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Robert S. Boyd[1,*] and Tanguy Jaffré[2]

Abstract - We investigated accumulation of elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, P, Pb, and Zn) in leaves of different ages for 11 evergreen woody plant species from serpentine soils of New Caledonia. Species were classified into four categories of Ni accumulation ability: one species was a non-accumulator (<100 mg Ni/kg), three were accumulators (100–1000 mg Ni/kg), two were hyperaccumulators (1000–10,000 mg Ni/kg), and five were hypernickelophores (>10,000 mg Ni/kg). We harvested leaves from each species, separating them into three (four in one case) relative age categories based upon their position along branches (younger toward the apex, older far from it). Leaf samples were dried, ground, and dry-ashed, and their elemental concentrations were determined by inductively coupled plasma spectrometry (all elements except Ni) or atomic absorption spectrophotometry (Ni). Great variation was found for most elements both within and among species, but Ni varied most (1050-fold between species for oldest leaves). Correlations between Ni and other transition metals showed no significant relationships within samples of any species, but, we found significant positive correlations between Ni and Pb (correlation coefficient = 0.97) and Ni and Fe (correlation coefficient = 0.87) among species. Leaf Ni concentrations varied significantly with leaf age for two species, the hypernickelophores *Geissois pruinosa* and *Homalium kanaliense*. We conclude that Ni concentration varies markedly between species, but generally does not vary with leaf age within species. We also suggest that four Ni accumulation category terms— non-accumulator, hemi-accumulator, hyperaccumulator, and hypernickelophore—be used to subdivide the wide variation found in Ni concentrations in plant leaves.

Introduction

Plants vary greatly in elemental makeup. Factors influencing this variation are many, including species differences, environmental conditions, plant physiological state, variation among plant organs, and others. The extremely large variation in the concentrations of metals in plant tissues has stimulated considerable scientific interest (Brooks 1987). Many high-metal plants grow on serpentine soils derived from ultramafic rocks (high in Mg and Fe, but low in Si and Ca), but other plants with much lower metal concentrations can be found growing side-by-side with high-metal plants (Alexander et al. 2007, Brooks 1987, Kruckeberg 2002).

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The wide variation in metal concentration of plant species has stimulated attempts to divide that variation into categories. For Ni, the term "hyperaccumulator" was coined by Brooks et al. (1977) to describe plants containing >1000 mg Ni/kg dry mass. The concept has since been extended to define hyperaccumulation for a number of metals and other elements (Reeves and Baker 2000), but Ni is the most commonly hyperaccumulated element, comprising about 75% of the >400 taxa of hyperaccumulator plants listed by Reeves and Baker (2000). Jaffré and Schmid (1974) used the term "hypernickelophore" for plants containing extremely large (>10,000 mg/kg) Ni concentrations. The term "accumulator" has been applied to plants that take up 100–1000 mg/kg (Berazaín 2004), whereas Reeves and Baker (2000) defined "normal" Ni concentrations as 1–10 mg/kg. Thus, the concentration of Ni in plant tissues shows extraordinary variation (>4 orders of magnitude), from as little as 1 mg Ni/kg in normal plants to as much as 60,000 mg Ni/kg in leaves of some hypernickelophore *Phyllanthus* species (Reeves and Baker 2000).

The enormous variation in plant Ni concentrations undoubtedly reflects different physiological uptake and sequestration mechanisms among species. Considerable effort is being made to understand these mechanisms (Salt 2004), including the relatively new effort termed "metallomics" (Szpunar 2004): the study of the location, identity, quantity, and complexation of metals (including Ni) in cells. Variation in plant Ni concentrations also must have ecological consequences (Boyd and Martens 1998), some of which stem from the toxicity of Ni to organisms that consume living high-Ni plant tissues (plant natural enemies). One consequence may be plant defense; Martens and Boyd (1994) suggested that high levels of metal in plant tissue provide an "elemental defense" against plant natural enemies, and research to date has shown defensive effects for several hyperaccumulated metals (Boyd 2007). Documenting how plant defenses vary in time and space is vital to our understanding of plant defense strategies (e.g., Brenes-Arguedas and Coley 2005), yet few studies have been conducted regarding such variation in elemental defenses (for exceptions, see Boyd et al. 1999, 2004; Galeas et al. 2007).

The effectiveness of elemental defenses may be influenced by levels of organic defenses or by high concentrations of other elements in plant tissues. Boyd (2007) suggested that these "joint effects" may be ecologically important and may have played a role in the evolution of hyperaccumulation by plants. Simultaneously elevated levels of more than one metal may enhance their protective effects against a single natural enemy or may protect against multiple natural enemies (Boyd 2007). Examining hyperaccumulators for concentrations of multiple metals provides information relevant to this relatively new concept.

Finally, plants with enhanced Ni uptake abilities have applied uses in phytoremediation (Pilon-Smits 2004, Raskin and Ensley 2000) or phytomining (Nicks and Chambers 1998). Typically, these technologies use hyperaccumulator plants to remove metal (such as Ni) from soils.

Knowledge of how metal accumulation varies within and among metal accumulating species is important basic information for the applied uses of these species (Whiting et al. 2004).

