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HYPERACCUMULATION, COMPLEXATION AND DISTRIBUTION OF NICKEL IN *SEBERTIA ACUMINATA**

SILVIA SAGNER†, RALF KNEER†, GERHARD WANNER‡, JEAN-PIERRE COSSON§, BRIGITTE DEUS-NEUMANN† and MEINHART H. ZENK†¶

† Lehrstuhl für Pharmazeutische Biologie, Universität München, Karlstrasse 29, D-80333 München; ‡ Botanisches Institut der Universität, Menzinger Strasse 67, D-80638 München, Germany; § C.N.R.S., I.C.S.N., F-91190 Gif-sur-Yvette, France

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Key Word Index—*Sebertia acuminata*; Sapotaceae; nickel hyperaccumulation; nickel complexation; nickel distribution; repellent effect.

Abstract—The nickel content in different parts of the hyperaccumulating tree *Sebertia acuminata* was analysed by atomic absorption spectroscopy. Nickel was found to be mainly located in laticifers. The total nickel content of a single mature tree was estimated to be 37 kg. By gel filtration and NMR spectroscopy, citric acid was unequivocally identified as counter ion for about 40% of this metal present. Nitrate was assumed to be a further partner for a complete ionic balance. Phytochelatins were not found to be involved in nickel detoxification in *Sebertia*. The localization of nickel complexes inside the laticifers was demonstrated by light microscopy as well as by scanning electron microscopy in combination with an EDX system for the analysis of elements. A repellent effect of the plant sap was observed on the fruit fly *Drosophila melanogaster* indicating that in hyperaccumulating plants nickel functions as an agent to prevent predation.

INTRODUCTION

Heavy metals, i.e. elements with a specific weight of higher than 5 g cm^{-3}, are found in soils all over the Earth. These elements are localized mainly in rocks and, as a consequence of weathering, continuously accumulate in the soil. Moreover, human activities such as industrial production lead to high anthropogenic emission of heavy metals into the biosphere [1].

While some metal ions such as Cu^{2+} and Zn^{2+} are essential micronutrients for plants, the same heavy metals, and even more potent the ions of Cd, Hg, Ag etc., can accumulate in the soil in phytotoxic concentrations. Nevertheless, these contaminated soils are not completely free of vegetation. In fact, plants growing in these environments have evolved mechanisms to tolerate toxic heavy metals [2, 3].

The complexation of heavy metal ions by phytochelatins is the general detoxification mechanism in higher plants [4, 5]. Most heavy metals were found to be active in provoking the synthesis of phytochelatins [6]. Therefore, these peptides seem to be responsible for an immediate and general detoxification of toxic metal ions.

Reduced uptake [7] and active efflux of metal ions or their deposition in the cell wall [8] can prevent the accumulation of toxic metal concentrations inside the cells. In contrast, a number of terrestrial plants are known which are able to accumulate large quantities of metals such as zinc, manganese, nickel, cobalt and copper [9]. The property of heavy metal accumulation is widespread among the members of the plant kingdom [10, 11] and the amount of heavy metal accumulation varies significantly. Contents of more than 1% of dry material can be found. For example, nickel accumulations in *Psychotria douarrei* approach 5% of dry weight [12]. This plant, therefore, belongs to the group of the so-called 'hyperaccumulators', that means plants which accumulate nickel in concentrations exceeding 0.1% of above-ground dry biomass in field samples [9, 11, 13, 14]. These hyperaccumulators are mainly found on ultramafic serpentine soils, which are characterized by their high content of magnesium ('ma'), iron ('f'), nickel, and chromium [15]. Plants that are growing in such an environment must be extraordinarily resistant against nickel. The highest nickel content ever determined in any plant tissue was found in a tree, *Sebertia acu-*

* Dedicated to Prof. B. Parthier, Halle, on the occasion of his 65th birthday.

¶ Author to whom correspondence should be addressed.

minata [16], which is endemic to serpentine soils of New Caledonia, a region with a versatile vegetation of hyperaccumulating plants [17]. The dried latex of *S. acuminata* contained 25.74% nickel while 1.17% was found in dry leaf material [16].

