Proc. R. Soc. Lond. B **205**, 385–394 (1979)
Printed in Great Britain

Nickel uptake by Flacourtiaceae of New Caledonia

BY T. JAFFRÉ†, W. KERSTEN‡, R. R. BROOKS‡ AND R. D. REEVES‡
†*Centre ORSTOM, Noumeá, New Caledonia*
‡*Department of Chemistry, Biochemistry and Biophysics, Massey University, Palmerston North, New Zealand*

(*Communicated by R. E. F. Matthews, F.R.S. – Received* 4 *October* 1978)

Herbarium and field specimens (over 300) of all of the Flacourtiaceae of New Caledonia were analysed for nickel in order to identify hyperaccumulators (> 1000 μg/g dry mass) and to assess nickel accumulation in relation to the evolutionary status of 'nickel plants' of New Caledonia. One hyperaccumulator was identified in the genus *Lasiochlamys*, ten among *Xylosma*, one among *Casearia* and seven among *Homalium*. Although these *Homalium* nickel plants had previously been recorded, fresh data for these and other *Homalium* are presented. The remarkable tolerance of Flacourtiaceae to ultrabasic rocks is shown by the fact that 75 % of the species are found on such substrates. The number of hyperaccumulators was greatest in the genera *Xylosma* and *Homalium*. The Flacourtiaceae are among the most primitive of all angiosperms and in common with other primitive hyperaccumulators, contain nickel as a complex with citric acid. The only advanced New Caledonian nickel plant (*Psychotria douarrei*) has most of its nickel bound with ligands other than citric acid, a feature of other advanced hyperaccumulators. It is postulated that nickel complexing with citric acid may be a primitive character. Most of the New Caledonian nickel plants belong to the order Violales of subclass Dilleniidae. It is suggested that hyperaccumulation of nickel is an evolutionary character which occurs in long-indisturbed floras such as that of New Caledonia.

1. Introduction

Although the nickel content of plants not growing over ultrabasic or other nickeliferous rocks is usually less than 1 μg/g (dry mass), significantly higher levels (10–100 μg/g) are found in serpentine floras. Occasionally plants are found with nickel concentrations in the range 1000–50000 μg/g (0.1–5.0 %). Such plants have been termed *hyperaccumulators* of nickel (Brooks *et al.* 1977). This classification is concerned only with the nickel concentration in the plant and does not substitute for more specific (though potentially confusing) terms indicating relations between plants and their substrates (see, for example, Duvigneaud & Denaeyer-De-Smet 1963). It also does not imply that all individuals of a given species will necessarily contain > 1000 μg/g nickel.

Since the discovery of the first 'nickel plant' (*Alyssum bertolonii* Desv.) in

Italy by Minguzzi & Vergnano (1948), another 70 such species (including a further 44 species of *Alyssum*) have been reported. The largest number of hyperaccumulator species, all in the genus *Alyssum*, occurs in the Eastern Mediterranean (Brooks & Radford 1978, Brooks *et al.* 1979). Five nickel plants have been discovered in Southeast Asia (Brooks & Wither 1977; Wither & Brooks 1977) and

TABLE 1. LIST OF COOPERATING HERBARIA

country	institution	abbreviation
France	Muséum National d'Histoire Naturelle, Paris	P
Netherlands	Rijksherbarium, Leiden	L
New Caledonia	Centre ORSTOM, Nouméa	NOU
Switzerland	Institut für Systematische Botanik, Zürich	Z
United Kingdom	Royal Botanic Garden, Edinburgh	E
	Royal Botanic Gardens, Kew	K

one in Western Australia (Severne & Brooks 1972; Cole 1973). Two hyperaccumulators have been reported from Africa (Wild 1970, 1974), but no species from The Americas has yet been found. The remaining 18 hyperaccumulators are confined to New Caledonia and include seven species of *Homalium* (Jaffé & Schmid 1974, Brooks *et al.* 1974, 1977), two *Hybanthus* (Jaffré & Schmid 1974), seven *Geissois* (Jaffré & Schmid 1974; Jaffré *et al.* 1979), one *Psychotria* (Jaffré & Schmid 1974), and one *Sebertia* (Jaffré *et al.* 1976).

