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Plants Which Accumulate Metals. Part III. A Further Investigation of Two Australian Species Which take up Zinc

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The zinc which remains insoluble in the plants *Crotalaria novae hollandiae* and *Polycarpaea glabra* after treatment with aqueous ethanol and water, has been shown to be associated with carbohydrates. It is concluded that zinc ions are stored in carbohydrate ion exchange sites in the cell walls and that these are not specific for zinc. Part of the zinc may be removed by Ca^{2+} ions and the plant residues from which zinc has been removed in this way show greater affinity for Cu^{2+} than for Zn^{2+} ions.

Introduction

We have shown [1] that zinc in *Polycarpaea glabra* and in *Crotalaria novae hollandiae* is largely water soluble. Other plants also have high proportions of soluble metal: *Hybanthus floribundus* [1, 2], *Astragalus stoloniferi* [3], and *Becium homblei* [4], are examples of this type. Other work, however has found more insoluble metals [5].

In both zinc accumulating plants examined in this work, a proportion of the zinc remains insoluble in water [1], and this paper reports on the nature of this insoluble zinc fraction. The specificity of the zinc sites is also of interest, and experiments were carried out to determine their exchange behaviour.

Experimental and Results

Extraction Studies

A known weight of dried milled plant material was extracted with 150 cm^3 each of aqueous ethanol and water. The residue from these extractions was treated with pronase by the method of Peterson [6]. Since pronase contains zinc, only 300 mg was used for each 1.5 g of plant material, and it was also added to the blank determinations. The residue was treated with pronase, 150 cm^3 buffer (pH 7.5) and 3 mg chloramphenicol. The procedure was repeated and the two extracts were pooled. The residue was divided into two equal parts and treated as shown (Scheme 1). The extracts were centrifuged, evaporated to dryness, wet ashed, and analysed for zinc by atomic absorption spectrophotometry.

Exchange with Ca^{2+} Ions followed by Extractions

Approximately 1 g dried, milled plant material was extracted with aqueous ethanol and water as before. To the residue was added 200 cm^3 of a solution containing 0.00027 mol dm^{-1} (aqueous) of Ca^{2+} ions. The mixture was shaken for 24 hours and the extraction was repeated. The residue was then extracted as in Scheme 1, and the extracts treated as before.

Scheme 1

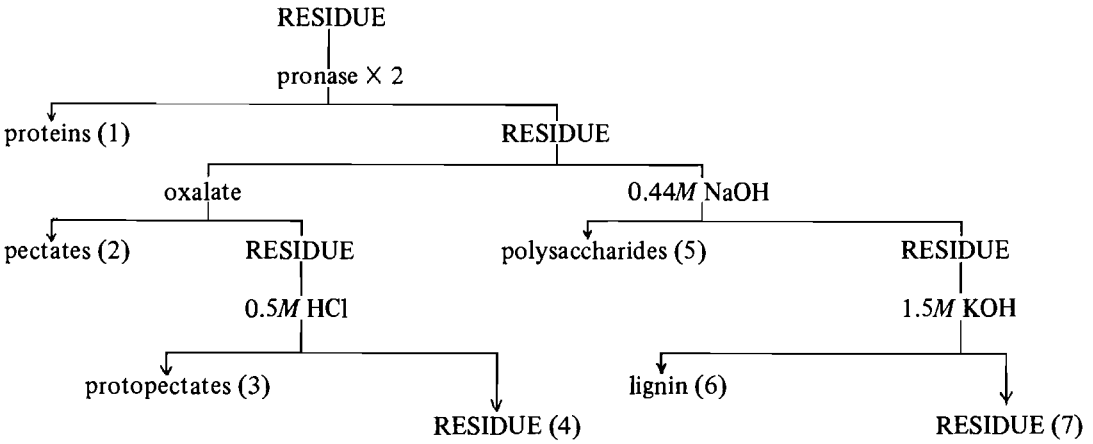

TABLE I. Concentration of Zinc (p.p.m. dry weight) in Various Fractions from Plant Residues[a] as in Scheme 1.