The archipelago of New Caledonia contains remarkably high levels of biological endemism in many groups of organisms (Mittermeier et al. 2005), including plants (Jaffré 2005). Nickel hyperaccumulators are particularly numerous in New Caledonia, which ranks second (behind Cuba) in hosting the greatest number of Ni hyperaccumulator taxa (Reeves 2003). New Caledonian species also are important to the historical development of the terms used in reference to hyperaccumulators, as both "hyperaccumulator" and "hypernickelophore" were developed from investigations that focused primarily on New Caledonian high-Ni plants. We selected 11 New Caledonian plant species varying greatly in leaf Ni concentration and asked the following sets of questions:

(1) What are the element concentrations in leaves of these 11 species, and do they vary significantly between species? We were particularly interested in documenting differences in Ni levels between species to subdivide them into categories of Ni accumulation (normal or non-accumulator, accumulator, hyperaccumulator, hypernickelophore).

(2) Given the wide range in mature leaf Ni concentrations documented in answer to question 1, are there significant relationships between the concentrations of Ni and other metals in mature plant leaves? Correlations between metals may reflect the specificity of underlying physiological mechanisms of metal uptake, transport, and sequestration. They also may have ecological ramifications: positive correlations of concentrations of two metals may lead to enhanced plant defense due to joint effects (Boyd 2004, 2007). For example, Jhee et al. (2006) demonstrated additive toxicity of Ni and Zn to caterpillars of a leaf-chewing insect.

(3) Do leaf metal levels vary with leaf age within species, and are there consistent patterns among plant species from different categories of Ni accumulation? This question is relevant to determine if metal-based defenses vary during leaf lifespans (Boyd 1998), as illustrated by research into similar variation in the organic defense compounds of some tropical forest species (e.g., Brenes-Arguedas et al. 2006).

Methods

We selected 11 species of plants (Table 1) growing on serpentine soils at the southern end of Grand Terre, the main island of the New Caledonian archipelago. Species were selected to include a diversity of Ni accumulation categories. Samples of five species: *Psychotria baillonii, Casearia silvana, Homalium guillainii, Hybanthus austrocaledonicus,* and *Sebertia acuminata* (Sève Bleue) (see Table 1 for nomenclatural authorities) were collected from a site within the Parc Territorial de la Rivière Bleue, which contains areas of humid tropical forest. Jaffré and Veillon (1991) provide a description of the vegetation of this forest type. These samples were collected from a site located at Kaori Géant (a very large *Agathis lanceolata* Linley ex Warb. tree). This site includes one of the study plots described by Jaffré

and Veillon (1991) and is remarkable because six high-Ni species co-occur in two layers of the vegetation (Boyd et al. 1999). Several other studies of hyperaccumulators and their ecological relationships (Boyd and Jaffré 2001; Boyd et al. 1999, 2006; Davis et al. 2001) have taken advantage of the large number of co-occurring Ni hyperaccumulators found at this site. The shrub layer contains the hypernickelophores *Psychotria douarrei* (Beauvis.) Däniker (Rubiaceae), *Hybanthus austrocaledonicus*, and *Casearia silvana*. The overstory contains *Sebertia acuminata*, as well as *Homalium guillainii* and *Geissois hirsuta* Brongn. and Gris.

The remaining six species (Table 1) were sampled from serpentine sites outside of the park. Leaves of two species, *Grevillea gillivrayi* and *Geissois pruinosa*, were collected from plants growing at several serpentine sites along the main road from Noumea to Yaté, before reaching the junction of that road with the road leading northwest to the park entrance. Two other species, *Agatea longipedicillata* and *Garcinia amplexicaulis*, were collected from a site at the junction of the road from Noumea to Yaté and the road leading to the park entrance. Samples of the remaining two species, *Xanthostemon aurantiacus* and *Homalium kanaliense*, were collected from sites along the road leading to the park entrance.

Species were classified into the following Ni accumulation categories: normal or non-accumulator (<100 mg Ni/kg), accumulator (100–999 mg Ni/kg), hyperaccumulator (1000–9999 mg Ni/kg), and hypernickelophore (≥10,000 mg Ni/kg). Following the criteria of Reeves (1992) for defining

Table 1. Species selected for sampling in this study. The "Reference" column provides the citation used to classify each species as either an accumulator of Ni (at least one sample contained 100–999 mg Ni/kg), hyperaccumulator of Ni (at least one sample contained 1000–9999 mg Ni/kg), or hypernickelophore (at least one sample contained >10,000 mg Ni/kg).

Species	Family	Reference
Non-accumulator (< 100 mg Ni/kg)		
Grevillea gillivrayi Hook. and Arn.	Proteaceae	Jaffré 1980
Ni accumulators (100–999 mg Ni/kg)		
Garcinia amplexicaulis Vieill.	Clusiaceae	This study
Pyschotria baillonii Schltr.	Rubiaceae	This study
Xanthostemon aurantiacus (Brongn. and Gris) Schltr.	Myrtaceae	This study
Ni hyperaccumulators (1000–9999 mg Ni/kg)		
Agatea longipedicillata Baker f. (referred to as *A. deplanchei* by Jaffré)	Violaceae	Jaffré 1980
Casearia silvana Schltr.	Flacourtiaceae	Jaffré 1980
Hypernickelophores (>10,000 mg Ni/kg)		
Geissois pruinosa Brongn.	Cunoniaceae	Jaffré et al. 1979
Homalium guillainii Briq.	Flacourtiaceae	Jaffré 1980
Homalium kanaliense (Vieill.) Briq.	Flacourtiaceae	This study
Hybanthus austrocaledonicus (Vieill.) Schinz and Guillamin ex Melchior	Violaceae	Brooks et al. 1977
Sebertia acuminata Pierre ex Baillon	Sapotaceae	Jaffré et al. 1976

hyperaccumulation, which he defined as "a plant in which a nickel concentration of at least 1000 mg/kg has been recorded in the dry matter of any above-ground tissue in at least one specimen growing in its natural habitat," we classified each species into Ni accumulation categories (Table 1) based on either values reported in the literature or the greatest Ni value generated among all samples in our dataset.