How can a plant live with such enormous and potentially toxic heavy metal concentrations? Since metal hyperaccumulation in plants was discovered, it has been assumed that the ability to tolerate toxic concentrations is feasible by production of an appropriate high-affinity ligand [9]. In fact, Lee *et al.* found complexes of nickel with citric acid in leaf extracts and in the latex of *S. acuminata* [18, 19], a first indication for a possible role of this tricarboxylic acid in the complexation of heavy metals inside the plant cell. Later, a nickel-malate complex was found in the hyperaccumulator *Psychotria douarrei* [20]. In *Alyssum* leaf extracts, malic acid was supposed to bind nickel and transport it into the vacuole, where it could be bound to other acids [21]. In addition to tricarboxylic acids, nickel can be sequestered by amino acids as shown for nickel-resistant strains of *Saccharomyces cerevisiae* [22]. It could be demonstrated that histidine plays an important role in the complexation of nickel inside the yeast vacuole. Furthermore, Krämer *et al.* have shown recently that histidine functions as a metal chelator in nickel hyperaccumulating species of *Alyssum* [9].

The aim of our work was to find clues to the mechanism of nickel complexation in the hyperaccumulating species *S. acuminata* grown on a serpentine soil from the Rivière Bleue in New Caledonia. Especially the chelating agent of nickel should be verified and the nickel complexes within the plant tissue under investigation should be localized. Furthermore, a possible function of Ni^{2+} for the plant should be elucidated.

RESULTS

Analysis of soil samples

The plant material analysed in the course of this study was collected in the region of the Rivière Bleue in New Caledonia. In this place mainly serpentine soil is found with low content of plant nutrients like nitrogen, phosphate, potassium and calcium. Magnesium and silica are almost completely washed out. This soil is extraordinarily poor in nutrients [15] and allows only a specialized vegetation to survive.

A surface soil sample from this region in the immediate vicinity of a *S. acuminata* tree was analysed for its metal content by atomic absorption spectroscopy (AAS) and the results expressed as percentage of the dry soil material. About one third of the soil sample consisted of iron (36%), while the contents of chromium, nickel, magnesium and manganese amounted to 1.5, 0.7, 0.2 and 0.5%, respectively. The AAS analysis thus confirmed that the plant material collected had been grown on ultramafic soil with a high content of nickel.

Table 1. Heavy metal distribution in vascular bundles of *Sebertia acuminata* branches

	Heavy metal content (% dry wt)			
	Ni	Fe	Zn	Cu
Phloem and latex	1.15	0.01	0.006	0.001
Xylem	0.13	0.006	0.0008	0.001

Metal content in Sebertia acuminata

Sebertia acuminata Pierre ex Baill. is a rare tree species belonging to the Sapotaceae. It is endemic to New Caledonia where it is found mainly in the rainforest of the Grand Massif du Sud of the island along the Rivière Bleue. The tree reaches a height of about 15 m with little white flowers in March. It produces oval greenish-brown fruits (berries) having a length of 5–6 cm and containing the seed with about 2.5 cm in length.

Different parts of *S. acuminata* were analysed separately for their metal content. Table 1 shows the distribution of nickel, iron, zinc and copper in the phloem including the latex and in the xylem, respectively. The metals investigated were mainly accumulated in the inner bark of the tree, which contains the phloem with laticifers. Except for copper, the metal content of the xylem was distinctly lower.

Although spectral analysis of the soil, on which the tree under investigation grows, had demonstrated that this plant has to deal with extremely large quantities of iron, the examination of the plant material showed that only nickel accumulated in the phloem to an unusual and normally phytotoxic extent of 1.15% dry weight.

The total nickel content of deep frozen *Sebertia* fruits was determined to be 0.5%. The distribution of nickel among parts of a berry is depicted in Fig. 1. Except in the embryo, whose dry weight was too small for metal analysis, nickel was found in all parts of the fruit. The highest amount of 1.4% dry weight was measured in the rudimentary endosperm. While the seed peel contained 0.1%, the nickel content of all other fruit parts analysed was distinctly higher.

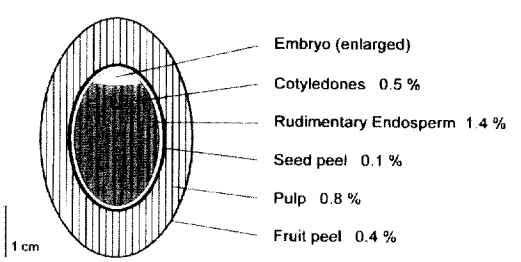

Fig. 1. Distribution of nickel (as % dry wt) in fruits of *Sebertia acuminata*. Because of the small size of the embryo its nickel content could not be determined.