Sample	Fraction	1 Proteins	2 Pectates	3 Protopectates	4 Residue	5 Polysaccharides	6 Lignin	7 α cellulose
Polycarpaea glabra (stem)								
A high zinc area[b]		100	356	55	–	334	100	–
B high zinc area[b]		100	251	52	–	289		5
C low zinc area[c]		80	80	40	–	110	–	–
B with Ca^{2+}[b]		105	201	58	2	260	2	5
Crotalaria novae hollandiae								
D leaf		583	1884	279	86	1574	427	101
D with Ca^{2+}		911	913	148	83	620	422	34
E stem		384	1448	119	53	1298	54	54
E + Ca^{2+}		677	666	353	36	487	427	70

[a]After treatment with aqueous ethanol and water. [b]Dugold River Lode, Queensland, Australia. [c]Mount Poly, Queensland, Australia.

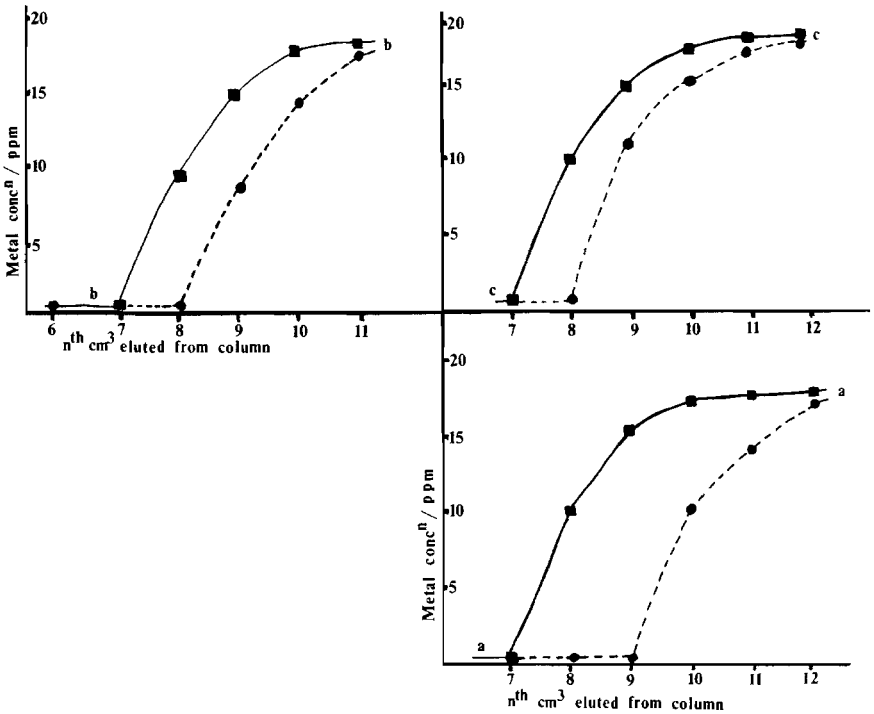

Figure. Ion exchange experiments with plant residues from which zinc has been removed by treatment with Ca^{2+}. ■, Zn; ●, Cu; in ppm in the n^{th} fraction from column. (a) *Crotalaria novae hollandiae*; (b) *Polycarpaea glabra* from high zinc area; (c) *Polycarpaea glabra* from low zinc area.

The results of the extraction studies are shown in Table I.

Column Exchange Experiments

About 5 g of *Polycarpaea glabra* stem material from both high and low zinc areas (see Table I) and of *Crotalaria novae hollandiae* were extracted with aqueous ethanol and water as before. The three residues were shaken separately with a solution of Ca^{2+} ions (50 ppm) for 8 hours per day for 4 days, the solution being changed each day. The residues were then packed in chromatography columns. The calcium solution was run through each column and the eluant was spotted on to dry dithiozone paper. No red colour was obtained, thus no Ca^{2+} exchan-

TABLE II. Concentration of Zinc in Cell Wall Material of *Polycarpaea glabra* after Separation by Ultracentrifugation and Treatment with ^{65}Zn.

	P. glabra from High Zinc Area		*P. glabra* from Low Zinc Area	
Mass of cell wall/g	0.3138	0.2482	0.2092	0.2233
Readings/cps	2793	2132	1820	2006
$Zn/10^8$ g	4.198	3.010	2.570	2.832
Conc. Zn 10^{-8} g Zn/g cell wall	13.37	12.13	12.28	12.68

geable Zn^{2+} ions were present. An equimolar (0.00027 mol dm^{-3}) solution of Cu^{2+} and Ca^{2+} ions was passed through each column and 1 cm^3 samples were collected. The samples were analysed for copper and zinc by atomic absorption spectrophotometric methods. The results are shown in the Figure.

