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Do Tropical Nickel Hyperaccumulators Mobilize Metals into Epiphytes? A Test Using Bryophytes from New Caledonia

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Abstract - Hyperaccumulator plants mobilize large amounts of certain elements from the soil into their tissues. Those elements then may be transferred to other organisms in those communities. Using a humid tropical forest site in New Caledonia, we tested whether epiphytes (mosses and liverworts) growing on Ni hyperaccumulator hosts contained greater levels of Ni (and seven other metals) than those growing on non-hyperaccumulator hosts. We selected two Ni hyperaccumulator species, *Psychotria douarrei* and *Hybanthus austrocaledonicus*, pairing individuals of each species with similar-sized non-hyperaccumulators and collecting epiphytes from each for elemental analysis. Samples of epiphytes and host plant leaves were analyzed for concentrations of eight metals (Co, Cr, Fe, Mg, Mn, Ni, Pb, and Zn). Two-way ANOVA was used to assess the influence of host type (hyperaccumulator or non-hyperaccumulator), epiphyte group, and the interaction term. Leaves of both Ni hyperaccumulator species had greater Ni concentrations than the paired non-hyperaccumulator species, but leaf concentrations of other metals (Co, Cr, Fe, Pb, and Zn) were higher as well in one or both cases. The strongest influence on epiphyte elemental composition was found to be the host type factor for Ni. Epiphytes collected from hyperaccumulator hosts had significantly greater Ni concentrations than those collected from non-hyperaccumulator hosts. Epiphyte Ni concentrations often exceeded the threshold used to define Ni hyperaccumulation (1000 μg/g), showing that some epiphytes (in most cases those growing on Ni hyperaccumulators) also hyperaccumulate Ni. Six of the epiphytes we analyzed, four liverworts (*Frullania ramuligera*, *Schistochila* sp., Morphotype #1 and Morphotype #13) and two mosses (*Calyptothecium* sp. and *Aerobryopsis wallichii*), had at least one specimen containing more than 1000 μg Ni/g and hence qualify as Ni hyperaccumulators. We conclude that Ni could move from Ni hyperaccumulator hosts to their epiphytes, either from leachates/exudates from tissues or from accumulated external dust, thus potentially mobilizing Ni still further into the food webs of these humid tropical forests.

Introduction

Plants are crucial members of terrestrial communities because they provide habitat for other species and supply the energy and most of the elements that flow through food webs. Element concentrations of plant tissues can vary among species by several orders of magnitude. Studies of plant Ni concentrations, measured in μg Ni/g dry mass, have identified species that

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accumulate extraordinary concentrations of Ni in their tissues. These species have been called Ni hyperaccumulators (Brooks et al. 1977). Reeves (1992) defined a Ni hyperaccumulator as a species for which at least one aboveground sample has been reported to contain at least 1000 μg Ni/g dry mass. Hyperaccumulation has been described for a number of other elements, including Cd, Co, Cr, Cu, Mn, Pb, and Zn (Reeves and Baker 2000), Al (Jansen et al. 2002), As (Ma et al. 2001), B (Babaoglu et al. 2004), Fe (Rodríguez et al. 2005), and Se (Brooks 1987). Hyperaccumulation of Ni is most common, however, as about 75% of all known hyperaccumulator species are Ni hyperaccumulators (Baker et al. 2000).

Hyperaccumulator plants may influence their communities by mobilizing elements from the soil into their tissues and thence to other species with which they interact (Boyd and Martens 1998, Quinn et al. 2007). This transfer can occur directly via herbivores that consume high-Ni plant tissues, as well as through other interactions between hyperaccumulators and members of their communities. For example, Boyd (2009) listed 15 species of insect that have been reported to contain at least 500 μg Ni/g on a whole-body dry-mass basis. These "high-Ni insects" are generally herbivores that feed on Ni hyperaccumulator plants and thus move Ni from producer to consumer trophic levels. Effects of hyperaccumulation on other species interactions involving Ni hyperaccumulators—such as detritivory (Gonçalves et al. 2007), decomposition (Boyd et al. 2008), elemental allelopathy (Morris et al. 2008), etc.—have rarely been investigated.

Epiphytism is a widespread and ecologically important species interaction (Benzing 1990). Epiphytes are often sensitive to host chemical composition and may absorb elements from their host (e.g., Zotz and Heitz 2001), thus participating in nutrient cycles in their communities. There is practically no information available on the ecological relationships between epiphytes and hyperaccumulator plants. Boyd and Martens (1998) suggested that Ni may move from hyperaccumulator plants to epiphytes that grow on them. Boyd et al. (1999) reported that a sample of leafy liverwort epiphytes removed from leaves of the New Caledonian Ni hyperaccumulator *Psychotria douarrei* (Beauvis.) Däniker contained a relatively high level of Ni (400 μg Ni/g). As far as we know, however, no study has yet performed a comparison of element levels of epiphytes collected from hyperaccumulator and non-hyperaccumulator plants.

