Coordination Chemistry Reviews, 16 (1975) 1—8
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

THE CHEMISTRY OF PLANTS WHICH ACCUMULATE METALS

M.E. FARAGO, A.J. CLARK and M.J. PITT
Department of Chemistry, Regent's Park, London, NW1 4NS (Great Britain)

INTRODUCTION

Copper, iron and zinc have been found to be essential to the life of plants in trace quantities. Zinc, copper and nickel are toxic at high concentrations, so that plants growing on mineralised soils containing these metals must possess tolerance mechanisms in order to restrict access of these metals to their metabolic reaction sites. Some plants restrict their uptake of metal from the soil, so that little or no metal is found in their tissues. Other plants effect tolerance by storage of metal at a particular site, where it may be sequestered by complexation with naturally occurring ligands.

Antonovics, Bradshaw and Turner have reviewed the possible mechanisms of tolerance (Table 1) [1].

Information on the complexing of metal ions in plants has come largely from the study of metal translocation within the plant. Tiffin [2] has suggested association between iron and citrate in exudates of sunflower and soya bean. In a study of metal ion translocation in tomato [3] and other plants, it was shown using ^{63}Ni (at physiological levels) that the metal is transported as a negatively charged species, which was not identified but had considerable thermodynamic stability. This behaviour contrasts with that of manganese, cobalt and zinc in tomato exudate, where the metals are largely in the form of the aquo ions [4].

Work by Reilly [5] on copper accumulating species has suggested the presence of copper amino acid complexes, in particular a copper cysteine complex which is water extractable. Reilly et al suggest that copper is removed by binding as a copper peptide complex, and have further shown that much of the copper is bound in the plant in a non-extractable form.

Metal complexes as part of the structure of cell walls has also been suggested [6]. The importance of the cell wall in the tolerance mechanism has been demonstrated in the case of the metal tolerant grass, *Agrostis tennuis* [7]. Peterson [8] has found that the ^{65}Zn in the pectate extract of this grass is higher in zinc-tolerant than in the non-tolerant plants, and suggests that the mechanism of tolerance is the deactivation of zinc by cation binding sites in the cell wall.

Histochemical studies [9] of the nickel containing plant *Alyssum bertolonii* located the nickel in the epidermal regions and between the vascular bundles.

TABLE 1

Possible mechanisms of metal tolerance (from ref. 1)

A. External	B. Internal
(i) Form of metal is not directly soluble in water and/or if dissolved then rapidly diluted by surrounding water. (ii) Actual amount of freely diffusable metal ions is small compared to total amount present. (iii) Lack of permeability to heavy metals under specific conditions. (iv) Metal ion antagonisms.	(v) Differential uptake of ions. (vi) Removal of metal ions from metabolism by deposition in vacuole. (vii) Removal of metal ions from metabolism by pumping from cell. (viii) Removal of metal ions from metabolism by rendering into an innocuous form. (ix) Excretory mechanisms—removal of "metal storage organ". (x) Greater requirement of enzyme systems for metal ions. (xi) Alternative metabolic pathway by-passing inhibited site. (xii) Increased concentration of metabolite that antagonizes inhibitor. (xiii) Increased concentration of enzyme that is inhibited. (xiv) Decreased requirement for products of inhibited system. (xv) Formation of altered enzyme with decreased affinity for inhibitor or increased relative affinity for substrate compared to the competitive inhibitor. (xvi) Decreased permeability of cell or subcellular units to metal ions. (xvii) Alteration in protoplasm so that enzymes may function even when toxic metals replace physiological metals.

Studies by Ernst have suggested that the cell vacuoles may be important sites of heavy metal accumulation [10]. Using electron microscope and biochemical techniques, Ernst et al found that 50 % of the total zinc in a zinc accumulating plant was in the vacuoles. The zinc in the cell walls (about 10

TABLE 2

Some nickel indicator species

Plant species	Occurrence	Ref.
Hybanthus floribundus	W. Australia	13
Alyssium bertolonii	Europe	15
Dicoma nicolifera	South Africa	10
Agrostis tennius	Europe	16

TABLE 3

Some zinc accumulator species

	Zinc content (p.p.m. dry wt)
Thlaspi alpestre ssp. calaminaria	7757
Armeria maritima ssp. halleri	3328
Minuartia verna ssp. hereynica	3007
Silene cucubalus (=vulgaris) var. humilis	1719
Armeria maritima ssp. calaminaria	1895
Viola calaminaria	686

%) was in a non-crystalline form. Modification of the cell wall phosphatase enzyme has been suggested [12] in the metal tolerant species.

