (1981): The chemical form and physiological function of nickel in some Iberian *Alyssum* species. *Physiol. Plant.* **51:** 167–170.

Butler, M., Haskew, A.E.J. & Young, M.M. (1980): Copper tolerance in the green alga *Chlorella vulgaris*. *Plant Cell Environ.* **3:** 119–126.

Cakmak, J. & Horst, H. (1988): Increase in membrane permeability and exudation in roots of zinc deficient plants. *J. Plant Physiol.* **132:** 356–361.

Cataldo, D.A., Garland, T.R. & Wildering, R.E. (1983): Cadmium uptake kinetics in intact soybean plants. *Plant Physiol.* **73:** 844–848.

Cathala, N. & Salsac, L. (1975): Absorption du cuivre par les racines de mais (*Zea mais* L.) et de tournesol (*Helianthus annuus* L.). *Plant Soil* **42:** 65–83.

Clijsters, H., Van Assche, F.& Gora, L. (1991): Physiological responses of higher plants to soil contamination with metals. In: Rozema J. and Verkleij

J.A.C. (eds): *Ecological Responses to Environmental Stresses*. 32–39. Kluwer Academic Publishers, Dordrecht.

Cook, S.C.A., Lefèbre, C. & McNeilly, T. (1972): Competition between metal tolerant and normal plant populations on normal soil. *Evolution* **26:** 366–372.

Coombes, A.J., Phipps, D.A. & Lepp, N.W. (1977): Uptake patterns of free and complexed copper ions in excised roots of barley (*Hordeum vulgare* L. cv Zephyr). *Z. Pflanzenphysiol.* **82:** 435–439.

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

Cox, M.R., Thurman, D.A. & Brett, M. (1976): Some properties of the soluble acid phosphatases of roots of zinc-tolerant and non-tolerant clones of *Anthoxanthum odoratum*. *New Phytol.* **7:** 547–552.

Cox, R.M. & Thurman, D.A. (1978): Inhibition by zinc of soluble and cell wall acid phosphatases of zinc-tolerant and non-tolerant clones of *Anthoxanthum odoratum*. *New Phytol.* **80:** 17–32.

Craig, G.C. (1977): A method of measuring heavy metal tolerance in grasses. *Trans. Rhod. Sci. Assoc.* **58:** 9–16.

Davies, M.S., Francis, D. & Thomas, J.D. (1991a): Rapidity of cellular changes induced by zinc in a zinc-tolerant and non-tolerant cultivar of *Festuca rubra* L. *New Phytol.* **117:** 103–108.

Davies, M.S. & Snaydon, R.W. (1973): Physiological differences among populations of *Anthoxanthum odoratum* L. collected from the Park Grass Experiment Rothamsted. II. Response to aluminium. *J. Appl. Ecol.* **10:** 47–55.

Davies, R.L., Davies, M.S. & Francis, D. (1991b): Zinc-induced vacuolation in root meristematic cells of *Festuca rubra* L. *Plant Cell Environ.* **14:** 399–406.

De Filippis, L.F. (1979): The effect of heavy metal compounds on the permeability of *Chlorella* cells. *Z. Pflanzenphysiol.* **92:** 39–49.

De Koe, T. (1991): Arsenic in water, sediment and vegetation of the Jales gold mine, North Portugal. In: Rozema J. and Verkleij J.A.C. (eds): *Ecological Responses to Environmental Stresses*, 42–47. Kluwer Academic Publishers, Dordrecht.

Delhaize, E., Jackson, P.J., Lujan, L.D. & Robinson, N.J. (1989): Poly(γ-glutamyl-cysteinyl) glycine synthesis in *Datura innoxia* and binding with cadmium. *Plant Physiol.* **89:** 700–706.

De Miranda, J.R., Thomas, M.A., Thurman, D.A. & Tomsett, A.B. (1990): Metallothionein genes from the flowering plant *Mimulus guttatus*. *FEBS Letters* **260:** 277–280.

De Vos, C.H. (1991): *Copper-induced oxidative stress and free radical damage in roots of copper tolerant and sensitive* Silene cucubalus. Doctoral Thesis, Vrije Universiteit Amsterdam.

De Vos, C.H.R., Schat, H., De Waal, M.A.M., Vooijs, R. & Ernst, W.H.O. (1991): Increased resistance to copper-induced damage of the root cell plasmalemma in copper-tolerant *Silene cucubalus*. *Physiol. Plant* **82:** 523–528.