In terms of density of occurrence and generic diversity, the hyperaccumulators of New Caledonia are unique. The development of so many species and genera of nickel-tolerant plants in New Caledonia probably results from two main factors: the relatively large area of ultrabasic rocks upon the island (i.e. one third of its 16750 km^2) and the isolation of New Caledonia from continental masses and a subsequent development of a flora with 80% endemism at the species level.

Sleumer (1974) has recognized 53 species and 4 genera among the Flacourtiaceae of New Caledonia and the Loyalty Islands. They include *Lasiochlamys* (13 species), *Xylosma* (18 species), *Casearia* (6 species) and *Homalium* (16 species). Because investigations among most of the New Caledonia *Homalium* species have already revealed the existence of seven hyperaccumulators (Jaffré & Schmid 1974; Brooks *et al.* 1974, 1977), it would obviously be of interest to examine the whole of the *Homalium* genus in the island as well as other genera of Flacourtiaceae within New Caledonia, in the expectation of discovering further nickel plants in a family well represented in New Caledonia. The data would be of additional significance for attempting to explain more fully, the reasons for the concentration of so many hyperaccumulators upon this island. The results of such a survey are presented in this paper.

2. Material and methods

(a) *Plants sampled*

Plant material was supplied from the herbaria listed in table 1. Over 300 specimens of all the 53 species listed by Sleumer (1974) were analysed for nickel. These species are listed in table 2 together with the number analysed.

(b) *Analytical procedures*

Dried leaf samples with an average mass of about 0.03 g (about 1 cm^2) were placed in 5 cm^3 borosilicate test-tubes and ignited at 500 °C in a muffle furnace. The ash in each tube was then dissolved in 1 cm^3 of 2 M HCl. The solutions were analysed for nickel by atomic absorption spectrophotometry. Automatic corrections for non-atomic absorption were made using a hydrogen continuum lamp. All concentration data were expressed on a dry mass basis.

Although analytical data were presented to three significant figures, there was an approximately 20% intraleaf, and 30% interleaf variability in the nickel content of a given herbarium specimen. Although this variation was appreciable, it was far less than the interspecific variations of the nickel contents of the plants and can therefore be considered as relatively unimportant.

Since both nickel and chromium are present in comparable concentrations in New Caledonian soils (Jaffré *et al.* 1971), and since chromium uptake by plants is usually very slight, the chromium content of samples high in nickel was used as an index of possible contamination because dried herbarium material is impossible to wash. Specimens with chromium contents exceeding 10 μg/g were therefore assumed to be contaminated and were rejected. On occasions, plants may accumulate significant amounts of chromium. There was therefore the possibility that some of the rejected specimens were ignored unwarrantedly. However since only three or four specimens were in fact rejected, this possibility does not affect the data to any significant degree.

3. Results and discussion

Nickel concentrations exceeding 1000 μg/g (dry mass) were found in one species of *Lasiochlamys*, ten of *Xylosma*, one of *Casearia* and seven of *Homalium*. Concentration data are shown in table 2. This table also includes information on the type of substrate and on the collection localities. Collection localities are coded in table 2 and are shown in full in figure 1. As far as possible 'type' material (including types, isotypes, holotypes and lectotypes) was used. In all cases, the plants represented type material and authenticated specimens originally studied by Sleumer (1974) in his revision of the family (numbers indicated in table 2). The use of such material ensured a minimum of misidentifications (a common problem with herbarium specimens). The relatively large number of specimens of *Homalium*