This paper reports some of our investigations of the metal accumulating plants *Hybanthus floribundus* [13] and *Polycarpia glabra* [14]. In general, nickel levels in the aerial parts of plants are quite low, however a number of plants have been cited as nickel tolerant, thus as indicators of nickel mineralisation (Table 2), and some may be classed as nickel accumulators.

There has been considerable study of the uptake of zinc by plants and of zinc tolerant species [1,14,17]. Some zinc accumulator plants are given in Table 3.

RESULTS

Nickel accumulation by Hybanthus floribundus

Analytical studies

Hybanthus floribundus is a shrub occurring in the Eastern goldfields area of Western Australia. The geobotanical and biogeochemical investigations in this area which have established the relationships between the metal content, the distribution of plant species and bedrock geology have been described in detail [13(a)]. Samples of leaves and stems of *Hybanthus floribundus* have been shown to contain over 200,000 p.p.m. of metal per ash weight, i.e. nearly 10,000 p.p.m. (23%) expressed as oven dried weight.

Samples of *Hybanthus* were analysed for carbon, hydrogen and metal content (Table 4), but there appears to be little correlation in the figures.

The results of the analysis of leaf samples for Ni, Ca, Mg, Fe, Co, Pb, and K are shown in Table 5.

High nickel content appears to be associated with high calcium content, and there appeared to be a relationship between Ni and Ca + Mg. Approximately 90 % of the nickel in the leaf is extractable in water and aqueous acid (Table 6).

TABLE 4

Elemental content (dried weight) of samples of *Hybanthus floribundus*

Sample	C(%)	H(%)	N(%)	Ni (p.p.m. dry wt)
1	46.8	6.7	1.96	11,000
2	46.5	6.7	2.26	8,000
3	46.9	6.7	2.54	6,000
4	48.5	6.8	3.08	2,000
5	47.0	6.6	3.20	6,800

TABLE 5

Average metal content (p.p.m. dry weight) of leaf samples of *Hybanthus Floribundus*

Sample number	Ni	Ca	Mg	Fe	Co	Pb	K
7461	8,000	6,100	4,980	146	125	<4	5,000
7509	7,900	7,600	3,270	113	20	<5	5,300
7465	6,000	4,600	4,240	130	90	<4	6,000
7514	4,500	6,900	3,350	113	40	<2	8,200
7518	2,000	3,800	5,170	145	40	<4	9,700
7517	3,000	4,700	5,010	167	46	<4	10,680
7494	6,800	5,150	2,600		40		6,280

TABLE 6

Percentages of total nickel extracted sequentially

Solvent	Leaf	Green leaf stem	Green stem	Small twig	Old twig
Ether	~0.1	~0.1	~0.2	~0.1	~0.1
Alcohol	12	5	4	1	1
Water	48	54	63	11	18
Acid	43	41	33	87	81
Residue	<1	<0.1	<0.1	<0.1	<0.1

TABLE 7

Metal content (p.p.m. dry weight) of Polycarpia glabra

	An	Fe	Pb	Cu
Flowers	216	180	41	14
Green stem	484	65	61	
Brown stem	613	216	307	

Histochemical Studies

Sections of the leaf and stem of *Hybanthus* were stained and viewed under a high powered microscope. The stains used were: (1) dithioxamide which gives a blue colour with nickel; (2) dimethylglyoxime which gives a red colour with nickel; (3) ruthenium red which gives a pink colour with pectin.