Leaf sampling and analysis

We searched sample sites for plants with leafy branches low enough to be reached from the ground. In the case of tree species, we often used relatively young individuals growing in roadside disturbed areas. Leaves were divided into relative age categories, basing leaf-age on the position of leaves along a branch. Samples were collected by clipping a leafy branch (at a point closer to the trunk from the attachment point of the oldest leaf) and removing the leaves in order from the tip to the cut end of the branch, dividing the leaves into three groups of approximately equal biomass. Those closest to the cut end were labeled "old," those at the apex were "young," and those in between were "intermediate" in age. For one species, *Geissois pruinosa*, we were able to define four leaf age categories due to the presence of rapidly expanding light-colored young leaves at the distal ends of branches, these last being considered "very young" leaves.

Leaf samples were dried for at least 72 h at 60 °C, ground, dry-ashed at 485 °C, and further oxidized using 1 M HNO_3, and the residue then was re-dissolved in 1 M HCl. Concentrations of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, P, Pb, and Zn were determined by an inductively coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000). Nickel concentrations were determined using an atomic absorption spectrophotometer (Instrumentation Laboratory, IL 251).

Data analysis

Data from old leaves were used to compare element concentrations among species and to investigate associations among levels of elements. We focused these analyses on old leaves because our definition of intermediate and young leaves was contingent upon our initial identification of leaves comprising the "old" category; thus, old leaves provide a relatively consistent leaf-age category for comparisons between species. Element concentrations of old leaves were compared among species using a separate one-way analysis of variance (ANOVA) for each element. Fisher's protected least significant difference (PLSD) test was used for post-hoc mean separations.

We searched for associations between concentrations of Ni and concentrations of other transition metals (Co, Cr, Cu, Fe, Mg, Mn, Pb, Zn) using correlations of values for old leaves of each species. Again, we used data from old leaves because we believed that the "old" age category was defined most consistently among species. These correlations were conducted for data from each species separately, and then for all species using the mean metal concentrations of old leaves for each species. Finally, we examined the influence of leaf age on the concentrations of all transition metals analyzed

(Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn) using separate one-way ANOVAs for each species followed by Fisher's PLSD test.

Statistical analyses used StatView 5.0 (Abacus Concepts 1998). Because of the large number of statistical tests comprising all the preceding analyses, we used a more conservative alpha (0.001) as our criterion for statistical significance for all analyses and mean separations to decrease our probability of committing Type I error (Zar 1996).

Results

Elemental concentrations

Considerable variation in concentration was observed for all elements (Table 2). The highest mean value of an element in any species was 20,000 mg Ni/kg in *Hybanthus austrocaledonicus*, although some high values for Ca were found for some species (16,000 mg/kg for *Grevillea gillivrayi* and *Geissois pruinosa*; Table 2). Comparing mean values by dividing the greatest species' mean by the smallest mean in Table 2 showed Ni to vary more between species than any other element (1050-fold). Means of two metals (Co and Pb) could not be compared in that way because the lowest mean values were below detection limits for at least one species.

All elements excepting P varied significantly among species (Table 2). Calcium and K varied comparatively little, 4–fold for Ca and 7–fold for K, and no general trends in levels of these elements among the Ni accumulator categories were discernable (Table 2). Among transition metals, variation was least for Cu and greatest for Ni, with Mn, Co, and Cr also varying greatly (60–fold or greater). As expected, mean Ni values increased from non-accumulator to accumulator to hyperaccumulator to hypernickelophore categories (Table 2), but no trends in other metal concentrations emerged from inspection of Table 2. There was considerable variation in Ni values within and among species, so that there were no clear divisions of species' Ni concentrations that corresponded to Ni accumulation categories (non-accumulator, accumulator, etc.). Besides the extremely high Ni concentrations of some hypernickelophores (*Hybanthus austrocaledonicus* with 20,000 mg/kg and *Sebertia acuminata* with 14,000 mg/kg), we documented extremely high concentrations of Mn in *Garcinia amplexicaulis* (6300 mg/kg) and *Grevillea gillivrayi* (3500 mg/kg), of Co in *Homalium kanaliense* (450 mg/kg) and of Cr in *Agatea longipedicillata* (170 mg/kg).

Relationships between concentrations of Ni and other metals

Comparing correlations of leaf Ni concentrations against concentrations of other metals in old leaves of each species revealed no significant relationships for any species (results were $P > 0.001$ in all cases: data not shown). On the other hand, testing correlations of the mean Ni concentrations of old leaves of all species against concentrations of other metals showed two significant positive relationships ($P < 0.001$): with Fe and Pb. Correlation coefficients were 0.87 for Ni and Fe, and 0.97 for Ni and Pb. Correlations

of Ni against all other transition metals (Co, Cr, Cu, Mg, Mn, Zn) were not significant ($P = 0.034$ for Co, $P > 0.26$ in all other cases; data not shown).