TABLE 2. NICKEL CONCENTRATIONS (µg/g DRY MASS) IN THE FLACOURTIACEAE OF NEW CALEDONIA

species	number	mean Ni	range	substrate	collection locations (see figure 1)	authenticated material analysed
		Genus *Lasiochlamys*				
(1) *L. koghiensis* (Guillaumin) Sleumer	5	388	284–563	U	38, 39, 44	S (5)
(2) *L. peltata* Sleumer†	1	1000	3–7	U	29	T
(3) *L. coriacea* Sleumer	2	5	3–7	NU	4, 12	S (2)
(4) *L. pseudocoriacea* Sleumer	1	111	—	U	17, 46	T
(5) *L. rivularis* Sleumer	3	97	71–138	U	27	T
(6) *L. trichostemona* (Guillaumin) Sleumer	2	21	3–39	NU	39	T
(7) *L. fasciculata* (Guillaumin) Sleumer	1	1	—	NU	4	S (1)
(8) *L. cordifolia* Sleumer	1	17	—	NU	14*a*	T
(9) *L. hürlimannii* (Guillaumin) Sleumer	1	320	—	U	40	T
(10) *L. grossecrenata* Sleumer	1	162	—	NU	49	T
(11) *L. reticulata* (Schlechter) Pax & Hoffman	2	1	—	NU	6, 16	S (1)
(12) *L. mandjeliana* Sleumer	1	3	—	NU	4	S
(13) *L. planchonellaefolia* (Guillaumin) Sleumer	6	96	42–225	U	23, 27, 28, 34, 41	S (5)

Table 2 (*cont.*)

			Genus *Xylosma*			
(1) *X. orbiculatum* (J. R. & G. Forster) G. Forster	2	3	1–4	NU	10, 30	S (1)
(2) *X. pinisulare* Guillaumin†	2	902	538–1280	U	31*a*	S (1)
(3) *X. pancheri* Guillaumin†	14	510	29–1130	M	2, 5, 9, 36, 50, 55	S (9)
(4) *X. dothioense* Guillaumin†	4	686	10–1780	M	18, 19, 45	S (2)
(5) *X. vincentii* Guillaumin†	10	2830	22–3750	U	11, 14, 25, 27, 49	S (5)
(6) *X. capillipes* Guillaumin	1	873	—	U	28	T
(7) *X. nervosum* Guillaumin	9	313	74–465	U	12, 18, 20, 21, 41, 42	S (5)
(8) *X. serpentinum* Sleumer†	7	755	147–1490	U	52, 57, 58, 59	S (6)
(9) *X. lancifolium* Sleumer	4	257	21–545	M	12	S (2)
(10) *X. gigantifolium* Sleumer	1	4	—	NU	59	T
(11) *X. inaequinervium* Sleumer	2	323	314–333	U	15	T
(12) *X. bernardianum* Sleumer	2	2	1–3	NU	4	S (2)
(13) *X. boulindae* Sleumer†	4	1260	722–1930	U	47	T
(14) *X. kaalense* Sleumer†	3	1700	1430–1900	U	54	S (1)
(15) *X. confusum* Guillaumin†	9	637	70–1630	U	25, 27, 43	S (2)
(16) *X. molestum* Sleumer†	3	892	565–1140	U	15	S (1)
(17) *X. tuberaculatum* Sleumer†	5	1010	615–1600	U	15, 47, 48	S (2)
(18) *X. lifuanum* Guillaumin	2	3	2–3	NU	10*a*	S (2)
			Genus *Casearia*			
(1) *C puberula* Guillaumin	3	13	10–19	M	27	S (3)
(2) *C. coriifolia* Lescot & Sleumer	3	75	10–178	U	44	S (1)
(3) *C. kaalensis* Lescot & Sleumer	1	12	—	U	54	S (1)
(4) *C. deplanchei* Sleumer	4	88	1–162	U	57	T
(5) *C. lifuana* Däniker	1	4	—	NU	10	T
(6) *C. melistaurum* Sprengel†	18	252	8–1490	U	17, 22, 26, 27, 37, 41, 47, 51	S (11)