Our study tested whether epiphyte metal levels were influenced by the hyperaccumulation ability of their host. We compared elemental concentrations of epiphytes collected from Ni hyperaccumulator and non-hyperaccumulator species at a New Caledonian humid forest site. We hypothesized that epiphytes growing on hyperaccumulator hosts would have elevated levels of Ni, and possibly other heavy metals such as Co and Cr that might also be at greater levels in Ni hyperaccumulator plants, when compared with epiphytes growing on non-hyperaccumulators.

Field-site Description

Our study took place in the Parc Provincial de la Rivière Bleue, which protects humid tropical forest (Jaffré and Veillon 1991) close to the southern end of Grande Terre (the main island). Much of the southern end of Grande Terre is covered by serpentine soils, which have relatively high concentrations of Ni and other metals (Jaffré 1980). The study location was a site in the Park called Kaori Géant, named for a very large *Agathis lanceolata* Lindl. (Araucariaceae) tree. This site has been used for several studies of Ni hyperaccumulator ecology (Boyd et al. 1999, Boyd and Jaffré 2001, Davis et al. 2001). Boyd et al. (1999) reported six Ni hyperaccumulator species grow at this site: *Psychotria douarrei*, *Hybanthus austrocaledonicus* (Vieill.) Schinz & Guillamin ex Melchior, and *Casearia silvana* Schltr. (Flacourtiaceae) grow in the shrub layer, and there are three Ni hyperaccumulator tree species: *Homalium guillainii* (Vieill.) Briq., *Geissois hirsuta* Brongn. & Gris (Cunoniaceae), and *Sebertia acuminata* Pierre ex Baillon (Sapotaceae).

Methods

Our study focused on two of the Ni hyperaccumulator species: the shrubs *Psychotria douarrei* and *Hybanthus austrocaledonicus*. Jaffré (1980) reported very high leaf Ni concentrations (high even among Ni hyperaccumulators) for these species, with values ranging from 15,000–26,000 μg Ni/g for *H. austrocaledonicus* and from 23,000–45,000 μg Ni/g for *P. douarrei*, making these species likely candidates for detection of Ni mobilization into epiphytes collected from them.

Psychotria and *Ficus* hosts

The first Ni hyperaccumulator species, the serpentine endemic shrub (Baker et al. 1985) *Psychotria douarrei*, was matched with the non-hyperaccumulator shrub *Ficus webbiana* Miq. (Moraceae). Both species are relatively small (<3 m tall) shrubs scattered in the understory of the forest. We haphazardly selected eleven *P. douarrei* shrubs and a like number of similar-sized and nearby *F. webbiana* shrubs. The trunk and branches of each shrub were examined for epiphytes, and samples of epiphyte morphotypes ("morphotype" being defined as an apparently distinct species using field characteristics) were collected from those epiphytes that could be easily separated from the host bark. We attempted to obtain at least 1 g of material from each morphotype sampled from an individual shrub. Because of variable abundance of epiphytes, the numbers of samples of each morphotype collected from each host species varied. To document element levels in host plant leaves, a sample of mature leaves was collected from each of 20 *P. douarrei* and *Ficus webbiana* shrubs for elemental analysis.

Samples of each morphotype also were collected for later identification to the lowest practical taxonomic level. Liverwort samples were identified by Barbara Thiers (New York Botanical Garden). Moss samples were identified by Bruce Allen and Marshall Crosby (Missouri Botanical Garden).

Some samples were not identified: these are listed by the morphotype number we assigned to them in the field.

Hybanthus/other hosts

The second Ni hyperaccumulator species used was *Hybanthus austrocaledonicus*, a shrub species 1–3.5 m tall (Kelly et al. 1975). Each of 18 individuals was paired with an individual of another woody plant species that does not hyperaccumulate Ni. Non-hyperaccumulator individuals included shrubs as well as saplings of tree species. The trunk of each plant (within 3 m of the ground) was examined for epiphytes and samples of epiphyte morphotypes were collected as described above for *Psychotria/Ficus* sampling. As with the *Psychotria/Ficus* study, additional epiphyte samples were also collected for submission to experts for identification.

A sample of mature leaves was collected from five *H. austrocaledonicus* individuals and from individuals of four of the five non-hyperaccumulator taxa for elemental analysis. Two of the non-hyperaccumulator taxa were not identifiable, but the other three were identified as *Dysoxylum roseum* (Baill.) C. DC. (Meliaceae), *Guettarda* sp. (Rubiaceae), and *Phyllanthus* sp. (Phyllanthaceae).

