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Plants which Accumulate Metals. Part I. The Metal Content of Three Australian Plants Growing over Mineralised Sites

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Analysis of the nickel accumulating plant Hybanthus floribundus *from serpentenite regions shows the plant to be deficient in calcium. Two zinc accumulating species:* Polycarpaea glabra *and* Crotalaria novae-hollandiae *have also been analysed. The first takes up significant amounts of zinc, lead and iron into aerial parts, while the second accumulates up very high concentrations of zinc and some iron. Nickel and zinc are largely water soluble, and most of the remainder of the metal can be displaced from the plant tissues by dilute acid.*

Introduction

Some plant species and ecotypes have been found to grow and thrive on mineralised sites. Thus they are tolerant to soils which contain high concentrations of metal ions, normallly toxic to plant growth. Some of these plants take up metals into their aerial parts, and are known as metal accumulators. This phenomenon raises questions as to how the metals are bonded within the tissues so that the metabolic disruption normally associated with high metal concentrations is avoided. Further, the mechanism of specificity whereby tolerance to one metal does not confer tolerance to another, has not yet been elucidated. The area of metal tolerance was reviewed extensively by Bradshaw and co-workers in 1971 [1].

The aim of the present work is to elucidate the tolerance mechanism by seeking to establish the chemical bonding of the heavy metals both in storage and in translocation in the plant. This paper is concerned with the concentrations of certain metals and their solubilities in three Australian species. The species, which grow over mineralised soils are: *Hybanthus floribundus, Polycarpaea glabra* and *Crolataria novae-hollandiae.*

Stet Background

Hybanthus floribundus was first found in the Eastern Goldfields area of Western Australia [2, 3]. Elkington reported that the species grew only over serpentenite outcrops. It was subsequently reported over a wider area of Western Australia growing over nickel toxic soils South of Coolgardie, in Widgiemooltha and in several other areas [3] including Queensland [4]. Cole [3] has established the relationships between plant distribution, bedrock geology, and environmental factors in the Eastern Goldfield area, in particular for *Hybanthus floribundus.* Brooks and co-workers have recently reported data on *Hybanthus species,* particularly from New Caledonia, [4–6]. The plant is thus well established as a nickel accumulator.

Polycarpaea glabra was found growing over the Dugald River lode, and in the Little Eva Mine and Turkey Greek areas, all in Western Queensland [7]. The Dugald River lode is a lead–zinc ore body, whereas there is copper mineralisation at Little Eva Mine and Turkey Creek. *Polycarpaea glabra* was found [7] to take up little copper: plants growing up to 10,000 ppm of copper contained less than 20 ppm in the leaves. It can therefore be considered as an indicator rather than an accumulator of copper. However, it does not restrict the uptake of zinc, which does not appear to be toxic.

The Dugald River lode area was subsequently studied again [8, 9] both by plant and soil sampling and by aircraft and satellite imagery. This second investigation revealed *Crotalaria novae-hollandiae* as a zinc accumulator.

Experimental

Spectrophotometric Analysis

Samples of dried plant material (1g) were ground using a Glen Creston agate mill. The ground samples were finally dried to constant weight. Duplicate samples were dissolved by heating in either nitric acid or nitric–perchloric mixtures* until the mixtures were clear and colourless. Each sample was then made up to a known volume with doubly distilled water for analysis by atomic absorption spectrophotometry. A

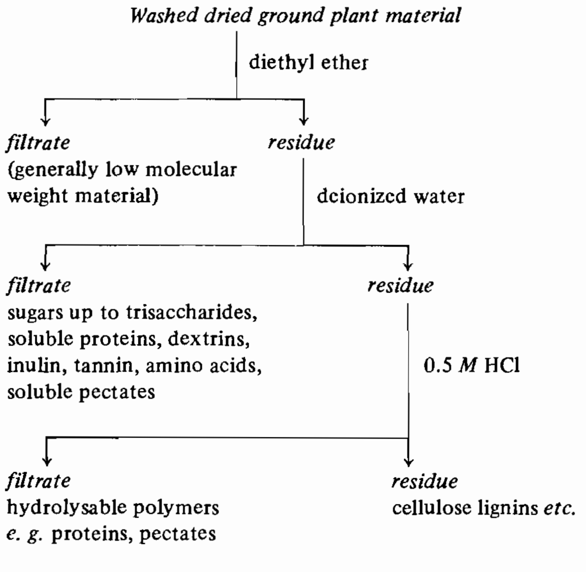
*Initially nitric–perchloric acid mixtures were used, but change was made to nitric acid alone on safety grounds.