De Vos, C.H.R., Vooijs, R., Schat, H. & Ernst, W.H.O. (1989): Copper-induced damage to the permeability barrier in roots of *Silene cucubalus*. *J. Plant Physiol.* **135:** 165–169.

De Vos, C.H.R., Vonk, M.J., Vooijs, R. & Schat, H. (1992): Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. *Plant Physiol.* **98:** 853–858.

Dueck, Th.A., (1986): *Impact of heavy metals and air pollutants on plants*. Doctoral Thesis, Vrije Universiteit, Amsterdam.

Dueck, Th.A., Ernst, W.H.O., Faber, J. & Pasman, F. (1984): Heavy metal emission and genetic constitution of plant populations in the vicinity of two metal emission sources. *Angw. Bot.* **58:** 47–59.

Dueck, Th.A., Visser, P., Ernst, W.H.O. & Schat, H. (1986): Vesicular–arbuscular mycorrhizae decrease zinc-toxicity to grasses growing in zinc-polluted soil. *Soil Biol. Biochem.* **18:** 331–333.

Duvigneaud, P. (1958). La végétation du Katanga et de ses sol métallifères. *Bull. Soc. r. Bot. Belg.* **90:** 127–286.

Ernst, W.H.O. (1964): *Okologisch-soziologische Untersuchungen in den Schwermetall-Pflanzengesellschaften Mitteleuropas unter Einschluss der Alpen*. Inaugural-Dissertation Universität Münster.

Ernst, W.H.O. (1968): Der Einfluss der Phosphatversorgung sowie die Wirkung von ionogenem und chelatisiertem Zink auf die Zink- und Phosphataufnahme einiger Schwermetallpflanzen. *Physiol. Plant.* **21:** 323–333.

Ernst, W.H.O. (1969): Zur Physiologie der Schwermetallpflanzen—Subzelluläre Speicherungsorte des Zinks. *Ber. Deutsch. Bot. Ges.* **82:** 161–164.

Ernst, W.H.O. (1972a): Ecophysiological studies on heavy metal plants in South Central Africa. *Kirkia* **8:** 125–145.

Ernst, W.H.O. (1972b): Schwermetallresistenz und Mineralstoffhaushalt. *Forschungsber. Land. Nordrhein-Westfalen* **2251:** 1–38. Westdeutscher Verlag, Opladen.

Ernst, W.H.O. & Weinert, H. (1972): Lokalisation von Zink in der Blättern von *Silene cucubalus* Wib. *Z. Pflanzenphysiol.* **66:** 258–264.

Ernst, W.H.O., Mathys, W. & Janiesch, P. (1975): Physiologische Grundlagen der Schwermetallresistenz. Enzymaktivitäten und organische Säuren. *Forschungsber. Land. Nordrhein Westfalen*, **2469:** 1–38. Westdeutscher Verlag, Opladen.

Ernst, W.H.O. (1974): *Schwermetallvegetation der Erde*. G. Fischer Verlag Stuttgart.

Ernst, W.H.O. (1975): Physiology of heavy metal resistance in plants. *Int. Conf. Heavy Metals in the Environment*, Toronto 1975, pp. 121–136.

Ernst, W.H.O. (1976): Physiological and biochemical aspects of metal tolerance. In: Mansfield T.A. (ed.) *Effects of Air Pollutants in Plants*, 115–133. Cambridge University Press, Cambridge.

Ernst, W.H.O. (1980): Biochemical aspects of cadmium in plants. In: Nriagu J.O. (ed.) *Cadmium in the Environment I*, 639–653. John Wiley & Sons, New York.

Ernst, W.H.O. (1982): Schwermetallpflanzen. In: Kinzel H. (ed.) *Pflanzenökologie und Mineralstoffwechsel*, 472–506. Ulmer Verlag, Stuttgart.

Ernst, W.H.O. (1983): Ökologische Anpassungsstrategien an Bodenfaktoren. *Ber. Deutsch. Bot. Ges.* **96:** 49–71.

Ernst, W.H.O. (1984): Metal tolerance in *Plantago lanceolata*. *Nieuwsbrief* 15–19.

Ernst, W.H.O. (1990): Element allocation and (re)-translocation in plants and its impact on representative sampling. In: Lieth H. and Markert B. (eds): *Element concentration cadasters in ecosystems*, 17–40. VCH Verlagsgesellschaft, Weinheim.

Ernst, W.H.O., Schat, H. & Verkleij, J.A.C. (1990): Evolutionary biology of metal resistance in *Silene vulgaris*. *Evol. Trends Plants.* **4,** 45–51.