Effect of leaf age on metal concentrations

Leaf age affected concentrations of at least one transition metal for eight of the 11 species examined (Table 3). Species for which no age effect was found for any metal included one accumulator (*Garcinia amplexicaulis*), one hyperaccumulator (*Casearia silvana*), and one hypernickelophore (*Sebertia acuminata*), revealing no clear trend of leaf-age patterns among species in different Ni accumulation categories. Two hypernickelophores had significant leaf-age effects for a large number of metals (six metals for *Geissois pruinosa* and five for *Homalium kanaliense*), whereas all other species for which we detected significant leaf-age effects had significant patterns for only one or two metals (Table 3).

Among metals, Fe was most frequently affected by leaf age, as five species (*Grevillea gillivrayi*, *Xanthostemon aurantiacus*, *Geissois pruinosa* and both species of *Homalium*) had significantly greater Fe concentrations in older leaves (Table 3). Four species, the Ni hyperaccumulator *Agatea longipedicillata* and three hypernickelophores (*Geissois pruinosa*, *Homalium kanaliense*, and *Hybanthus austrocaledonicus*), showed significant increases in Pb concentration with increased leaf age. Effects of leaf age on Co, Cr, and Cu concentrations were found for three species each; these relationships showed increases in Co and Cr concentrations with increased leaf age, but decreases in Cu concentrations as leaf age increased (Table 3). Two species (*Geissois pruinosa* and *Homalium kanaliense*, both hypernickelophores) showed increases in leaf Ni with age, although these were not the species with greatest leaf Ni concentrations (those were *Hybanthus austrocaledonicus* and *Sebertia acuminata*). One species (*Geissois pruinosa*) had increased leaf Zn concentrations with leaf age. Two metals, Mg and Mn, did not vary significantly with leaf age for any species examined.

Discussion

We found wide differences in the levels of variation of different elements. Levels of Ni varied more among species than for any other element documented (Table 2), showing that we captured considerable variability among the species chosen for sampling. Variation also was high for most elements, including Ni, among samples within a species, indicating that other factors besides leaf age and plant identity affect leaf element concentrations. These other factors may include plant size, rooting depths, variation in soil properties between sites, or genetic variability within species.

Our results for leaf age (Table 3) were generally consistent with what is known about the mobility of the elements examined (Kabata-Pendias 2000). For example, Fe was the metal for which a significant leaf-age effect was found for five of the 11 species, and Fe is generally considered an immobile element (Kabata-Pendias 2000). Departures from these expectations may be

Table 2. Mean element concentrations of old leaves for all sampled species (in mg/kg dry mass, SE in parentheses below each mean). Mean values for elements that differ significantly between species (Fisher's PLSD test, $\alpha = 0.001$) are denoted by differing superscripts. Sample sizes (number of plants sampled) for each species' name. Concentrations of Co and Pb that were below detection limits (<1 mg/kg) are denoted by "N.D." (not detected).

Species							Element					
	Ca	K	P	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Non-accumulator												
Grevillea gillivrayi	16,000[C]	2000[A]	210	N.D.	3.9[A]	2.2[AB]	130[AB]	3300[AB]	3500[B]	19[A]	1.1[A]	15[A]
(n = 6)	(2300)	(340)	(17)		(1.2)	(0.29)	(17)	(560)	(820)	(6.7)	(0.70)	(3.3)
Ni accumulators												
Garcinia amplexicaulis	7000[AB]	3800[AB]	160	3.9[A]	14[AB]	3.8[ABC]	180[AB]	1300[A]	6300[C]	110[AB]	6.4[AB]	130[AB]
(n = 7)	(1100)	(480)	(21)	(2.8)	(9.5)	(0.32)	(84)	(170)	(990)	(25)	(2.9)	(17)
Pyschotria baillonii	15,000[C]	11,000[DE]	570	N.D.	22[AB]	6.5[C]	330[AB]	8400[DE]	34[A]	550[AB]	2.9[A]	93[AB]
(n = 7)	(1000)	(1800)	(17)		(4.3)	(0.26)	(55)	(1300)	(13)	(110)	(0.77)	(49)
Xanthostemon aurantiacus	12,000[BC]	4400[ABC]	140	N.D.	3.4[A]	1.9[A]	140[AB]	1200[A]	110[A]	160[AB]	N.D.	24[A]
(n = 7)	(2100)	(480)	(7.8)		(1.2)	(0.22)	(17)	(170)	(64)	(37)		(3.9)
Ni hyperaccumulators												
Agatea longipedicillata	8400[ABC]	9100[CDE]	510	7.7[A]	170[D]	13[D]	180[AB]	11,000[DE]	1100[A]	1000[ABC]	6.8[ABC]	230[AB]
(n = 4)	(450)	(1000)	(12)	(1.1)	(22)	(1.3)	(66)	(2000)	(59)	(270)	(1.1)	(20)
Casearia silvana	10,000[BC]	5900[ABCD]	1800	3.1[A]	2.8[A]	5.5[ABC]	320[AB]	12,000[E]	130[A]	1100[ABC]	5.1[AB]	260[B]
(n =7)	(820)	(1900)	(1300)	(0.87)	(0.49)	(0.53)	(97)	(1000)	(20)	(220)	(0.81)	(82)

Table 2, continued.