Shandon Southern A30 spectrophotometer fitted with an external recorder was used for measurements. Since no ash weights were recorded all results in these studies were given on a dry weight basis.

Extraction Scheme

This scheme shown below was a modification of that devised by Bowen *et al.* [10]. It is not considered as a rigid analysis, but rather an indication of the chemical character of the compounds with which the metal is associated. Duplicate samples of dried and ground plant material were weighed and placed in a soxhlet thimble. A third apparatus was always set up as a blank. The samples and blanks were left syphoning for 24 hours using 150 cm^3 of solvent. The 250 cm^3 round bottomed flask was then replaced with an identical flask containing 150 cm^3 of the next solvent. After the aqueous extraction the thimble was dried at 90 °C to constant weight. A plug of plant material was carefully removed from the thimble and placed in a weighed 250 cm^3 flask. The weight of the transferred material could thus be calculated. 150 cm^3 of 0.5 *M* hydrochloric acid was added to the plant material and the mixture was shaken for 24 hours. The mixture was allowed to settle and the clear solution was drawn off. Each of the solutions after extraction was evaporated to dryness using a rotary evaporator. The residues and the final residue were treated as before for analyses.

EXTRACTION SCHEME



Polycarpaea glabra was grown from seed collected at the Dugald River lode, in John Innes Potting Compost No. 1 and watered with 20 ppm zinc solution. After 12 weeks the aerial parts were bulked and analysed.

Results and Discussion

Some analytical results for *Hybanthus floribundus* have already been published [3,11]. Table I gives some figures for metal content of leaf material, and Table II gives some results for various aerial parts. Table III gives the percentages of total nickel extracted sequentially for various aerial parts. Table IV gives some analysis results for *Polycarpaea glabra* and *Crotalaria novae-hollandiae* from the Dugald River lode. Tables V and VI show the percentages of total metal extracted sequentially in the last two species.

TABLE I. Average Metal Content (in ppm) of Leaf Samples of *Hybanthus floribundus* from Western Australia (dry weight basis).

Sample No.	Area[a]	Ni	Ca	Mg	Ca/Mg
1	Dordie West	5210	4380	4090	1.07
2[b]	Coolgardie	7900	7600	3270	2.32
3[c]	Coolgardie	4500	6900	3350	2.05
4[d]	Mount Thirsty	6000	4600	4240	1.08
5[e]	Kurnalpi	2000	3800	5170	0.73
6[f]	Kurnalpi	3000	4700	5010	0.94
7[g]	Kurrajong	6800	5150	2600	1.98
8[h]	Widgiemooltha	8000	6100	4980	1.22
9	Widgiemooltha	7180	6080	5400	1.12
10	Widgiemooltha	13440	6730	6090	1.10
11	Widgiemooltha	9950	5860	4540	1.29
12	Widgiemooltha	9370	8000	6500	1.23
13	Widgiemooltha	7630	5600	4700	1.19

[a]For further details of areas see reference 3. Sample numbers given in reference 11; [b]7509; [c]7514; [d]7465; [e]7518; [f]7517; [g]7494; [h]7461.

TABLE II. Average Metal Content of Aerial Parts of *Hybanthus floribundus*[a].

	Leaf	Green Leaf Stem	Green Stem	Small Twig	Old Twig
Ni	8000	5710	5080	3390	3080
Mg	4980	5050	2010	860	400
K	5000	5260	2830	1850	2860

[a]Sample No. 8 from Table I.

The species discussed in this paper come from two widely differing Australian habitats. *Hybanthus floribundus* was collected from the Eastern Goldfield of Western Australia. Elkington [2a] reported that *Hybanthus floribundus* grew over serpentenite outcrops where the major elemental concentrations were shown to be: Ni, 1230 ppm; Na, 560 ppm; K, 1430 ppm; Ca, 2000 ppm; Mg, 9000 ppm. In contrast

TABLE III. Average Percentages of Nickel Extracted Sequentially from *Hybanthus floribundus*[a].

	Ether	Ethanol	Water	Dilute Acid	Residue
Leaf	~0.1	12	48	43	–
Green Leaf Stem	~0.1	5	54	41	–
Green Stem	~0.2	4	63	33	–
Small Twig	~0.1	1	11	87	–
Old Twig	~0.1	1	18	81	–

[a]Results from reference 11.

TABLE IV. Average Metal Content of Plants from Dugald River lode[a].