Ernst, W.H.O. & Verkleij, J.A.C. (1991): Can phytochelatins be used for estimation of metal availability? In: Farmer J.G. (ed.): *Heavy Metals in the Environment* (Edinburgh 1991) 1: 111–114. CEP Consultant, Edinburgh.

Evans, P.M., Gatehouse, L.N., Gatehouse, J.A., Robinson, N.J. & Greg, R.R.D. (1990): A gene from pea (*Pisum sativum* L.) with homology to metallothionein genes. *FEBS Letters* **266:** 29–32.

Farago, M.E. & Pitt, J. (1977): Plants which accumulate metals. Part III. A further investigation of two Australian species which take up zinc. *Inorg. Chim. Acta.* **24:** 211–214.

Godbold, D.L., Horst, W.J., Collins, J.C., Thurman, D.A. & Marschner, H. (1984): Accumulation of zinc and organic acids in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitosa*. *J. Plant Physiol.* **116:** 59–69.

Graham, R.D. (1981): Absorption of copper by plant roots. In: Goneragan J.F., Robson A.D. and Graham A.D. (eds): *Copper in Soils and Plants*, 141–163. Academic Press, Sydney.

Gregory, R.P.G. (1965): *Heavy metal tolerance in grasses*. PhD. Thesis University of Wales.

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

Gries, B. (1965): *Zellphysiologische Untersuchungen über die Resistenz gegen Zink an Galmeiökotypen und der Normalform von* Silene cucubalus. Inaugural-Dissertation Universität Münster.

Gries, B. (1966): Zellphysiologische Untersuchungen über die Zinkresistenz bei Galmeiökotypen und Normalformen von *Silene cucubalus* Wib. *Flora* **B156:** 271–290.

Griffioen, W.A.J. & Ernst, W.H.O. (1990): The role of VA mycorrhiza in the heavy metal tolerance of *Agrostis capillaris* L. *Agric. Ecosyst. Environ.* **29:** 173–177.

Grill, E., Winnacker, E.L. & Zenk, M.H. (1985a): Phytochelatins: the principal heavy-metal complexing peptides of higher plants. *Science* **230:** 674–676.

Grill, E., Winnacker, E.G. & Zenk, M.H. (1987): Phytochelatins, a class of heavy-metal binding peptides from plants, are functionally analogous to metallothioneins. *Proc. Natl. Acad. Sci.* USA **84:** 439–443.

Grill, E., Zenk, M.H. & Winnacker, E.L. (1985b): Induction of heavy metal-sequestering phytochelatin by cadmium in cell cultures of *Rauvolfia serpentina*. *Naturwissenschaften* **72:** 432–433.

Gupta, S.C. & Goldsbrough, P.B. (1991): Phytochelatin accumulation and cadmium tolerance in selected tomato cell lines. *Plant Physiol.* **97:** 306–312.

Gutknecht, K. (1983): Cadmium and thallous ion permeabilities through lipid bilayer membranes. *Biochim. Biophys., Act* **735:** 185–188.

Hall, A., Fielding, A.H. & Bulter, M. (1979): Mechanism of copper tolerance in the marine fouling alga *Ectocarpus siliculosus*—Evidence for an exclusion mechanism. *Mar. Biol.* **54:** 195–199.

Harmens, H., Verkleij, J.A.C., Koevoets, P. & Ernst, W.H.O. (1989): The role of organic acids and phytochelatins in the mechanisms of zinc tolerance in *Silene vulgaris* (= *Silene cucubalus*). In: Vernet J.P. (ed.): *Heavy Metals in the Environment* (Geneva 1989), **2:** 178–181. CEP Consultant, Edinburgh.

Harrison, S.L., Lepp, N.W. & Phipps, N.A. (1979): Uptake of copper by excised roots. II. Copper desorption from the free space. *Z. Pflanzenphysiol.* **94:** 27–34.

Hassen, M.M. & Tang Han Hai (1976): Kinetics of zinc uptake by citrus roots. *Z. Pflanzenphysiol.* **79:** 177–181.

Heuillet, E., Moreau, A., Halpern, S., Jeanne, N., Puiseux-Dao, S. (1986): Cadmium binding to a thiole-molecular in vacuoles of *Dunaliella bioculata* contaminated with Cd Cl_2: electron probe analysis. *Biol Cell* **58:** 79–86.

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

Hille, B. (1984): *Ionic channels of excitable membranes*. Sinauer Associates, Sunderland, MA.

Höfner, W. (1970): Eisen- und manganhaltige Verbindungen im Blutungssaft von *Helianthus annuus*. *Physiol. Plant.* **23:** 673–677.