Species	Element											
	Ca	K	P	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Hypernickelophores												
Homalium guillainii	12,000[BC]	5100[ABC]	490	20[A]	12[A]	6.0[C]	570[BC]	6900[BCD]	80[A]	6300[CD]	21[DE]	150[AB]
(n = 7)	(740)	(850)	(16)	(2.8)	(1.9)	(0.37)	(92)	(440)	(27)	(720)	(1.5)	(12)
Homalium kanaliense	11,000[BC]	14,000[E]	230	450[C]	4.3[A]	5.5[ABC]	390[AB]	1700[A]	560[A]	9900[DE]	17[CD]	510[C]
(n = 6)	(2700)	(1800)	(21)	(54)	(1.5)	(0.33)	(36)	(300)	(83)	(1000)	(1.8)	(110)
Geissois pruinosa	16,000[C]	7900[BCD]	280	37[A]	41[ABC]	5.5[ABC]	300[AB]	4200[ABC]	280[A]	8800[DE]	16[BCD]	67[AB]
(n = 6)	(3100)	(330)	(18)	(6.0)	(12)	(0.47)	(53)	(380)	(73)	(1800)	(2.6)	(8.0)
Hybanthus austrocaledonicus	8200[ABC]	5000[ABC]	630	150[B]	87[C]	6.3[C]	1700[C]	7900[CDE]	280[A]	20,000[F]	41[F]	120[AB]
(n = 6)	(910)	(930)	(38)	(54)	(18)	(0.33)	(580)	(470)	(37)	(1600)	(2.9)	(7.6)
Sebertia acuminata	3700[A]	3800[ABC]	420	32[A]	58[BC]	6.9[C]	1600[C]	3000[A]	250[A]	13,000[E]	30[E]	93[AB]
(n = 8)	(660)	(750)	(70)	(6.8)	(16)	(1.7)	(460)	(780)	(64)	(2000)	(4.2)	(11)
Highest species mean	16,000	14,000	1800	450	170	13	1700	12,000	6300	20,000	41	510
Lowest species mean	3700	2000	140	N.D.	2.8	1.9	130	1200	34	19	N.D.	15
Comparison (high/low)†	4×	7×	13×	≥450×[††]	61×	7×	13×	10×	185×	1050×	≥41×[††]	34×

†This row compares the highest species mean for an element to the lowest species mean. Since the lowest species means for Co and Pb were below detection limits (<1 mg/kg), definite comparisons of highest and lowest species means could not be made for these elements.

††These values are estimates because the lowest values for these metals were below detection limits (<1 mg/kg), thus comparisons of highest and lowest species means for these metals are probably underestimates.

Table 3. Mean metal concentrations (in mg/kg dry mass, SE in parentheses after each mean). Means for which a significant effect of leaf age was detected are denoted with a superscripted letter. Mean values that differ significantly between leaves of different age for a species (Fisher's PLSD test, $\alpha = 0.001$) are denoted by differing superscripts. Sample sizes (number of plants sampled) are reported for each species.

Species	Metal								
	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
Grevillea gillivrayi ($n = 6$; Non-accumulator)									
Young	0.0 (0.0)	0.0 (0.0)	4.2 (0.28)	50^A (3.5)	2200 (150)	870 (250)	10 (3.2)	0.0 (0.0)	17 (2.4)
Intermediate	0.0 (0.0)	1.0 (0.64)	2.9 (0.52)	98^{AB} (14)	3100 (350)	2300 (620)	15 (3.3)	0.33 (0.19)	12 (1.0)
Old	0.0 (0.0)	3.9 (1.2)	2.2 (0.29)	130^B (17)	3300 (560)	3500 (820)	19 (7.8)	1.1 (0.70)	15 (3.3)
Psychotria baillonii ($n = 7$; Ni accumulator)									
Young	0.0 (0.0)	1.6^A (0.73)	8.3 (0.52)	130 (20)	5200 (860)	0.0 (0.0)	210 (63)	0.0 (0.0)	140 (34)
Intermediate	0.0 (0.0)	13^{AB} (2.1)	7.0 (0.75)	250 (40)	9100 (1500)	4.0 (2.0)	290 (93)	1.8 (0.87)	270 (140)
Old	0.0 (0.0)	22^B (4.3)	6.5 (0.26)	330 (55)	8400 (1300)	34 (13)	550 (110)	2.9 (0.77)	93 (49)
Xanthostemon aurantiacus ($n = 7$; Ni accumulator)									
Young	0.0 (0.0)	0.0 (0.0)	5.5^B (0.69)	41^A (1.9)	1800 (270)	7.2 (3.6)	31 (9.8)	0.0 (0.0)	23 (1.6)
Intermediate	0.0 (0.0)	0.60 (0.46)	3.7^{AB} (0.62)	93^{AB} (15)	1400 (190)	21 (9.9)	78 (12)	0.0 (0.0)	26 (2.7)
Old	0.0 (0.0)	3.4 (1.2)	1.9^A (0.22)	140^B (17)	1200 (170)	110 (64)	160 (37)	0.0 (0.0)	24 (3.9)
Garcinia amplexicaulis ($n = 7$; Ni accumulator)									
Young	0.45 (0.45)	1.2 (0.51)	6.8 (0.89)	45 (5.7)	1300 (110)	4300 (600)	37 (8.0)	1.5 (0.74)	69 (19)
Intermediate	1.5 (1.5)	2.7 (0.58)	5.0 (0.84)	63 (7.1)	1100 (180)	5800 (410)	140 (87)	2.9 (0.94)	110 (15)
Old	3.9 (2.8)	4.8 (1.4)	3.8 (0.32)	180 (84)	1300 (170)	6300 (990)	110 (25)	6.4 (2.9)	130 (17)
Agatea longipedicillata ($n = 4$; Ni hyperaccumulator)									
Young	0.0^A (0.0)	20 (5.0)	37 (8.6)	75 (8.3)	5700 (340)	330 (57)	330 (61)	0.0^A (0.0)	91 (4.2)
Intermediate	4.7^{AB} (1.2)	140 (35)	13 (1.2)	250 (110)	9900 (1400)	720 (220)	600 (150)	5.5^{AB} (0.55)	190 (25)
Old	7.7^B (1.1)	170 (22)	13 (1.3)	180 (66)	11,000 (2000)	1100 (59)	1000 (270)	6.8^B (1.1)	230 (20)