Table 2 (*cont.*)

Genus *Homalium*

(1) *H. serratum* Guillaumin	6	47	10–116	U	20, 55, 58	S (1)
(2) *H. buxifolium* Däniker	2	1	1–1	NU	59	T
(3) *H. kanaliense* (Vieillard) Briquet†	81	4400	2100–9420	U	32, 47	S (2)
(4) *H. rubiginosum* (Vieillard) Warburg	2	636	397–875	U	47	T
(5) *H. austrocaledonicum* Seemann†	6	1100	432–1800	U	19, 20	S (11)
(6) *H. deplanchei* (Vieillard) Warburg†	10	522	10–1850	M	36, 47, 55, 56, 57	S (1)
(7) *H. decurrens* (Vieillard) Briquet	5	84	42–176	M	47	S (1)
(8) *H. rubrocostatum* Sleumer†	2	816	476–1160	U	47, 48	S (1)
(9) *H. guillainii* (Vieillard)† Briquet	27	8100	4500–11700	U	25, 27	S (1)
(10) *H. francii* Guillaumin†	7	7330	1500–14500	U	39	S (1)
(11) *H. intermedium* (Vieillard) Briquet	2	9	1–17	NU	4, 49	S (1)
(12) *H. rivulare* (Vieillard) Briquet	2	28	4–52	NU	14*a*	S (1)
(13) *H. mathieuanum* (Vieillard)†	3	576	16–1690	U	1, 54	T
(14) *H. juxtapositum* Sleumer	2	66	32–99	U	51	T
(15) *H. polystachyum* (Vieillard) Briquet	2	45	1–90	U	8	T
(16) *H. le-ratiorum* Guillaumin	6	172	23–643	U	41, 48, 49	S (1)

† Hyperaccumulator status.

S, Including specimens (number in parentheses) examined by Sleumer (1974); T, authenticated material including types, isotypes, holotypes or lectotypes; U, ultrabasic rocks; NU, non-ultrabasic rocks; M, ultrabasic and other rocks.

kanaliense (81) and *H. guillainii* (27) represented mainly material collected by the authors in the field.

The seven hyperaccumulators in the genus *Homalium* (table 2) have previously been reported by Jaffré & Schmid (1974), and Brooks *et al.* (1974, 1977), but the present data include many more specimens (160 instead of 36) and encompass all sixteen species (instead of 11). Data for *Lasiochlamys*, *Xylosma* and *Casearia* are entirely new.