Species	Cu	Fe	Pb	Zn	Ca
Polycarpaea glabra[b]	8	100	31	600	19,125
Polycarpaea glabra					
Green Stem	d	65	30	485	c
Brown Stem	d	215	306	810	c
Flowers		180	40	215	c
Crotalaria novae-hollandiae					
Stem	8	787	d	4745	c
Leaf	6	760	d	8975	c

[a]For further details of area see references 7 and 9. [b]Grown from seed collected at Dugald River lode. [c]Not measured. [d]Very low content.

TABLE V. Average Percentages of Total Metal Extracted Sequentially from *Polycarpaea glabra*.

	Ether	Ethanol	Water	Dilute Acid	Residue
Zinc					
Green Stem	5	10	25	58	2
Total Stem	3	10	47	34	5
Flower Heads	5	36	19	36	4
Lead					
Green Stem	–	–	–	28	72
Total Stem	–	19	13	39	27
Flower Heads	–	–	–	100	–
Iron					
Green Stem	26	14	7	14	39
Total Stem	–	4	2	25	68
Flower Heads	–	–	–	61	39

TABLE VI. Average Percentages of Total Metal Extracted Sequentially from *Crotalaria novae-hollandiae*.

	Ether	Ethanol	Water	Dilute Acid	Residue
Zinc					
Stem	–	–	60	29	10
Leaf	1	3	70	26	1
Iron	6	6	11	46	31
Leaf	8	6	10	50	26

metabasalt from the same area had: Ni, 120 ppm; Na, 560 ppm; K, 1430 ppm; Ca, 16000 ppm; Mg, 12600 ppm. The calcium content of *Hybanthus floribundus* appears low, but above the critical value where deficiency symptoms occur. The plants appear to be able to tolerate low calcium without significant accumulation of magnesium which is more common in the soil [2a]. Table I shows that nickel does not replace calcium in this plant since the plant calcium concentrations rise with nickel concentrations.

The effect of calcium upon the uptake of toxic metals has been the topic of discussion for many years. The lack of fertility of serpentenite soils has been considered to be primarily a lack of calcium [12]. Addition of calcium to serpenite soils has been shown to decrease the acidity [13, 14] which in turn decreases the absorption of nickel and cobalt [14]. Conversely the addition of ammonium nitrate reduced the pH of such soils and increased the availability of toxic metals [15]. There may be some relationship between nickel and calcium; Wiltshire [15] has shown that the addition of calcium to serpentenite soils did not decrease the total amount of nickel taken up, but the plants were larger and thus contained lower *concentrations* of nickel. Similarly Simon [16] showed that grasses from Rhodesian serpentenite soils show ability to take up calcium from nickel/chromium toxic soils, yet yielded less than grasses from control soils.

It is interesting to speculate on the reasons for stunted growth of plants grown in nickel rich soils particularly commented upon by Brooks *et al.* [6], Wiltshire [15] and Simon [16]. Albersheim [17] has suggested that a calcium-ion dependent ATPase might be activated by auxin. In this way auxin might activate a hydrogen-ion pump in the cell wall, reducing the pH giving elongation growth. If the ATPase is inhibited by nickel ions then the poor yields might be accounted for.

Other elements should also be taken into account, for example the calcium/magnesium ratio from Hybanthus floribundus shown in Tables I and II are near unity. However other *Hybanthus* specimens show a high Ca/Mg ratio [4] and from the results of Kelly *et al.* [4] larger leaves may be correlated with a high Ca/Mg ratio.

More than half of the nickel in the green parts of the plant is water soluble, the rest being released by acid. In the twig material, considerably less nickel was water soluble, more than 80% of the nickel appearing in the acid fraction.

The other two species are from the Dugald River area and are associated with reddish brown stony clays [7–9]. It can be seen from Table IV that both plants take zinc into their tissues with the *Crotalaria species* taking near to 9000 ppm zinc. The leaves of *Polycarpaea glabra* are very small spines, and this study did not examine their behaviour.

It can be seen from Table V that for the stem material of *polycarpaea,* 47% of the total zinc is water soluble, but less in the case of green stems and flowerheads, however in all cases the remainder of the zinc can almost completely be displaced by acid. The behaviour contrasts with those of iron and lead, where little of the metal is water soluble and a large amound remains in the residue. For *Crotalaria novae-hollandiae,* most of the zinc (60% in the stem material and 70% in the leaf material) is water soluble; again the behaviour of iron is contrasted, where only 10% is water soluble and a substantial amount remains in the solid residue.

Thus it appears in the three plants studied that both zinc and nickel are largely soluble in water and in dilute acid, whereas iron and lead are much more likely to be associated with cellulose or lignin and thus be insoluble in aqueous media.

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