Rinsing test

Elemental analysis of plant samples can be complicated by dust adhering to the surface of samples (Reeves 1992), as well as by leaching of materials from samples that are washed or rinsed. We conducted a limited test of the effect of rinsing epiphyte samples on elemental analysis results. After removing material from the samples collected for the elemental analyses described above, five samples of a particularly abundant epiphytic liverwort (Morphotype #13) collected from *P. douarrei* had considerable dried material remaining. We combined that material into a single sample, tearing it into small pieces (<5 cm long), mixing it thoroughly, and subdivided it into six equal-sized portions. Each portion was placed into an envelope made of fine-mesh bridal veil material (about 30 mesh/cm) and the bags were stapled closed. Three bags were randomly selected for the rinsing treatment: each rinsed treatment bag was agitated gently for 1 minute in deionized water. Both rinsed and unrinsed bags were then oven-dried at 60 °C for 4 days. Contents of each bag were chopped finely using scissors (to pieces <1 cm long) and analyzed for element concentrations as described below.

Element analysis

Samples were finely ground, dry-ashed at 485 °C, additionally oxidized in 1 M HNO_3, and the residues dissolved in 1 M HCl. We analyzed concentrations of eight metals: Co, Cr, Fe, Mg, Mn, Ni, Pb, and Zn. An inductively coupled argon plasma spectrometer (Jarrell-Ash, ICAP 9000) was used to determine concentrations of all metals except Ni. Nickel concentrations were determined using an atomic absorption spectrophotometer (Instrumental Laboratory, IL 251).

Statistical analysis

Elemental concentrations of leaves of pairs of host taxa (*P. douarrei*/*F. webbiana*, *H. austrocaledonicus*/other) were compared with a *t*-test for each element. Epiphyte element concentrations were analyzed using two-way analysis of variance (ANOVA). If the ANOVA revealed a significant effect of any factor, Fisher's protected least significant difference (PLSD) test was used to compare mean values. Two-way ANOVA was used as the primary analysis because in each host study there were two experimental factors involved: host type (hyperaccumulator or non-hyperaccumulator) and epiphyte "group." For epiphytes from the *Psychotria/Ficus* study, epiphyte groups were Morphotype #13 and a composite group, "other epiphytes," made up of samples from various other epiphyte taxa. The latter group was formed because too few representatives of those taxa were present on both host species to allow a single taxon to be analyzed as a separate group. The taxa that composed this composite group, and the host(s) from which they were collected, are listed in Table 1. In the *Hybanthus*/other study, two relatively common epiphyte morphotypes (*Frullania ramuligera* and Morphotype #1) were found on both types of hosts, so that we were able to avoid creating a category made up of multiple morphotypes (as in the *Psychotria/Ficus* study). For the rinsing test, element concentration means were compared between rinsed and unrinsed samples using a *t*-test for each element analyzed.

Results

Rinsing test

Rinsing samples did not significantly decrease concentrations of any of the eight metals measured (Table 2).

Psychotria and *Ficus* hosts

Analysis of host leaves showed significant differences in element concentrations for most (six of eight) metals. All had greater concentrations in

Table 1. Identifications of samples of epiphytes included in the "other epiphytes" group for the *Psychotria*/*Ficus* hosts study and the number of samples of each epiphyte collected from each host.

	Number of samples collected	
Epiphyte	*P. douarrei*	*F. webbiana*
Mosses		
Calyptothecium sp. Mitter	1	3
Ectropothecium zollingeri (C. Müller) Jaeger	0	1
Warburgiella sp. C. Müller ex Brotherus	0	1
Liverworts		
Frullania ramuligera (Nees) Mont.	6	1
Unidentified		
Morphotype #1 (liverwort)	1	3
Morphotype #19	0	1
Morphotype #14	0	2

P. douarrei leaves than in *F. webbiana* leaves: means are shown in Table 3 for the three metals for which no significant differences were found in epiphytes (Co, Cr, Fe) between the two host trees, whereas the three metals for which epiphytes differed significantly from *Psychotria* leaves (Ni, Pb, Zn) are presented in Figures 1–3. *Psychotria douarrei* leaves contained 182-fold more Ni (Fig. 1), 13-fold more Co (Table 3), 6.5-fold more Pb (Fig. 2), 2.4-fold more Zn (Fig. 3), 1.8-fold more Fe (Table 3), and 1.7-fold more Cr (Table 3) than *Ficus webbiana* leaves.

Two-way ANOVAs of data from epiphyte samples showed no significance for either the host species or the epiphyte group factor, or the interaction, for five heavy metals: Co, Cr, Fe, Mg, and Mn. Significant effects of

Table 2. Metal concentrations of rinsed (1 minute in DI H_2O) and unrinsed samples of Morphotype #13 collected from the Ni hyperaccumulator *Psychotria douarrei*. Values are means (SE) expressed in μg/g; $n = 3$ for all means. *P*-value is result of comparing means using an unpaired *t*-test.

Metal	Unrinsed	Rinsed	*P*-value
Cr	48 (3.2)	43 (4.3)	0.379
Co	6.7 (3.7)	2.7 (0.88)	0.349
Fe	1800 (220)	1700 (150)	0.653
Mg	2500 (60)	2600 (0)	0.158
Mn	110 (9.2)	120 (5.5)	0.433
Ni	1700 (190)	1300 (35)	0.119
Pb	4.3 (2.3)	3.3 (3.3)	0.818
Zn	32 (2.0)	38 (1.7)	0.076

Table 3. Mean metal concentrations (μg/g, dry mass basis; SE in parentheses) of leaves of *Psychotria douarrei* (Ni hyperaccumulator) and *Ficus webbiana* (non-hyperaccumulator) and epiphytes collected from them. The column labeled *P* contains results of *t*-tests comparing elemental concentrations in mature leaves of the two host species. Composition of the "other epiphytes" category is presented in Table 1.