The leaves of the *Hybanthus* plant have large epidermal cells and ridges of large cells which continue along the leaf stem and on to the main stem of the bush. It was found that nickel, as shown by stains (1) and (2), could be located in these large cells. In some cases so much nickel was present that nickel dimethylglyoxime crystallised out under the microscope. The presence of nickel in the ridge cells was confirmed by an electron probe technique using a scanning electron microscope. These same areas showed high concentration of pectins, in agreement with the results of Peterson [8], and Gambi [9].

Extraction Studies

Extraction of *Hybanthus* leaves with ammonium oxalate solution removed pectins and nickel. Paper chromatography of the extract showed the presence of a nickel containing substance with R_f value similar to that of nickel pectinate but different from that of ionic nickel. Two dimensional paper chromatography of simple aqueous extract of the leaves showed the presence of amino acids and again possibly nickel pectinate; Nickel spots corresponded with those from aspartic and cysteine. The identification of nickel amino acid complexes by chromatographic methods however, is complicated by the fact that dissociation occurs in many of the usual solvents, further work on this point is in progress.

Zinc accumulation by Polycarpia glabra

Extraction studies

Since the *Polycarpia* species contains, by comparison to *Hybanthus*, little metal, the histochemical methods were not suitable for the study of this plant. The samples collected were rich in flowers and stems and initial extraction techniques were carried out on these aerial parts.

The concentration of zinc, iron, lead and copper in the flowers and stems of *polycarpia* are shown in Table 7. A little more than 30 % of the zinc present in the flowers is soluble in 80 % aqueous ethanol, whereas 10 % of the zinc in the stem is soluble in this medium. 60 % of the zinc present in the stem is extracted in the acid fraction which is considered to contain proteins and pectins.

Chromatographic study of the purified ethanolic extracts showed that each contained a zinc complex which was not ionic zinc but which was different in the two extracts. A mixture of the two extracts showed two distinct spots. Thus we have concluded that two zinc complexes exist and from their chromatographic behaviour [18] it is deduced that the one in the stems is of low mobility and relatively low stability, while that in the flowers is of

higher mobility and stability in the butanol acetic acid water solvent. Further investigation of the stem compound indicates that it is possibly a zinc protein complex.

Most of the remaining zinc in the stems comes down in the pectin containing fractions. Further work is continuing on the zinc containing compounds.

Metal pectates

Pectates of some metals were prepared according to literature methods [19]. The visible spectra (diffuse reflectance, dry material) of the nickel and copper compounds showed bands at 25,000, 15,200, (13,800 sh), 8,500 cm^{-1} and 12,500 (9,400 sh) cm^{-1} respectively, indicating octahedral coordination.

DISCUSSION

The problem of the accumulation of metals by plants can be divided into three sections: (a) the intake of metal through the root, (the availability of metal in the immediate vicinity of the root wall will also be involved); (b) the transport of the metal through the plant tissue and (c) the deposition of the metal in the aerial part of the plant.

Up to the present, two main methods have been used to attack the problem. One area has been the attempted elucidation of the nature of chelating agents, first proposed by Jowett [20] in 1958. Work in this connection has concentrated on plant exudates [2—4]. The main area of investigation has been the localisation of the immobilised metals and suggestions have been made that the metals become structural components of the cell wall [5—9]. This process must also involve ligation of some kind. The present state of knowledge, however, does not allow us to say if a particular metal is accumulated by various plants by a similar mechanism or if plants of related species accumulate metals by similar mechanisms.

Turner and Gregory [6] came to the conclusion that the root cell wall was very important in the accumulation of zinc by tolerant clones of the grass *Agrostis tennuis*. Further studies have tended to confirm this conclusion. Suggestions have also been made that the cell wall functions as a cationic exchange site.

The work of Peterson [8] using ^{65}Zn and *Agrostis tennuis* showed that zinc is extracted with the pectate fraction of the root residue which suggested that zinc accumulation is associated with the carbohydrate component of the cell wall.

Metal components of pectates are well known [19], however their stability constants lie in a narrow range [19(b)] and appear to bear out the objections to a simple carbohydrate mechanism of accumulation [21]. The main objection put forward was that carbohydrate binding sites would not be specific for any particular metal, and proteins were suggested as being more likely specific binding sites. However, if specificity is at the primary

entry of the metal into the root, then there is no objection to a carbohydrate mechanism of accumulation, since cell wall sites will simply store that metal ion introduced at root level.