Humphreys, M.D. & Nicolls, M.K. (1984): Relationships between tolerance to heavy metals in *Agrostis capillaris* L. (*A. tenuis* Sibth.). *New Phytol.* **98:** 177–190.

Ietswaart, J.H., Griffioen, W.A.J. & Ernst, W.H.O. (1992): Seasonality of VAM infection in three populations of *Agrostis capillaris* (Gramineae) on soil with or without heavy metal enrichments. *Plant Soil* **139:** 67–73.

Jackson, P.J., Unkefer, P.J., Delhaize, E., Robinson, N.J. (1990): Mechanism of trace metal tolerance in plants. In: Katterman F. (ed.): *Environment Injury to Plants*, 231–255. Academic Press, San Diego.

Jellinek, H.H.G. & Sangal, S.P. (1972): Collection of metal ions from water by polygalacturonic acids. *Water Res.* **6:** 305–307.

Jowett, D. (1958): Populations of *Agrostis* ssp. tolerant of heavy metals. *Nature* **182:** 816–817.

Jowett, D. (1959): *Genecology of heavy metal tolerance in* Agrostis *spp.* Ph.D. Thesis University of Wales.

Kennedy, C.D. & Gonsalves, F.A.N. (1987): The action of divalent zinc, cadmium, mercury, copper and lead on the trans-root potential and H^+ efflux of excised roots. *J. exp. Bot.* **38:** 800–817.

Krotz, R.M., Evangelun, B.P. & Wagner, G.J. (1989): Relationships between cadmium, zinc, Cd-peptide, and organic acid in tobacco suspension cells. *Plant Physiol.* **91:** 780–787.

Lane, S.D., Martin, E.S. & Garrod, J.F. (1978): Lead toxicity effects of indole-3yl acetic acid induced cell elongation. *Planta* **144:** 79–84.

Laurie, S.H, Tancock, N.P., McGarth, S.P. & Sanders, J.R. (1991). Influence of complexation on the uptake by plants of iron, manganese, copper and zinc. 1. Effect of EDTA in a multi-metal and computer simulation study. *J. exp. Bot.* **42:** 500–513.

Lee, J.R., Reeves, R., Brooks, R.R. & Jaffré, T. (1978): The relationship between nickel and citric acid in some nickel accumulating plants. *Phytochemistry* **17:** 1033–1035.

Lolkema, P.C. (1985): *Copper resistance in higher plants*. Doctoral Thesis, Vrije Universiteit Amsterdam.

Lolkema, P.C., Donker, M.H., Schouten, A.J. & Ernst, W.H.O. (1984): The possible role of metallothioneins in *Silene cucubalus*. *Planta* **162:** 174–179.

Lolkema, P.C. & Vooijs, R. (1986): Copper tolerance in *Silene cucubalus*. Subcellular distribution of copper and its effects on chloroplasts and plastocyanin synthesis. *Planta* **167:** 30–36.

Maathuis, F.J.M. & Prins, H.B.A. (1991): Patch-clamp studies in cell membranes of higher plants. *Acta Bot. Neerl.* **40:** 197–209.

Macnair, M.L. (1981): Tolerance of higher plants to toxic materials. In: Bishop J.M. and Cook L.M. (eds): *Genetic Consequences of Man-Made Changes*, 137–199. Academic Press, London.

Macnair, M.R. (1983): The genetic control of copper tolerance in the yellow monkey flower, *Mimulus guttatus*. *Heredity* **50:** 283–293.

Marquenie-van der Werff, M. & Ernst, W.H.O. (1979): Kinetics of copper and zinc uptake by leaves and roots of an aquatic plant, *Elodea nuttallii*. *Z. Pflanzenphysiol.* **92:** 1–10.

Mathys, W. (1975a): *Physiologische Aspekte der Zinktoleranz bei* Silene cucubalus *Wib. und anderen Arten*. Inaugural-dissertation, Universität Münster.

Mathys, W. (1975b): Enzymes of heavy-metal-resistant and non-resistant populations of *Silene cucubalus* and their interaction with some heavy metals in vitro and in vivo. *Physiol. Plant.* **33:** 161–165.

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

Matsumoto, Y., Okado, Y., Min, K.S., Onosaka, S. & Tanaka, K. (1990): Amino acids and peptides. XXVII. Synthesis of phytochelatin-related peptides and examination of their metal-binding properties. *Chem. Pharm. Bull.* **38:** 2364–2368.

McBrien, D.C.H. & Hassall, R.A. (1965): Loss of cell potassium by *Chlorella vulgaris* after contact with toxic amounts of copper sulphate. *Physiol. Plant.* **18:** 1053–1065.