Table 3, continued.

Species	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
					Metal				
Casearia silvana (n = 7; Ni hyperaccumulator)									
Young	1.1 (0.57)	1.9 (1.7)	6.4 (0.93)	140 (21)	8400 (920)	95 (21)	460 (88)	2.2 (0.93)	72 (8.5)
Intermediate	2.2 (0.44)	2.1 (1.2)	5.4 (0.24)	270 (64)	11,000 (650)	150 (32)	850 (150)	5.1 (0.45)	100 (22)
Old	3.1 (0.87)	2.8 (0.49)	5.5 (0.53)	320 (97)	12,000 (1000)	130 (20)	1100 (220)	5.1 (0.81)	260 (82)
Homalium guillainii (n = 7; Ni hyperaccumulator)									
Young	8.2 (2.7)	0.0^A (0.0)	7.9 (0.94)	73^a (12)	4200 (990)	43 (22)	3100 (530)	14 (5.5)	110 (16)
Intermediate	20 (3.5)	2.2^A (0.86)	6.9 (0.28)	210^{a,b} (33)	5900 (580)	85 (29)	5800 (440)	22 (0.91)	160 (14)
Old	20 (2.8)	12^B (1.9)	6.0 (0.37)	570^b (92)	6900 (440)	80 (27)	6300 (720)	21 (1.5)	150 (12)
Geissois pruinosa (n = 6; hypernickelophore)									
Very young	4.2^A (0.82)	1.4 (1.4)	19^B (1.1)	100^A (28)	4500 (440)	180 (26)	960^A (180)	0.16^A (0.16)	30^A (3.1)
Young	8.3^A (2.1)	3.2 (2.7)	15^B (1.2)	82^A (15)	4600 (230)	200 (54)	1900^A (430)	1.8^A (1.5)	30^A (2.1)
Intermediate	31^B (4.2)	34 (8.4)	8.4^A (0.63)	180^{AB} (22)	5200 (400)	330 (76)	5400^{AB} (720)	8.6^{AB} (1.5)	47^{AB} (4.1)
Old	37^B (6.0)	41 (12)	5.5^A (0.47)	300^B (53)	4200 (380)	280 (73)	9100^B (2000)	16^{B,C} (2.6)	67^{BC} (8.0)
Homalium kanaliense (n = 6; hypernickelophore)									
Young	85^A (15)	0.0 (0.0)	8.7^B (0.49)	65^A (6.5)	3100 (310)	210 (33)	2300^A (340)	0.58^A (0.22)	190 (9.6)
Intermediate	330^B (30)	1.4 (0.81)	6.1^B (0.32)	240^B (28)	2900 (320)	470 (86)	8000^B (480)	13^B (1.3)	470 (63)
Old	450^B (46)	4.3 (1.3)	5.3^A (0.32)	380^C (31)	1800 (260)	510 (83)	9900^B (890)	17^B (1.6)	490 (91)
Hybanthus austrocaledonicus (n = 6; hypernickelophore)									
Young	39 (14)	5.7^A (1.4)	7.2 (0.40)	120 (17)	6800 (970)	120 (21)	17,000 (590)	26^A (2.5)	110 (11)
Intermediate	98 (27)	49^{AB} (10)	6.7 (0.31)	660 (170)	9300 (640)	250 (42)	20,000 (1100)	45^B (0.86)	130 (7.2)
Old	150 (54)	87^B (18)	6.3 (0.33)	1700 (580)	7900 (470)	280 (37)	20,000 (1800)	41^B (2.9)	120 (7.6)
Sebertia acuminata (n = 8; hypernickelophore)									
Young	14 (1.5)	9.6 (3.5)	8.4 (0.56)	190 (42)	2200 (280)	140 (26)	14,000 (1400)	22 (2.5)	63 (8.9)
Intermediate	18 (0.73)	25 (4.1)	6.6 (1.3)	590 (110)	2200 (660)	150 (28)	12,000 (2300)	25 (3.4)	72 (12)
Old	36 (6.3)	63 (17)	7.4 (1.9)	1800 (500)	3200 (880)	290 (64)	14,000 (2100)	33 (2.6)	98 (10)

due to physiological differences between species, but also may stem from other factors. These other factors may include differences in growth rate of the species examined. Because we defined leaf age in a relative manner, effects of leaf age on element concentrations may have been difficult to detect for species with considerably long-lived leaves.