	Mature leaves ($n = 20$)			Morphotype #13		Other epiphytes	
Metal	*Psychotria*	*Ficus*	*P*	*Psychotria* ($n = 10$)	*Ficus* ($n = 7$)	*Psychotria* ($n = 10$)	*Ficus* ($n = 11$)
Co	17 (0.05)	1.3 (0.1)	<0.0001	5.2 (1.0)	4.9 (0.8)	6.6 (1.2)	4.5 (0.62)
Cr	12 (1.0)	7.1 (0.5)	<0.0001	34 (5.5)	41 (4.6)	36 (8.1)	35 (3.9)
Fe	210 (150)	120 (39)	0.015	1300 (220)	1500 (180)	1200 (330)	1300 (180)
Mg	6200 (130)	5900 (150)	0.19	2000 (64)	2000 (150)	2200 (190)	2200 (87)
Mn	42 (2.0)	45 (1.7)	0.32	82 (13)	80 (16)	100 (20)	69 (9.3)

Figure 2 (bottom of opposite page). Mean Pb concentrations of host leaves and epiphytes from the *Psychotria*/*Ficus* host comparison. Error bars are standard errors. Different capital letters show significant ($P < 0.0001$) differences between host leaves (*t*-test), and different lower case letters show epiphyte means that differ significantly (Fisher's PLSD test) at $P < 0.05$. Sample sizes (*n*) are: 20 for both *Psychotria* (hyperaccumulator) and *Ficus* (non-hyperaccumulator) leaves, 10 for Morphotype #13 from *Psychotria*, 7 for Morphotype #13 from *Ficus*, 10 for other epiphytes from *Psychotria*, and 11 for other epiphytes from *Ficus*. Morphotype #13 is abbreviated as "Morph 13" in the x-axis legend.

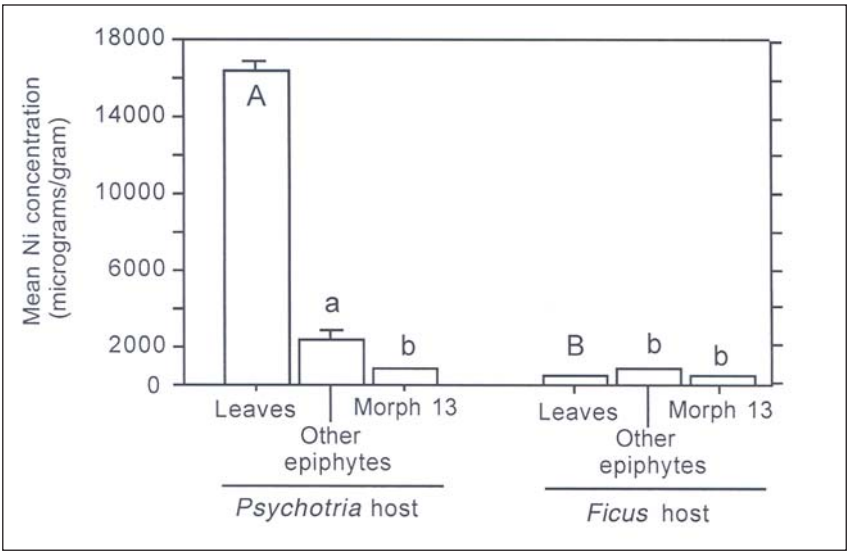
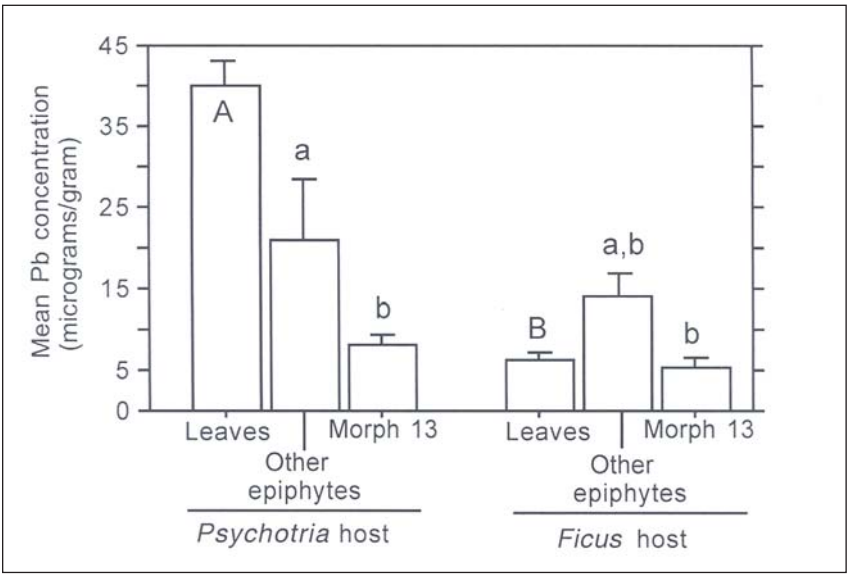