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

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

Meharg, A.A. & Macnair, M.R. (1990): An altered phosphate uptake system in arsenate-tolerant *Holcus lanatus* L. *New Phytol.* **116:** 29–35.

Meharg, A.A. & Macnair, M.R. (1991a): Uptake, accumulation and translocation in arsenate tolerant and non-tolerant *Holcus lanatus* L. *New Phytol.* **117:** 225–231.

Meharg, A.A. & Macnair, M.R. (1991b): The mechanism of arsenate tolerance in *Deschampsia cespitosa* (L.) Beauv. and *Agrostis capillaris* L. Adaptation of the arsenate uptake system. *New Phytol.* **119:** 291–297.

Mench, M., Morel, J.L., Cuckert, A. & Guillet, B. (1988): Metal binding with root exudates of low molecular weight. *J. Soil Sci.* **33:** 521–527.

Mukherji, S. & Maitra, P. (1977): Growth and metabolism of germinating rice (*Oryza sativa* L.) seeds influenced by toxic concentrations of lead. *Z. Pflanzen. Physiol.* **81:** 26–33.

Mullins, M., Hardwick, K. & Thurman, D.A. (1985): Heavy metal location by analytical electron microscopy in conventionally fixed and freeze-substituted roots of metal tolerant and non-tolerant ecotypes. In: Lekkas T.D. (ed.): *Heavy Metals in the Environment* (Athens 1985), 43–50. CEP Consultants Edinburgh.

Murasugi, A., Wada, C. & Hayashi, Y. (1981): Purification and unique properties in UV and CD spectra of CD-binding peptide I from *Schizosaccharomyces pombe*. *Biochem. Biophys. Res. Commun.* **103:** 1021–1028.

Muzzarelli, R.A.A. (1973): *Natural chelating polymers*. Pergamon Press, Oxford.

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

Nissen, P. (1973): Multiphasic uptake in plants. II. Mineral cations, chloride, and boric acid. *Physiol. Plant.* **29:** 298–354.

Pelosi, P., Fiorentini, R. & Galoppini, C. (1976): On the nature of nickel compounds in *Alyssum bertolonii* Desv. *Agric. Biol. Chem.* **40:** 1641–1642.

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

Pickering, D.C. & Puia, I.L. (1969): Mechanism for the uptake of zinc by *Fontinalis antipyretica*. *Physiol. Plant.* **22:** 653–661.

Porter, E.K. & Peterson, P.J. (1975): Arsenic accumulation by plants on mine waste (United Kingdom). *Sci. Total Environ.* **4:** 365–371.

Poulter, A., Collin, H.A., Thurman, D.A. & Hardwick, K. (1985): The role of the cell wall in the mechanism of lead and zinc tolerance in *Anthoxanthum odoratum* L. *Plant Sci.* **42:** 61–66.

Powell, M.J., Davies, M.S. & Francis, D. (1986a): The influence of zinc on the cell cycle in the root meristem of a zinc-tolerant and a non-tolerant cultivar of *Festuca rubra* L. *New Phytol.* **102:** 419–428.

Powell, M.J., Davies, M.S. & Francis, D. (1986b): Effects of zinc on cell, nuclear and nucleolar size, and on RNA and protein content in the root meristem of a zinc-tolerant and a non-tolerant cultivar of *Festuca rubra* L. *New Phytol.* **104:** 671–679.

Powell, M.J., Davies, M.S. & Francis, D. (1986c): Effects of zinc on meristem size and proximity of root hairs and xylem elements to the root tip in a zinc-tolerant and a non-tolerant cultivar of *Festuca rubra* L. *Ann. Bot.* **61:** 723–726.

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

Qureshi, J.A., Collin, H.A., Hardwick, K. & Thurman, D.A. (1981): Metal tolerance in tissue culture of *Anthoxanthum odoratum*. *Plant Cell Reports* **1:** 80–82.

Rai, L.C., Mallick, N., Singh, J.B. & Kumar, H.D. (1991): Physiological and biochemical characteristics of a copper tolerant and a wild type strain of *Anabaena doliolum* under copper stress. *J. Plant Physiol.* **138:** 68–74.

Rauser, W.E. (1991): Cadmium-binding peptides from plants. *Methods in enzymology* **205:** 319–333.

Rauser, W.E. & Ackerley, C.A. (1987): Localization of cadmium in granules within differentiating and mature root cells. *Can. J. Bot.* **65:** 643–646.