Nickel levels for some species reported here differed from those reported in earlier literature; as a result, we classified some species into Ni accumulation categories that differ from earlier reports. For example, Jaffré (1980) reported a value of 21 mg Ni/kg for *Xanthostemon aurantiacus*, but we found old leaves had a mean value of 160 mg/kg and thus classified that species as a Ni accumulator. Jaffré (1980) also reported *Homalium kanaliense* as a Ni hyperaccumulator, but we found some samples of old leaves to contain >10,000 mg Ni/kg (Table 2) and so classified it as a hypernickelophore in Table 1. In another case, we found lower Ni values than expected: Jaffré (1980) reported *Homalium guillainii* as a hypernickelophore, but our mean values were between 3100 and 6300 mg Ni/kg (Table 3), putting our specimens into the hyperaccumulator range.

As illustrated above, the Ni-concentration boundaries used to categorize species are difficult to use consistently. We agree with Reeves (1992) and Macnair (2003) that the boundaries that separate categories of Ni accumulation are artificial and are not based upon natural discontinuities. For example, our data (Tables 2 and 3) did not show a clear separation of species into Ni hyperaccumulator and hypernickelophore categories. On the other hand, we think it is helpful to divide the wide range of Ni (and other metal) concentrations of plants into categories to aid in discussions of their properties (such as their physiologies or their ecological effects on other organisms). Using orders of magnitude to define category boundaries is particularly convenient, and we suggest doing so with Ni even though this approach probably does not reflect clear biological boundaries. For Ni, we suggest non-accumulator (<100 mg/kg), hemi-accumulator (100–999 mg/kg), hyperaccumulator (1000–9999 mg/kg) and hypernickelophore categories (≥10,000 mg/kg). We particularly suggest that hypernickelophore be retained, as plants with Ni concentrations of that magnitude may be particularly important for their ecological effects, such as mobilizing metals into terrestrial food webs (Boyd 2004). The use of "accumulator" for plants that accumulate to below hyperaccumulator status is problematic because the term "accumulator" has been used generically to denote plants with relatively high metal concentrations (e.g., Reeves and Adigüzel 2004). If accumulator is used generally to refer to plants with greater than normal Ni concentrations, then another term is needed for those in the sub-hyperaccumulator range. Therefore, we suggest use of a new term, "hemi-accumulator," to describe those plants with 100–999 mg Ni/kg. The Greek-derived prefix means "half" and thus conveys that these plants accumulate Ni, but not to the extremes of hyperaccumulators or hypernickelophores.

To be operationally useful, Ni accumulation categorical terms need stringent definition. Following the criteria of Reeves (1992), in which he defined a Ni hyperaccumulator as "a plant in which a nickel concentration of at least 1000 mg/kg has been recorded in the dry matter of any above-ground tissue in at least one specimen growing in its natural habitat," we suggest that the terms "non-accumulator," "hemi-accumulator," and "hypernickelophore" be similarly defined based upon records of Ni concentration of <100 mg/kg, 100–999 mg/kg, and ≥10,000 mg/kg, respectively, in the dry matter of any above-ground tissue in at least one specimen growing in its natural habitat. We recognize that, as pointed out by Macnair (2003), only a single specimen is needed to elevate the classification of a species' Ni accumulation category, and thus these categories can become "over-reported." Still, we conclude it is necessary to have a discrete definition so that use of these terms can be standardized.

The inadvertent uptake hypothesis for metal hyperaccumulation (Boyd and Martens 1992) suggests that metal hyperaccumulation might have evolved from physiological mechanisms that target uptake and sequestration of one or more other soil ions. This hypothesis has received some support in the literature on Zn hyperaccumulation (Macnair 2003, Taylor and Macnair 2006) but to our knowledge, there is no direct evidence of shared uptake and sequestration mechanisms for Ni and other elements (Callahan et al. 2006). However, this hypothesis is consistent with strong positive correlations between tissue levels of Ni and another soil constituent. In our study, correlations showed significant positive relationships between the concentrations of Ni and Fe, and Ni and Pb, in leaves of the studied species. These results may stem from similar uptake and sequestration pathways for these metals. Because Pb is not known to be an essential element for plants (Pais and Jones 1997), it is difficult to envision a functional reason for enhancing Pb uptake (of course, Pb might be taken up inadvertently along with Ni). Iron, however, is a required plant micronutrient (Pais and Jones 1997), and our results may reflect linked uptake and sequestration pathways of Ni and Fe in these species. It is also plausible that Ni uptake and sequestration increase the Fe requirement of hyperaccumulator and hypernickelophore plants, although we know of no data that bear directly on this question.

We note that the concept of inadvertent uptake has previously focused on explaining the high concentrations of hyperaccumulated metals in plants, suggesting that another element was the target of uptake and sequestration mechanisms. Our results suggest another possibility: the correlations we found between Ni and both Pb and Fe may reflect inadvertent uptake not of Ni, but of Fe and Pb. It is possible that the extremely active Ni uptake and sequestration mechanisms characteristic of hyperaccumulators and hypernickelophores (Callahan et al. 2006) result in inadvertent uptake of other elements (e.g., Pb and Fe). In this scenario, the small amounts of Pb and Fe (relative to Ni) in these plants (Tables 2, 3) may be viewed as being inadvertently captured during the acquisition and transport of Ni.