Fruit and latex of the *Sebertia* tree was already analysed by Jaffré *et al.* [16] and the latex was found to have an extremely high nickel content of 25.74%. To verify this finding in the tree under investigation, the isolated latex was examined by AAS. In our study we determined a somewhat lower nickel content of 18.5%. The difference between the data of both studies may reflect variations between individual *Sebertia* trees. Nevertheless, the nickel amount found now in the latex confirmed that *S. acuminata* is not only a hyperaccumulating plant but belongs to the so-called 'hypernickelophors', a name coined by Jaffré [23] for plants that contain nickel in concentrations of more than 1% of dry material. The nickel content in different parts of *S. acuminata* as determined in our investigations compared with the nickel content of the respective soil is depicted in Fig. 2. The highest amount of nickel, by far, was accumulated in the latex of the tree which seems to be the main storage site for this metal.

On the basis of total biomass, the nickel content of a *Sebertia* tree with a height of 15 m and an estimated weight of 1980 kg (Prof. H.-D. Löffler, Faculty for Forestry, University of Munich, pers. comm.) was calculated to be 37 kg. What mechanism allows the plant to accumulate such a high quantity of nickel?

Investigation of the latex

To prove whether phytochelatins might participate in the sequestration of nickel in *Sebertia*, samples of the latex were subjected to HPLC analysis [6, 24]. Neither phytochelatins nor glutathione were, however, found in the latex. In addition, phytochelatin synthase, isolated from *Silene cucubalus* cell cultures, is not able to chelate Ni ions in the presence of glutathione (A. Hochberger and M. H. Zenk, publication in preparation). Phytochelatins, therefore, are not responsible for the detoxification of nickel in hypernickelophors.

Lee *et al.* had already found that nickel–citrato-complexes were involved in the complexation of nickel in *S. acuminata* [18, 19]. To confirm this assumption, the latex was analysed for its citrate content using the enzyme citrate lyase. In several independent assays an average citrate content of 0.6 M was determined.

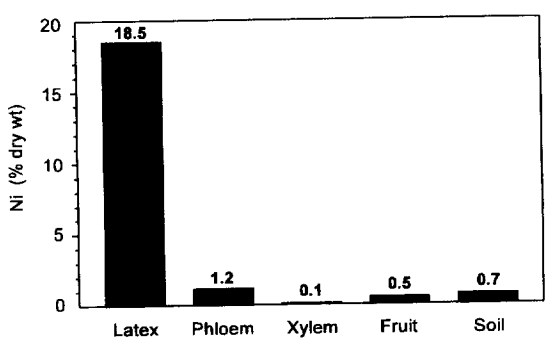

Fig. 2. Nickel content in latex and different parts of *Sebertia acuminata* compared with the nickel content of the respective soil.

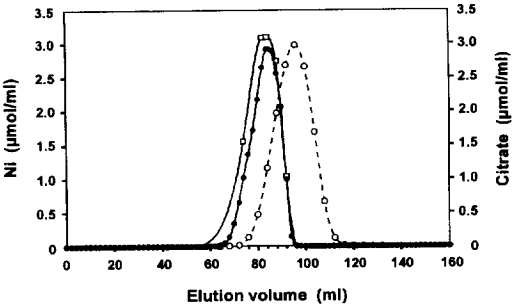

Fig. 3. Elution profile of *Sebertia acuminata* latex on Sephadex G10. A 100 µl aliquot of the latex was diluted with H_2O (1:10), applied to the column (50 × 3 cm) and eluted with double-distilled H_2O at a flow rate of 40 ml hr^{-1} (nickel, ●—●; citrate □—□). Fractions of 2 ml were collected and analysed for their nickel and citrate content. Free nickel ions (○—○) subsequently eluted with 20 mM citrate.

In order to examine whether nickel is indeed complexed by citrate, 100 µl of a diluted (1:10) latex sample was subjected to gel filtration on a Sephadex G10-column. Fractions of 2 ml were collected and analysed for their nickel and citrate content (Fig. 3). Prior to this analysis, the column had been calibrated using free nickel ions as well as a mixture of nickel and citrate (1:10). The nickel citrate complex eluted around fraction 88 ml (with H_2O) while free nickel ions were eluted with 20 mM citrate around fraction 96 ml.

Table 2 compares the nickel content determined in the latex of *S. acuminata* with the amount of nickel bound to citric acid and the concentration of free nickel ions. This nickel balance confirms that nickel is, indeed, sequestered by citric acid although the complexation was, however, not complete and about half of the nickel was eluted in free form.