Figure 1. Mean Ni concentrations of host leaves and epiphytes from the *Psychotria*/*Ficus* host comparison. Error bars are standard errors and are absent when too small to be shown (values <200 µg/g). Different capital letters show significant ($P < 0.0001$) differences between host leaves (*t*-test), and different lower case letters show epiphyte means that differ significantly (Fisher's PLSD test) at $P < 0.05$. Sample sizes (*n*) are: 20 for both *Psychotria* (hyperaccumulator) and *Ficus* (non-hyperaccumulator) leaves, 10 for Morphotype #13 from *Psychotria*, 7 for Morphotype #13 from *Ficus*, 10 for other epiphytes from *Psychotria*, and 11 for other epiphytes from *Ficus*. Morphotype #13 is abbreviated as "Morph 13" in the x-axis legend.



at least one factor were found for three metals: Ni, Pb, and Zn. Of all the ANOVA results, the strongest statistical effect (indicated by the highest *F*-value) was for the host factor for Ni. The most complex results also were found for Ni, for which host species, epiphyte group, and the interaction all were statistically significant. Host species was significant because more Ni was found in samples from *P. douarrei*, and epiphyte group was significant because less Ni was found in Morphotype #13 than in the "other epiphytes" category (Fig. 1). The significant interaction term probably stemmed from differences in the relative values of Ni concentrations from the two host species: Morphotype #13 from *P. douarrei* contained 4.9-fold more Ni whereas "other epiphytes" contained 5.3-fold more Ni compared to samples taken from *Ficus webbiana* (non-hyperaccumulator) plants.

Two other metals (Pb and Zn) showed a significant result from the two-way ANOVAs. In both cases, host was not a significant factor, but epiphyte group was. Values of both Pb (Fig. 2) and Zn (Fig. 3) were greater in samples from the "other epiphytes" category than for samples of Morphotype #13.

Hybanthus/other hosts

Comparison of host leaf metal concentrations showed differences for five metals (Co, Mg, Mn, Ni, and Zn; Table 4). Means are shown in

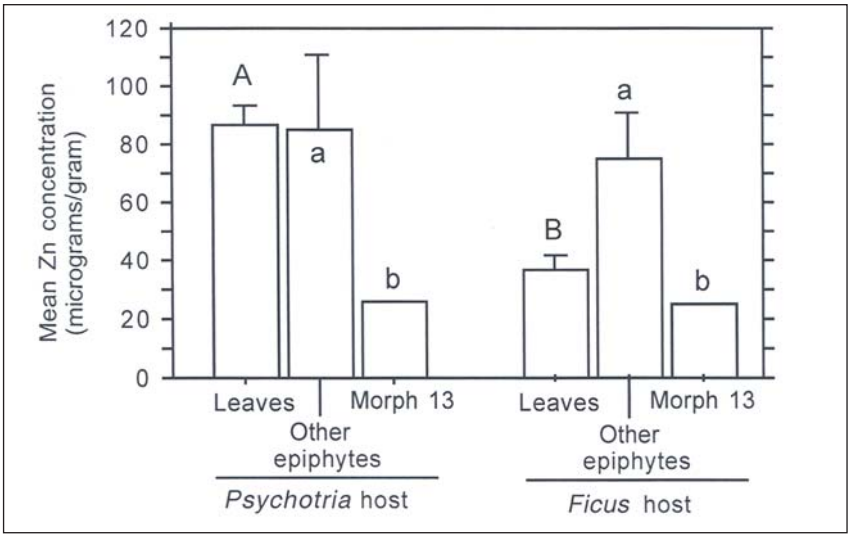


Figure 3. Mean Zn concentrations of host leaves and epiphytes from the *Psychotria*/*Ficus* host comparison. Error bars are standard errors and are absent when too small to be shown (values <2 µg/g). Different capital letters show significant ($P < 0.0001$) differences between host leaves (*t*-test), and different lower case letters show epiphyte means that differ significantly (Fisher's PLSD test) at $P < 0.05$. Sample sizes (*n*) are: 20 for both *Psychotria* (hyperaccumulator) and *Ficus* (non-hyperaccumulator) leaves, 10 for Morphotype #13 from *Psychotria*, 7 for Morphotype #13 from *Ficus*, 10 for other epiphytes from *Psychotria*, and 11 for other epiphytes from *Ficus*. Morphotype #13 is abbreviated as "Morph 13" in the x-axis legend.