Rauser, W.E. & Curvetto, N.R. (1980): Metallothionein occurs in roots of *Agrostis* tolerant to copper. *Nature* **287:** 563–564.

Reese, R.N. & Wagner, G.J. (1987): Properties of tobacco (*Nicotiana tabacum*) cadmium-binding peptide(s). *Biochem. J.* **241:** 641–647.

Reese, R.N, White, C.A. & Winge, D.R. (1992): Cadmium-sulfide crystallites in Cd-$(\gamma$-EC$)_n$G peptide complexes from tomato. *Plant Physiol.* **98:** 225–229.

Reilly, C. (1967): Accumulation of copper by some Zambian plants. *Nature* **215:** 667–668.

Reilly, C., Rowel, J. & Stone, J. (1970): The accumulation and binding of copper in leaf tissue of *Becium homblei* (De Wild.) Duvign. & Plancke. *New Phytol.* **69:** 993–997.

Repp, G. (1963): Die Kupferresistenz des Protoplasmas höherer Pflanzen auf Kupfererzböden. *Protoplasma* **57:** 643–659.

Robinson, N.J. & Jackson, P.J. (1986): Metallothionein-like metal complexes in angiosperms; their structure and function. *Physiol. Plant.* **67:** 499–506.

Robinson, N.J. & Thurman, D.A. (1986): Involvement of a metallothionein-like complex in the mechanism of copper tolerance in *Mimulus guttatus*. *Proc. R. Soc. London* **B227:** 493–501.

Rüther, F. (1966): *Vergleichende physiologische Untersuchungen über die Zinkresistenz von Schwermetallpflanzen*. Inaugural-Dissertation Universitat Münster.

Rüther, F. (1967): Vergleichende physiologische Untersuchungen über die Resistenz von Schwermetallpflanzen. *Protoplasma* **64:** 400–425.

Salt, D.E., Thurman, D.A., Tomsett, A.B. & Sewell, A.K. (1989): Copper phytochelatins of *Mimulus guttatus*. *Proc. R. Soc. Lond.* **236:** 79–89.

Sasse, F. (1976): *Okophysiologische Untersuchungen der Serpentinvegetation in Frankreich, Italien, Österreich und Deutschland*. Inaugural-Dissertation, Universität Münster.

Schat, H. & Kalff, M. (1992): Are phytochelatins involved in differential metal tolerance, or do they merely reflect metal-imposed strain? *Plant Physiol.* (in press).

Schat, H. & Ten Bookum, W.M. (1992a): Genetic control of copper tolerance in *Silene vulgaris*. *Heredity* **68:** 219–229.

Schat, H. & Ten Bookum, W.M. (1992b): Metal-specificity of metal tolerance syndromes in higher plants. *Serpentine Ecology* (in press).

Scheller, H.V., Huang, B., Hatch, E. & Goldbrough, P.B. (1987): Phytochelatin synthesis and glutathione levels in response to heavy metals in tomato cells. *Plant Physiol.* **85:** 1031–1035.

Searcy, K.B & Mulcahy, D.C. (1985a): Pollen tube competition and selection for metal tolerance in *Silene dioica* (Caryophyllaceae) and *Mimulus guttatus* (Scrophulariaceae). *Amer. J. Bot.* **72:** 1695–1699.

Searcy, K.B. & Mulcahy, D.C. (1985b): Pollen selection and the gametophytic expression of metal tolerance in *Silene diocia* (Caryophyllaceae) and *Mimulus guttatus* (Scrophulariaceae). *Amer. J. Bot.* **72:** 700–706.

Searcy, K.B. & Mulcahy, D.C. (1985c): The parallel expression of metal tolerance in pollen and sporophytes of *Silene dioica* (L.) Clairv., *S. alba* (Mill.) Krause and *Mimulus guttatus* DC. *Theor. Appl. Genetics* **69:** 597–602.

Slivinskaya, B.B. (1991): Nickel effect on sunflower leaf cell membranes. *Acta Bot. Neerl.* **40:** 133–138.

Smirnoff, N. & Stewart, G.R. (1987): Glutamine synthetase and ammonium assimilation in roots of zinc-tolerant and non-tolerant clones of *Deschampsia cespitosa* (L.) Beauv. and *Anthoxanthum odoratum*. *New Phytol.* **107:** 659–670.

Steffens, J.C. (1990): The heavy metal-binding peptides of plants. *Annu. Rev. Plant Physiol. Mol. Biol.* **41:** 553–575.

Steffens, J.C, Hunt, D.F. & Williams, B.G. (1986): Accumulation of non-protein metal-binding polypeptides (γ-glutamyl-cysteinyl)$_n$ glycine in selected cadmium-resistant tomato cells. *J. Biol. Chem.* **261:** 13879–13882.