    Previous studies (e.g., Brooks and Yang 1984, Yang et al. 1985) investigating elemental correlations in Ni hyperaccumulators have reported positive correlations between Ni and some elements (e.g., Ni with Co and Cr). Yang et al. (1985) reported a relatively weak positive Ni-Fe correlation. We found no significant correlations of Ni with other element concentrations when the analyses were done within species, but significant correlations when we used mean values for our species from differing Ni accumulation categories. This result suggests that uptake of all three metals (Fe, Ni, and Pb) differs based upon the Ni accumulation category of these species. We know of few physiological studies of Ni hyperaccumulators that have investigated the specificity of Ni transport and sequestration mechanisms. For example, Gabbrielli et al. (1991) reported competition of Ni with Co and Zn in roots of the Ni hyperaccumulator *Alyssum bertolonii* Desv (Brassicaceae). Assunção et al. (2003) reported variability of Cd, Ni, and Zn uptake among populations of *Thlaspi caerulescens* (Alpine Penny-cress) (Brassicaceae), and concluded this might be due to variable expression of multiple metal transporters.

    Elevated leaf metal levels also can result from the presence of dust on leaf surfaces (Reeves et al. 1999, 2007). Reeves et al. (2007) pointed out that samples containing more than 1500 mg Fe/kg (as well as high Cr and Ni) might indicate dust contamination. Fortunately, in our study, such high Fe values were found for only two species (Table 2), both hypernickelophores (*Hybanthus austrocaledonicus*, *Sebertia acuminata*). Although we did not wash our samples, Reeves et al. (1999, 2007) pointed out that dust contamination is not always readily removed in this way. We do not think the Ni-Fe correlation we observed across species is due to dust contamination, but it is possible that dust accumulation may have varied among species in such a way that those species with greatest Ni concentrations had greater amounts of dust on their leaves. This possibility seems improbable, however, given the relatively strong relationship we found between Ni and Fe and the fact that species with differing Ni accumulation categories were collected at each field site. Thus, it is unlikely that site-specific contamination differences would have resulted in the relationship we observed between Ni and Fe among species in different Ni accumulation categories.

    Metal hyperaccumulation may function as an elemental defense against plant natural enemies (Martens and Boyd 1994). Unfortunately, we do not know what metal concentration is sufficient to protect against plant natural enemies (Boyd 2004); it likely varies based on tissue and cellular level metal distribution in the plant, natural enemy feeding mode, and natural enemy physiology (Boyd 2007). For Ni, concentrations at hyperaccumulator levels suffice against some natural enemies but are ineffective against others (Jhee et al. 2006). In the context of plant elemental defense, our data suggest several conclusions. First, it is clear that leaf Ni concentrations vary greatly (1050-fold among old leaves in this study) among species growing on New Caledonian serpentine soils. If Ni is defensively valuable against some natural enemies, then some species are much better protected by this defense than others. Studies of organic chemical defenses in tropical forest trees have illus-

trated that variability in defense levels can help explain patterns of herbivore damage and plant defense strategies (Kursar and Coley 2003). It is also clear that, in general, leaf age does not significantly affect leaf Ni concentration (Table 3). Thus, in most species, young and old leaves are defended to an equal degree by this elemental defense. Exceptions did occur, such as *Geissois pruinosa* and *Homalium kanaliense* (Table 3), in which young leaves contained significantly less Ni and thus may have been less well defended. Similar cases of reduced Ni concentrations of young leaves have been reported by Boyd et al. (1999) for the New Caledonian hypernickelophore *Psychotria douarrei* and by Anderson et al. (1997) and Boyd et al. (2004) for the South African hypernickelophore *Berkheya coddii* Roessler (Asteraceae).

Our data are relevant to another aspect of elemental plant defense: combination effects of chemical defenses (Boyd 2007). Boyd (2004) hypothesized that elemental defenses may not act alone in generating defensive benefits to plants. The effects of one element may combine with those of another element, or with those of an organic defense compound, to generate a greater defensive effect in combination than either chemical alone. To our knowledge, the study of Jhee et al. (2006) is the only one to experimentally address this issue (using an artificial insect diet system). They found significant joint defensive effects between Ni and several other metals (Cd, Pb, Zn). Our data show that some New Caledonian species can have elevated concentrations of multiple metals, and we suggest these may enhance the defensive effect of Ni in some species. For example, we found: *Homalium kanaliense* with 9900 mg Ni/kg, 450 mg Co/kg, and 510 mg Zn/kg; *Hybanthus autrocaledonicus* with 20,000 mg Ni/kg, 7900 mg Mg/kg, and 150 mg Co/kg; *Casearia silvana* with 1100 mg Ni/kg, 12,000 mg Mg/kg, and 170 mg Cr/kg; and *Agatea longipedicillata* with 1000 mg Ni/kg and 11,000 mg Mg/kg (Table 2). Defensive effect enhancement by multiple metals may occur in two ways. First, a combination of metals may provide a greater defensive effect against a single natural enemy, as shown by Jhee et al. (2006) against a leaf-chewing insect. On the other hand, if enemies differ in their sensitivity to metals, each metal in a combination may provide a defensive benefit against a different natural enemy. In a hypothetical example, the high level of Co in *Homalium kanaliense* leaves may defend against a pathogenic bacterium, whereas the high level of Ni might defend against a folivorous insect. In this sense, a combination of metals may extend elemental defenses by being effective against a broader collection of natural enemies. Additional research testing these defensive effects, guided by the levels of metals revealed by our study, may illuminate these functions of metal accumulation by plants.

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