Table 4 for metals for which no significant differences were found in epiphytes (i.e., all except Ni), whereas Ni concentrations are presented in Fig. 4. In all cases, concentrations were greater in *H. austrocaledonicus* leaves than in leaves of "other hosts:" 63-fold for Ni (Fig. 4), 27-fold for

Table 4. Metal concentrations (μg/g, dry mass basis; SE in parentheses) of leaves of *Hybanthus austrocaledonicus* (Ni hyperaccumulator) shrubs, non-hyperaccumulators and epiphytes collected from each category of tree. The column labeled *P* contains results of *t*-tests comparing elemental concentrations in mature leaves of the two host categories.

	Mature leaves			Epiphyte taxa			
				Frullania ramuligera		Morphotype #1	
Metal	*Hybanthus* (*n* = 4)	Other (*n* = 3)	*P*	*Hybanthus* (*n* = 18)	Other (*n* = 3)	*Hybanthus* (*n* = 4)	Other (*n* = 3)
Co	56 (30)	2.1 (3.4)	0.028	11 (4.5)	15 (6.4)	8.4 (3.5)	15 (6.8)
Cr	85 (28)	6.5 (8.8)	0.065	97 (41)	140 (46)	59 (24)	100 (36)
Fe	210 (36)	280 (240)	0.72	4200 (2100)	6300 (2100)	2100 (1400)	4700 (1800)
Mg	7200 (250)	3500 (900)	0.0061	2400 (290)	1900 (240)	2100 (260)	2100 (230)
Mn	300 (48)	45 (20)	0.0078	150 (38)	170 (68)	110 (30)	170 (53)
Pb	<0.05	<0.05	-	11 (2.3)	9.1 (1.7)	7.6 (1.8)	7.0 (1.8)
Zn	82 (6.1)	14 (2.1)	0.0003	42 (3.6)	37 (16)	27 (4.3)	22 (2.9)

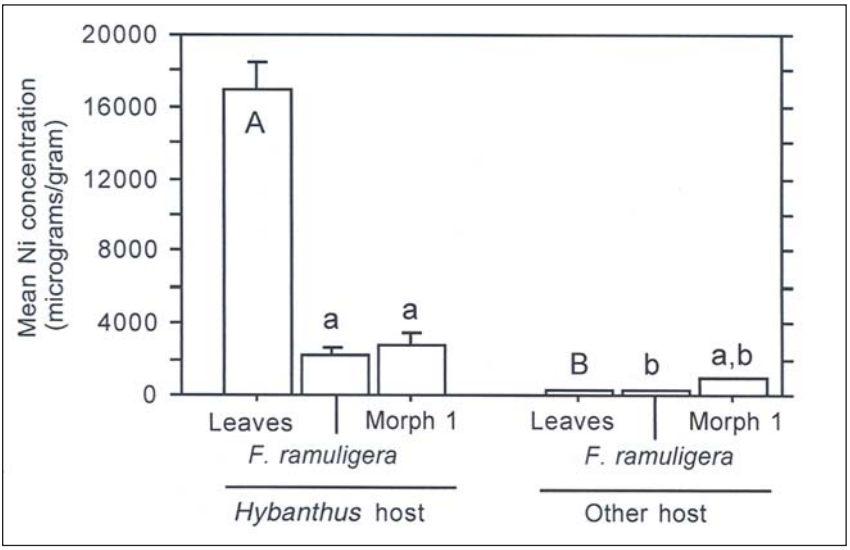


Figure 4. Mean Ni concentrations of host leaves and epiphytes from the *Hybanthus*/other host comparison. Error bars are standard errors and are absent when too small to be shown (values <280 μg/g). Different capital letters show significant ($P < 0.0001$) differences between host leaves (*t*-test), and different lower case letters show epiphyte means that differ significantly (Fisher's PLSD test) at $P < 0.05$. Sample sizes (*n*) are: 4 for *Hybanthus* (hyperaccumulator) leaves and 3 for other host leaves, 18 for *Frullania ramuligera* from *Hybanthus,* 3 for *F. ramuligera* from other hosts, 4 for Morphotype #1 from *Hybanthus,* and 3 for Morphotype #1 from other hosts. Morphotype #1 is abbreviated as "Morph 1" in the x-axis legend

Co (Table 4), 6.7-fold for Mn (Table 4), 5.9-fold for Zn (Table 4), and 2-fold for Mg (Table 4).

Two-way ANOVAs of data from epiphyte samples showed only one significant result, much fewer than for the *Psychotria/Ficus* hosts study. The significant result was for the host species factor for Ni. Nickel concentrations were 7.6-fold greater for *Frullania ramuligera* collected from *H. austrocaledonicus* compared to samples collected from *Ficus webbiana* (Fig. 4). For Morphotype #1, samples from *H. austrocaledonicus* had 2.9-fold greater Ni concentrations, but this difference was not significantly different (Fig. 4). Host species was a significant factor for none of the other seven metals analyzed.