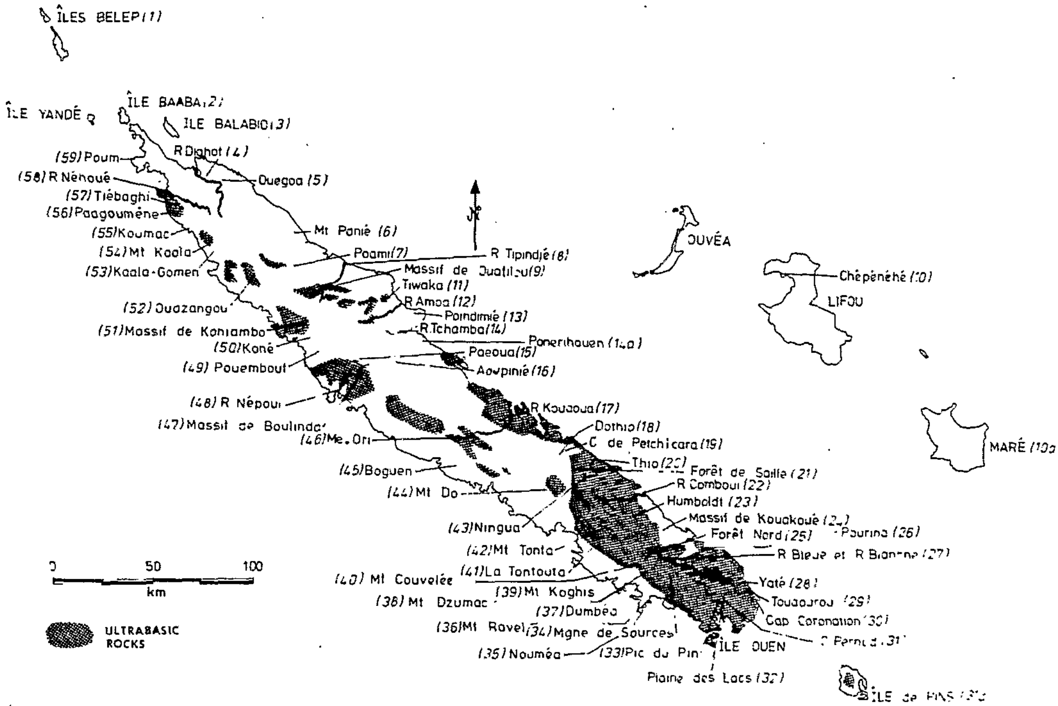

FIGURE 1. Map of New Caledonia showing areas of ultrabasic rocks. Numbers adjacent to place-names are referred to in table 2.

The Flacourtiaceae of New Caledonia have an inordinate tolerance of ultrabasic substrates. About 75 % of all species are found on ultrabasic rocks and 45 % are found on non-ultrabasic substrates. At least half of the species are exclusive to ultrabasics. This distribution is remarkable since ultrabasic rocks cover only one third of New Caledonia.

The proportion of hyperaccumulators is greatest among the *Xylosma* (10 out of 18) and *Homalium* (7 out of 16) species. Even among the remaining species of these genera, nickel uptake is very high. There are three species of *Lasiochlamys* with nickel contents in the range 100–1000 μg/g (an upper limit for most serpentine species, and four species of *Xylosma*, two of *Casearia* and four of *Homalium* in the same category. Even among plants growing on supposedly non-ultrabasic substrates, nickel levels are surprisingly high. Such examples are *L. trichostemona*

(39 μg/g), *L. grossecrenata* (162 μg/g) and *H. rivulare* (52 μg/g). However in some cases, these 'non-ultrabasic' soils consist of alluvia derived originally from the weathering of ultrabasic rocks and which still retain an appreciable nickel content.

The presence of so many hyperaccumulators (19 out of 53 species) in a single family raises interesting questions as to the mechanisms whereby nickel is not only tolerated, but is also accumulated by the New Caledonian plants. Phytochemical studies on: *Sebertia acuminata*, *Geissois pruinosa*, *Hybanthus austrocaledonicus*, *Hybanthus caledonicus*, and 12 species of *Homalium*, have shown a strong correlation between levels of nickel and of citric acid in leaves (Lee *et al.* 1978). Lee *et al.* (1977) have isolated a citrate complex of nickel from *Sebertia acuminata*, *Hybanthus austrocaledonicus*, *Hybanthus caledonicus*, *Homalium francii*, *Homalium guillainii*, and *Homalium kanaliense*. There is no reason to doubt that the other hyperaccumulators of nickel among the Homalium and probably in the other genera of the Flacourtiaceae also have their nickel completed with citric acid. From studies on seven of the New Caledonian hyperaccumulators it is apparent that with a single exception, most of the nickel is bound with citric acid. In the case of *Psychotria douarrei* (Rubiaceae), however, only a small part of the nickel is bound to citric acid. The remainder is present as a relatively low molecular weight cationic complex of unknown composition (Kelly *et al.* 1975).