Strange, J. & Macnair, M.R. (1991): Evidence for a role for the cell membrane in copper tolerance of *Mimulus guttatus* Fischer ex DC. *New Phytol.* **119:** 383–388.

Thurman, D.A. & Collins, J.C.L. (1983): Metal tolerance mechanisms in higher plants—review. In: *Proc. Int. Conf. Heavy Metals in the Environment*, Heidelberg, 298–300. CEP Consultants, Edinburgh.

Tommey, A.M., Shi, J., Lindsay, W.A., Urwin, P.E. & Robinson, N.J. (1991): Expression of the pea gene $PsMt_A$ in *E. coli*. Metal-binding properties of the expressed protein. *FEBS Letters* **292:** 48–52.

Turner, R.G. & Gregory, R.P.G. (1967): The use of radioisotopes to investigate heavy metal tolerance in plants. In: *Isotopes in Plant Nutrition and Physiology*. I.A.E.A. Vienna, 493–508.

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

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

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

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

Van Assche, F. & Clijsters, H. (1990): Effects of metals on enzyme activity in plants. *Plant Cell Environ.* **13:** 195–206.

Van der Werff, M. (1981): *Ecotoxicity of heavy metals in aquatic and terrestrial higher plants*. Doctorial Thesis, Vrije Universiteit Amsterdam.

Van Goor, B.J. & Wiersma, D. (1976): Chemical forms of manganese and zinc in phloem exudates. *Physiol. Plant.* **36:** 213–216.

Van Steveninck, R.F.M., Van Stevenick, M.E. & Fernando, D.R. (1992): Heavy metal (Zn, Cd) tolerance in selected clones of duck weed (*Lemna minor*). *Plant Soil* (in press).

Van Steveninck, R.F.M., Van Steveninck, M.E., Fernando, D.R., Edwards, L.B. & Wells, A.J. (1990a): Electron probe X-ray microanalytical evidence for two distinct mechanisms of Zn and Cd binding in a Zn tolerant clone of *Lemna minor* L. *C.R. Acad. Sci. Paris* **310:** Ser. III, 671–678.

Van Steveninck, R.F.M., Van Steveninck, M.E., Fernando, D.R., Horst, W.J. & Marschner, W.J. (1987): Deposition of zinc phytate in globular bodies in roots of *Deschampsia caespitosa* ecotypes; a detoxification mechanism? *J. Plant Physiol.* **131:** 247–257.

Van Steveninck, R.F.M., Van Steveninck, M.E., Wells, A.J. & Fernando, D.R. (1990b): Zinc tolerance and the binding of zinc as zinc phytate in *Lemna minor*. X-ray microanalytical evidence. *J. Plant Physiol.* **137:** 140–146.

Veltrup, W. (1976): Concentration-dependent uptake of copper by barley roots. *Physiol. Plant.* **36:** 217–220.

Veltrup, W. (1977): The uptake of copper by barley roots in the presence of zinc. *Z. Pflanzenphysiol.* **83:** 201–206.

Veltrup, W. (1978): Characteristics of zinc uptake by barley roots. *Physiol. Plant.* **42:** 190–194.

Veltrup, W. (1979): The effect of Ni^{2+}, Cd^{2+}, and Co^{2+} on the uptake of copper by intact barley roots. *Z. Pflanzenphysiol.* **93:** 1–9.

Verkleij, J.A.C. & Bast-Cramer, W.B. (1985): Co-tolerance and multiple heavy metal tolerance in

Silene cucubalus from different heavy metal sites. Lekkas, T.D. (ed.) *Heavy Metals in the Environment*, Athens 1985, 174–176. CEP Consultants, Edinburgh.

Verkleij, J.A.C., Bast-Cramer, W.B. & Koevoets, P. (1989a): Genetic studies in populations of *Silene cucubalus* occurring on various polluted and unpolluted areas. In: Scholz, F., Gregorius, H.R. and Rudin, D. (eds): *Genetic Effects of Air Pollutants*, 107–114. Springer Verlag Berlin.

Verkleij, J.A.C., Koevoets, P.L.M., van't Riet, J., de Knecht, J.A. & Ernst, W.H.O. (1990): The role of metal-binding compounds (phytochelatins) in the cadmium-tolerance mechanism of Bladder Campion (*Silene vulgaris*). In: Rennenberg, H., Brumold, C., de Kok, L.J. and Stulen, J. (eds): *Sulfur Nutrition and Sulfur Assimilation in Higher Plants*, 255–260. SPB Academic Publishing, The Hague.