Ni-hyperaccumulator bryophytes

Examination of the data from both studies allowed us to identify epiphyte taxa that meet the definition of Ni hyperaccumulator (Reeves 1992): collection of at least one sample from the field with a Ni concentration of 1000 μg Ni/g or greater. We surveyed our Ni analysis results from all epiphyte taxa and found six taxa to meet this definition (Table 5), including four leafy liverworts and two mosses. Two taxa (*F. ramuligera* and Morphotype #1) were collected from both Ni hyperaccumulator hosts, and both of these epiphyte taxa had at least some samples with Ni concentrations

Table 5. Epiphytes analyzed during this study in Parc Provincial de la Rivière Bleue (New Caledonia) that qualify for Ni hyperaccumulator status (based upon at least one field-collected sample containing >1000 μg Ni/g). The "data summary" column describes the Ni concentration data upon which hyperaccumulator status is based.

Taxa	Data summary
Liverworts	
Frullania ramuligera (Nees) Mont	Maximum values 4320 μg Ni/g from *P. douarrei*, 5120 μg Ni/g from *H. austrocaledonicus.*
Schistochila sp. Dumortier	Maximum value 1005 μg Ni/g from *H. austrocaledonicus*, not collected from *P. douarrei.*
Morphotype #1	Maximum values 4300 μg Ni/g from *P. douarrei*, 4460 μg Ni/g from *H. austrocaledonicus*. In the *Psychotria*/*Ficus* hosts study, two samples from *F. webbiana* had >1000 μg Ni/g (maximum value was 1700 μg Ni/g). In the *Hybanthus*/Other hosts study, two samples from Other hosts had >1000 μg Ni/g (maximum value was 1400 μg Ni/g).
Morphotype #13	Maximum value 1500 μg Ni/g from *P. douarrei*, not collected from *H. austrocaledonicus*.
Mosses	
Calyptothecium sp. Mitten	Maximum value 2700 μg Ni/g from *P. douarrei*, not collected from *H. austrocaledonicus*.
Aerobryopsis wallichii (Bridel) Fleischer	Maximum value 1005 μg Ni/g from *H. austrocaledonicus*, not collected from *P. douarrei.*

(>4000 μg Ni/g: Table 5) that were well above the hyperaccumulation definition threshold of 1000 μg Ni/g. For one epiphytic taxon (Morphotype #1), hyperaccumulator Ni concentrations were found for epiphyte samples collected from non-hyperaccumulator hosts. We note in Table 5 four samples of Morphotype #1 containing >1000 μg Ni/g which were collected from non-hyperaccumulator hosts.

Discussion

Measurements of elemental concentrations in plants can be complicated by adherence of dust to specimens (Reeves 1992). Since our study was conducted in a humid tropical forest, we suspect that dust contamination was less than in other (less rainy) habitats (Lee et al. 1977). Furthermore, our rinsing test did not show a significant reduction in concentration of the metals examined. This finding is similar to the result of Shotbolt et al. (2007), who found that Ni was removed much less (median loss 16%) than elements such as K during washing of herbarium samples of mosses. Our primary goal was to measure heavy metal values in epiphytes, and our finding of no significant rinsing effect on metal concentrations suggests that easily removable dust contamination did not contribute significantly to the metal values we measured in epiphytic bryophytes.

One weakness of our experimental approach for the *Psychotria*/*Ficus* hosts study was our combining epiphytes of a number of species into the "other epiphyte" category (Table 1). While we cannot show that the species being combined respond equivalently to host metal concentrations, the data for the composite samples showed a similar trend for Ni when compared to the data for Morphotype #13: samples collected from *Psychotria* had higher Ni concentrations (Fig. 1). The same result (a significant effect of host on epiphyte Ni concentrations) is also shown by the *Hybanthus*/other hosts study, bolstering our contention that host Ni concentration and epiphyte Ni concentration are causally connected. In each study, the strongest statistical effects (signified by the greatest *F*-value) measured in our two-way ANOVAs, for all metals examined, were for the host species factor for Ni concentration. This strong influence of host species on Ni concentration was supported by our analysis of element concentrations in host leaves, for which the greatest difference among all elements quantified was found for Ni concentration (Tables 3, 4; Figs. 1–4). We contend that it is likely that the elevated Ni in the epiphytes came from their hosts, either by leachates/exudates from tissues, or by washing from the leaves and transfer it through stem flow, but recognize we have not shown direct transfer of Ni. In fact, in some cases, we found very high Ni values for epiphytes from non-hyperaccumulator hosts (Table 5). One possible explanation for the latter finding is that we did not control for the distribution of overstory hyperaccumulators in the forest stand we studied. Because this site hosted several hyperaccumulator tree species, it is possible that litterfall or drip from overstory trees (Bates 1993) may carry Ni onto some non-hyperaccumulator shrubs in the understory, where it may be absorbed by epiphytes.

Other pathways of Ni entry into bryophytes, including through deposition of dust or through fungal connections between hosts and bryophytes (e.g., Wells and Boddy 1995), are also possible.