All the New Caledonian plants with nickel-citrate complexes are members of primitive families. The 'advancement index' as defined by Sporne (1969) is as follows for each family: Flacourtiaceae (*Lasiochlamys*, *Xylosma*, *Casearia*, *Homalium*) – 21, Cunoniaceae (*Geissois*) – 24, Violaceae (*Hybanthus*) – 42, Sapotaceae (*Sebertia*) – 42, Rubiaceae (*Psychotria*) – 70. The advancement index (based on morphological characters) extends from 21 (most primitive) to 100 (most advanced). It is clear that the advanced *Psychotria douarrei* is distinguished from all other New Caledonian hyperaccumulators by its behaviour in complexing nickel mainly with a ligand or ligands other than citric acid. It is postulated therefore that nickel complexing with citric acid may be a primitive character of hyperaccumulators, reaching its highest development in the Flacourtiaceae, one of the most primitive of all the angiosperms (Sporne 1969).

The postulate that nickel accumulation via citric acid complexing is a primitive character is strengthened by the findings of Lee *et al.* (1978) who showed that advanced hyperaccumulators of the family Cruciferae (advancement index 63) complexed their nickel with malic acid rather than citric acid. The same finding was originally made for A. *bertolonii* by Pelosi *et al.* (1976).

It is appropriate now to consider the taxonomic classification (Cronquist 1968) of the Flacourtiaceae and other New Caledonian families and genera of hyperaccumulators of nickel. With the exception of the advanced *Psychotria* (subclass Asteridae, order Rubiales) and the primitive *Geissois* (subclass Rosidae, order Cunoniae), all other New Caledonian nickel plants belong to subclass Dillenidae and the orders Ebenales (Sapotaceae) and Violales (Flacourtiaceae and Violaceae).

The occurrence of numerous nickel-accumulating species in the order Violales is not confined to New Caledonia. Brooks & Wither (1977) discovered hyperaccumulation of nickel in the South-East Asian *Rinorea bengalensis* (Violaceae) and Severne & Brooks (1972) discovered this character in the West-Australian species *Hybanthus floribundus* (see also Cole 1973). Within subclass Dilleniidae are also to be found (number of hyperaccumulating species in parentheses) the genera *Alyssum* (45 species), *Planchonella* (1 species), *Sebertia* (1 species), and *Trichospermum* (1 species), though only one of these is found in New Caledonia (*S. acuminata*).

The distribution of nickel plants among a very few subclasses and orders of the primitive Magnoliatae is highly unusual. It is also probable that hyperaccumulation of nickel is an evolutionary character which reaches its greatest development in relatively undisturbed floras such as that of New Caledonia. Many questions still remain unanswered, particularly in the phytochemistry and plant physiology of these species. Further work is needed to account for the limited uptake of nickel by the large number of non-accumulators found in ultrabasic areas, and to explain the detailed mechanisms of uptake and translocation of nickel in hyperaccumulator species. Such uptake mechanisms are also of interest to workers in the field of nickel extraction and refining, since there is an obvious benefit in being able to emulate technologically, the low-energy extraction of nickel by plants.

The authors would like to express their warm thanks to the institutions listed in table 1 and to the following individuals: Dr N. Hallé (P), Professor C. Kalkman, Mr A. K. Groenewegen, Dr H. Sleumer (L), The Director (NOU), Professor K. U. Kramer (Z), The Director (E), The Director (K).

References

Brooks, R. R., Lee, J. & Jaffré, T. 1974 Some New Zealand and New Caledonian plant accumulators of nickel. *J. Ecol.* **62**, 493–499.

Brooks, R. R., Lee, J., Reeves, R. D. & Jaffré, T. 1977 Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. geochem. Explor.* **7**, 49–57.

Brooks, R. R., Morrison, R. S., Reeves, R. D., Dudley, T. R. & Akman, Y. 1979 Hyperaccumulation of nickel by *Alyssum* Linnaeus (Cruciferae). *Proc. R. Soc. Lond.* B **203**, 387–403.