Investigation of Zinc Absorption Capacity of Cell Walls from Two Samples of Polycarpaea Glabra

It has been claimed [7] that for some species heavy metal toxicity is resisted by an increased specific metal absorption into cell walls. This postulation was tested for *P. glabra.* Seeds from plants from both the high and low zinc areas were germinated and grown in John Innes Potting Compost No. 1, and fed with Epstein one quarter strength nutrient solution [8]. After eleven weeks the aerial parts of the plants were harvested and homogenised. The cell debris were removed by filtration through cheesecloth and the remaining slurry was centrifuged down. The supernatant was drained off and the dry residue was devided into two parts and weighed into 10 cm^3 tubes. One cm^3 of Zn^{65} solution (80 μC) was added to each and the tubes were shaken for 2 hours. The mixtures were centrifuged, the residues were carefully washed and digested with 1 cm^3 concentrated nitric acid. The solutions were read using a scintillation counter. The results are shown in Table II.

Discussion

The results in Table I show that most of the zinc in the residue of the highly mineralised *Polycarpaea glabra* is associated with carbohydrates. Although the amount of zinc associated with protein in the soluble fractions was found to be low [1(b)] that associated with protein in the insoluble fraction is much higher. In contrast the *Polycarpaea glabra* plants from low zinc areas did not show an accumulation of zinc in association with carbohydrates, but had a much more even distribution.

Zinc is also associated with carbohydrates in the *Crotalaria novae hollandiae.* Table I shows that the residue, on treatment with Ca^{2+} ions after extraction with aqueous ethanol and water, released further quantities of zinc. This release seems to come almost entirely from the pectate and polysaccharide fractions. Some of it is taken up by the protein and amino acid fractions.

The results in Table I, thus lead to clear conclusions; first there appears to be a physiological difference between samples of *Polycarpaea glabra* plants from high and low zinc areas. Secondly, for plants from high zinc areas the metal is concentrated largely in association with the pectate fraction. Further histochemical work [9] on *Polycarpaea glabra* plants from high zinc areas has shown that zinc is accumulated in the pectate layer of leaf cell walls, a conclusion similar to that reached for *Agrostis Tenuis* [10] by a variety of workers.

Crotalaria novae hollandiae appears to resist the toxicity of zinc by a different mechanism. Zinc has been found mainly as the water soluble aquozinc(II) ion [1] particularly in the leaves. Superficially this is similar to the mechanism found by Ernst [11] where zinc is concentrated in the cell sap, restricted from transport and metabolic sites by the tonoplast. However, in the *Crotalaria species* zinc accumulates at the cell walls and at the phloem. In the copper containing *Becium homblei* the metal has been found in association with amino acids [12]. Copper is similarly associated with amino acids in *Armeria maritima* [13]. Thus there is evidence that the mechanisms of accumulation of copper and of zinc and nickel [14] are different. For both *Crotalaria* and *Hybanthus floribundus* [14] (a nickel accumulator) metal has been detected in the phloem in addition to the cell wall. It seems unlikely that zinc is travelling in the phloem in high concentrations. But it is more likely that the phloem is the repository for a significant fraction of the total zinc present. It seems likely that the phloem membrane restricts entry to zinc ions as it does to calcium ions. Thus large concentrations of the metal ions become immobile at the membrane; this mechanism perhaps becomes operational only when very large amounts of metal are accumulated, as in *Crotalaria novae hollandiae* and *Hybanthus floribundus.*

The Figure shows that the plant residues have greater affinity for Cu^{2+} ions than they do for Zn^{2+} ions. The separation of the two ions was very good, and marginally better in the case of *Crotalaria novae hollandiae*. Little difference was observed between the *Polycarpaea species* from high and low zinc areas. Similarly the results in Table II do not show any large differences in zinc absorptive capacity in progeny of plants from the two areas.

Thus we conclude the following: (i) zinc is accumulated in the two plants examined here in association with carbohydrates; (ii) the carbohydrates involved are in the cell walls; (iii) zinc ions are stored in ion exchange sites, which are not specific for zinc; (iv) when very high amounts of metal are accumulated, the phloem may be involved.

It therefor appears that the accumulation of zinc and probably of nickel [14] is an active process, and that specificity does not reside in the carbohydrate storage sites. Attention should now be turned to specific membrane carrier mechanisms.

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