Verkleij, J.A.C., Koevoets, P., van't Riet, J., van Rossenberg, M.C., Bank, R. & Ernst, W.H.O. (1989b): The role of metal-binding compounds in the copper tolerance mechanisms of *Silene cucubalus*. In: Winge, D. and Hamer, D. (eds): *Metal Ion Homeostasis: Molecular Biology and Chemistry*, 347–357. Alan R. Liss Inc., New York.

Verkleij, J.A.C. & Prast, J.E. (1989): Cadmium tolerance and co-tolerance in *Silene vulgaris* (Moench) Garcke (= *S. cucubalus* (L.) Wib.). *New Phytol.* **111:** 637–645.

Verkleij, J.A.C. & Schat, H. (1990): Mechanisms of metal tolerance in higher plants. In: Shaw, A.J. (ed.): *Heavy Metal Tolerance in Plants: Evolutionary Aspects*, 179–194. CRC Press, Boca Raton, Fl.

Vögeli-Lange, R. & Wagner, G.J. (1990): Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. Implications of a transport function for cadmium-binding peptides. *Plant Physiol.* **92:** 1086–1093.

Wagner, G.J. (1984): Characterization of a cadmium-binding complex of cabbage leaves. *Plant Physiol.* **76:** 797–805.

Wainwright, S.J. & Woolhouse, H.W. (1975): Physiological mechanisms of heavy metal tolerance. In: Chadwick, M.J. and Goodman, G.T. (eds): *The Ecology of Resource Degradation and Renewal*, 231–259. Blackwell Scientific Publications, Oxford.

Wainwright, S.J. & Woolhouse, H.W. (1977): Some physiological aspects of copper and zinc tolerance in *Agrostis tenuis* Sibth.: cell elongation and membrane damage. *J. exp. Bot.* **28:** 1029–1036.

Watkins, A.D. & Macnair, M.R. (1991): Genetics of arsenic tolerance in *Agrostis capillaris* L. *Heredity* **66:** 47–54.

Weigel, H.J. & Jäger, H.J. (1980): Subcellular distribution and chemical form of cadmium in bean plants. *Plant Physiol.* **65:** 480–482.

White, M.C., Decker, A.M. & Chaney, R.L. (1981a): Metal complexation in xylem fluid. 1. Chemical composition of tomato and soybean stem exudate. *Plant Physiol.* **67:** 292–300.

White, M.C., Chaney, R.L. & Decker, A.M. (1981b): Metal complexation in xylem fluid. 3. Electrophoretic evidence. *Plant Physiol.* **67:** 311–315.

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

Wilkins, D.A. (1978): The measurement of tolerance to edaphic factors by means of root growth. *New Phytol.* **80:** 623–633.

Wilson, J.B. (1988): The cost of heavy-metal tolerance: an example. *Evolution* **42:** 408–413.

Woolhouse, H.W. & Walker, S. (1981): The physiological basis of copper toxicity and copper tolerance in higher plants. In: Loneragan, J.F., Robson, A.D. and Graham, R.D. (eds): *Copper in Soils and Plants*, 235–262. Academic Press, Sydney.

Woolhouse, H.W. (1983): Toxicity and tolerances in the responses of plants to metals. In: Lange, O.L., Nobel, P.S., Osmond, C.B. and Ziegler, H. (eds): *Encyclopedia of Plant Physiology N.S. XII, C. Responses to the Chemical and Biological Environment*, 245–300. Springer Verlag, Berlin.

Wu, L. (1990): Colonization and establishment of plants in contaminated sites. In: Shaw, A.J. (ed.): *Heavy Metal Tolerance in Plants: Evolutionary Aspects*. 259–284. CRC Press, Boca Raton, Fl.

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

Wu, L. & Antonovics, J. (1978): Zinc and copper tolerance of *Agrostis stolonifera* L. in tissue culture. *Amer. J. Bot.* **65:** 268–271.

Wu, L., Thurman, D.A. & Bradshaw, A.D. (1975): The uptake of copper and its affect upon respiratory processes of copper-tolerant and non-tolerant clones of *Agrostis stolonifera*. *New Phytol.*, **75:** 225–229.

Wyn Jones, R.G., Sutcliffe, M. & Marshall, C. (1971): Physiological and biochemical basis for heavy metal tolerance in clones of *Agrostis tenuis*. In: Samish, R.M. (ed.): *Recent Advances in Plant Nutrition* **2:** 575–581. Gordon & Breach Science Publ., New York.