There is however, substantial evidence that proteins are involved in the accumulation of copper [5], and in this respect there appears to be a signigicant difference in the mechanisms of the accumulation of zinc and copper. Our work indicates that pectates are involved in the storage of zinc by *Polycarpia* and that protein species may be involved in transport of the metal.

Similiarly, it appears that in *Hybanthus floribundus* the pectate containing parts of the cell walls are involved in nickel storage. It has been suggested recently [22] that nickel either "stimulates the uptake of macronutrients" by nickel accumulating accumulating plants, or that it inhibits growth giving "a consequent increase of macronutrient values owing to the longer time needed for the plant organ to attain maturity". At present there is no evidence for either mechanism nor do the authors indicate what the mechanisms mean in a chemical sense.

There does, however, appear to be a real correlation between nickel and calcium content. Although nickel appears to be replacing calcium in the binding of the carbohydrates of the cell wall the total calcium content of the plant is not reduced as it might at first appear. Calcium appears to be deposited in the plant tissue as calcium oxalate crystals. The appearance of these crystals in *Hybanthus floribundus* and other accumulators is not satisfactorally explained.

ACKNOWLEDGEMENTS

A.J.C. and M.J.P. thank the S.R.C. for postgraduate studentships. We thank I.S.C. Ltd., Avonmouth for financial assistance, and Professor M.M. Cole for the collection of samples and for making available data prior to publication.

REFENCES

1 J. Antonovics, A.D. Bradshaw and R.G. Rurner, Advances in Ecol. Res. 1, 7, (1971) (and references therein).
2 O.L. Tiffin, Plant Physiol., 41 (1966) 510.; 45 (1970) 280.
3 O.L. Tiffin, Plant Physiol., 48 (1971) 273.
4 O.L. Tiffin, Plant Physiol., 42 (1967).
5 C. Reilly, New Phytol., 68 (1969) 1081; 69 (1970) 993; Zeit. Pflan. Physiol., 66 (1972) 294; A. Reilly and C. Reilly, Plant Soil, 38 (1973) 671.
6 R.G. Turner and R.P.G. Gregory, Uses of Isotopes in Plant Nutrition and Physiological Studies, IAEA, Vienna, 1967.
7 R.G. Turner, New. Phytol., 69 (1970) 725.
8 P.J. Peterson, J. Exp. Bot., 20 (1969) 863.
9 O.V. Gambi, G. Bot. Ital., 101 (1967) 59.
10 I. Ernst, Kirkia, 8, (2), 125, (1972).
11 W. Ernst and H. Weinart, Zeit. Pflan. Physiol., 66 (1971) 253.

12 H.W. Woolhouse, Ecological Aspects of the Mineral Nutrition of Plants, Brit. Ecol. Symp., 9 (1969) 357.
13 (a) M.M. Cole, J. App. Ecol., 10 (1973) 269 (b) B.C. Severne and P.R. Brooks, Planta, 91 (1972) 103.
14 O.W. Nicolls, D.M. Provan, M.M. Cole and J.S. Tooms, Trans Inst. Mining and Met, 74(II), 695, (1965).
15 C. Minguzzi and O. Vergnano, Nuovo G. Bot. Ital., 60 (1953) 287; O. Vergnano, G. Bot. Ital, 101 (1967) 59.
16 R.P.G. Gregory and A.D. Bradshaw, New Phytol., 64 (1964) 130.
17 (a) W. Ernst, Ber. Deut. Bot. Ges., 78 (1965) 205, (b) W. Baumaster, Angew Bot., 40 (1967) 185.
18 To be described in detail elsewhere.
19 (a) U.S. Patent, 2418865-6 BA(P) B11 361 (1950). (b) G. Wunsch, Makromol. Chem., 8 (1952) 124.
20 D. Jowett, Nature (London) 182, (1958) 816.
21 H.W. Woolhouse, Proc. Phytochem. Soc. Symp., Academic Press, (1969).
22 P.R. Brooks, J. Lee and T. Jaffré, J. Ecol. 62 (1974) 493.