Studies of the movement of pollutants (including metals) through food webs may find bioaccumulation (Laskowski 1991), which occurs when concentrations at a higher trophic level are greater than those at a lower trophic level. Although we found some samples of epiphytic bryophytes with hyperaccumulator levels of Ni, those levels were less than the Ni concentrations of leaves of the host species. Mean Ni values for leaves of both *H. austrocaledonicus* and *P. douarrei* were >16,000 µg Ni/g, whereas the greatest mean in any bryophyte sample was the 2800 µg Ni/g for Morphotype #1 collected from *H. austrocaledonicus* (Fig. 4). We did not collect bark samples from our host species, which probably would have been a more ecologically relevant measure of host Ni levels, but bark Ni levels also are reported to be high for these Ni hyperaccumulator species. Jaffré (1980) reported Ni values from *P. douarrei* bark ranging as high as 80,000 µg Ni/g, and values of 14,000 µg Ni/g for *H. austrocaledonicus* bark. Comparing either the leaf or bark Ni values to those we found in epiphytes, our results reveal no bioaccumulation of Ni in the epiphytes (defined as greater concentration in epiphyte than in host). This finding is in marked contrast to results reported by Lee et al. (1977), in which mean Cr concentrations of the New Caledonian moss *Aerobyropsis longissima* (Doz. et Molk.) Fleisch collected from the Ni hyperaccumulator *Homalium guillainii* (Vieill.) Briq. were 12-fold greater than the Cr concentrations of the host bark.

We found no metals other than Ni for which the host species factor had a significant effect on epiphyte element concentration. This result was despite our finding of statistically significant differences between host species in leaf metal concentrations of other metals, with more metals being found in the hyperaccumulators (Tables 3, 4: Figs. 1–4). Specifically, both hyperaccumulators had significantly more Co and Zn than the non-hyperaccumulators examined. We also found significantly more Mn in *H. austrocaledonicus*, compared to the "other host" category, and significantly more Pb, Fe, and Cr in *P. douarrei* than in *F. webbiana*. Concentrations of Ni were 182-fold (for *P. douarrei*) and 63-fold (for *H. austrocaledonicus*) more for the hyperaccumulators, whereas the next greatest difference was for Co (27-fold for *H. austrocaledonicus*, 13-fold for *P. douarrei*).

We found significant effects of epiphyte type (group or species) for at least one element in each of our studies. While it is not surprising that epiphyte species vary in elemental concentrations, it is interesting that they vary in metal concentrations when growing on the same host as this implies they have differing abilities to take up metals from their habitats. Other bryophytes (e.g., "copper mosses") are reportedly confined to areas containing high amounts of Cu and other heavy metals (Persson 1956). Our study was not extensive enough to determine if there are bryophytes that might be

restricted to Ni hyperaccumulator hosts, but this is an interesting question that should be explored in this habitat.

Our study documented the existence of six Ni hyperaccumulating bryophytes (Table 5). In defining Ni hyperaccumulator bryophytes, we have adopted the definition developed for vascular plants by Brooks et al. (1977) and Reeves (1992). Whether that definition is useful for bryophytes is an open question, for at least two reasons. First, the definition was developed based upon surveys of many vascular plant taxa: this knowledge base allowed recognition of 1000 μg Ni/g as a particularly high level of Ni in vascular plants. Similarly extensive data have not, to our knowledge, been generated for bryophytes, although our brief examination of the literature indicates that Ni values in mosses of more than a few hundred μg Ni/g are unusual (e.g., Empain 1985). Second, there are physiological differences in uptake processes between mosses and vascular plants (Bates 2000). Some authors have reported that dead bryophytes take up significant amounts of metals (e.g., Gstoettner and Fisher 1997). Other studies (e.g., Salemaa et al. 2004) have pointed out that, due to the differences between mosses and vascular plants, mosses may have greater metal concentrations than vascular plants in the same polluted habitat. Thus the same concentration of metal in plant tissues may not represent a similar environmental response for a bryophyte. Nevertheless, relative to vascular plants, these six bryophytes were hyperaccumulators.

In summary, our data suggest that elemental hyperaccumulation by plants may influence nutrient cycles by mobilizing Ni into epiphytes. Mobilization of Ni has been reported into the insect community of Ni hyperaccumulator plants (e.g., Boyd et al. 2006, Peterson et al. 2003), but to our knowledge this is the first report for epiphytes. The ramifications of these findings for other community components or processes are not known. For example, Ni hyperaccumulation may be an "elemental" plant defense (Boyd 2007) and, if so, the high levels of Ni in some epiphytes may defend them from their natural enemies. Similarly, hyperaccumulated Ni may be involved with drought resistance in some hyperaccumulator plants (Bhatia et al. 2005), although this is still an open question (e.g., Whiting et al. 2003), but this also might be a function for hyperaccumulated Ni in bryophytes. Furthermore, the distinctive chemical signature of Ni hyperaccumulators may influence their suitability as hosts for epiphytes, in which case epiphyte community composition may differ between hyperaccumulator and non-hyperaccumulator hosts. Further studies are needed to explore these possibilities.

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