Brooks, R. R. & Radford, C. C. 1978 Nickel accumulation by European species of the genus *Alyssum*. *Proc. R. Soc. Lond.* B **200**, 217–224.

Brooks, R. R. & Wither, E. D. 1977 Nickel accumulation by *Rinorea bengalensis* (Wall.) O.K. *J. geochem. Explor.* **7**, 295–300.

Cole, M. M. 1973 Geobotanical and biogeochemical investigations in the sclerophyllus woodland and scrub associations of the Eastern Goldfields area of Western Australia, with particular reference to the role of *Hybanthus floribundus* (Lindl.) F. Muell. as nickel indicator and accumulator plant. *J. appl. Ecol.* **10**, 269–330.

Cronquist, A 1968 *The evolution and classification of flowering plants*, Boston: Houghton Mifflin.

Duvigneaud, P. & Denaeyer-De Smet, S. 1963 Flore et végétation du cuivre au Katanga. *Bull. Soc. r. Bot. Belg.* **96**, 106–231.

Jaffré, T., Brooks, R. R., Lee, J. & Reeves, R. D. 1976 *Sebertia acuminata*: a nickel-accumulating plant from New Caledonia. *Science, N.Y.* **193**, 579–580.

Jaffré, T., Brooks, R. R. & Trow, J. M. 1979 Hyperaccumulation of nickel by *Geissois* species. *Pl. Soil* (in the press).

Jaffré, T., Latham, M. & Quantin, P. 1971 *Les sols des massifs miniers de Nouvelle Calédonie. et leur relation avec la végétation.* Nouméa: Spec. Rep. O.R.S.T.O.M.

Jaffré, T. & Schmid, M. 1974 Accumulation du nickel par une Rubiacée de Nouvelle Calédonie: *Psychotria douarrei* (G. Beauvisage) Däniker. *C. r. hebd. Séanc. Acad. Sci. Paris* **278**, 1727–1730.

Kelly, P. C., Brooks, R. R., Dilli, S. & Jaffré, T. 1975 Preliminary observations on the ecology and plant chemistry of some nickel-accumulating plants from New Caledonia. *Proc.R. Soc. Lond.* B **189**, 69–80.

Lee, J., Reeves, R. D., Brooks, R. R. & Jaffré, T. 1977 Isolation and identification of a citrato complex of nickel from nickel-accumulating plants. *Phytochemistry* **16**, 1503–1505.

Lee, J. Reeves, R. D., Brooks, R. R. & Jaffré, T. 1978 The relation between nickel and citric acid in some nickel-accumulating plants. *Phytochemistry* **17**, 1033–1035.

Minguzzi, C. & Vergnano, O. 1948 Il contenuto di nichel nelle ceneri di *Alyssum bertolonii* Desv. *Memorie Soc. tosc. Sci. nat.* A **55**, 49–77.

Pelosi, P., Fiorentini, R. & Galoppini, C. 1976 On the nature of nickel complexes in *Alyssum bertolonii* Desv. II. *Agric. biol. Chem.* **40**, 1641–1642.

Severne, B. C. & Brooks, R. R. 1972 A nickel-accumulating plant from Western Australia. *Planta* **103**, 91–94.

Sleumer, H. 1974 A concise revision of the Flacourtiaceae of New Caledonia and the Loyalty Islands. *Blumea* **22**, 123–147.

Sporne, K. R. 1969 The ovule as an indicator of evolutionary status in angiosperms. *New Phytol.* **68**, 555–562.

Wild, H. 1970 The vegetation of nickel-bearing soils. *Kirkia* (suppl. to vol. 7), pp. 1–62.

Wild, H. 1974 Indigenous plants and chromium in Rhodesia, *Kirkia* **9**, 233–241.

Wither, E. D. & Brooks, R. R. 1977 Hyperaccumulation of nickel by some plants of S. E. Asia *J. geochem. Explor.* 8, 579–583.