The presence of citric acid in nickel complexation was confirmed by ^{13}C NMR spectroscopy of the *Sebertia* latex. Latex samples were subjected to ion exchange chromatography (DOWEX 1-X8), the organic acid fractions desorbed with formic acid and subjected to ^{13}C NMR analysis (Fig. 4). The NMR spectra of both sample and reference coincided very well showing the following characteristics: ^{13}C NMR (D_2O): δ(ppm) = 44.32 (C4), 74.30 (C3), 174.41 (C2), 177.75 (C1). Only a minor impurity could be detected (δ(ppm) = 50.60), which, however, would by far not

Table 2. Sephadex G10-nickel-balance of *Sebertia acuminata* latex

Ni in latex	1188 mM	100%	Elution at
Ni, citrate bound	437 mM	37%	84 ml
Ni, free	589 mM	50%	96 ml
Ni, total recovered	1026 mM	87%	

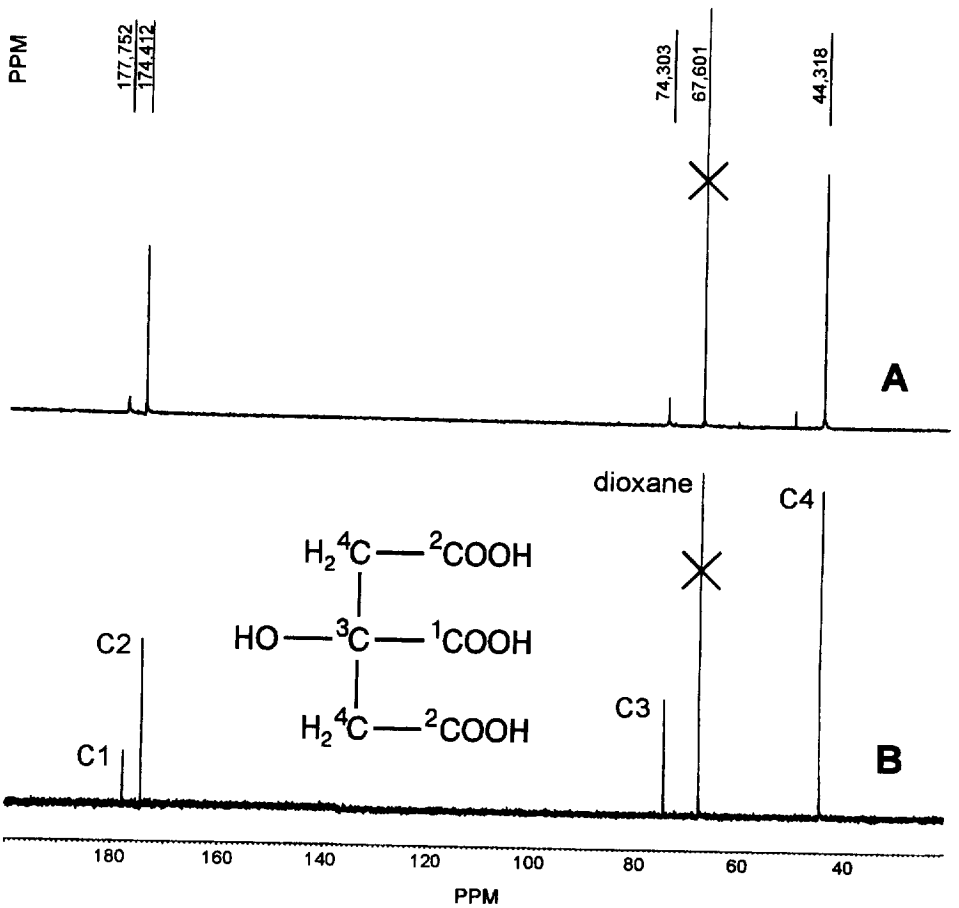

Fig. 4. ^{13}C NMR spectra of citric acid. A, isolate from the latex of *Sebertia acuminata*; B, authentic reference sample.

be sufficient to counterbalance 50% of the free Ni ions.

These data confirmed citric acid to be the ligand of nickel for complexation in *S. acuminata*. The complex formation of nickel (II) ions with citric acid in aqueous solutions was studied by Hedwig *et al.* [25], who characterized the equilibrium constants of four possible complexes. The main complex for metal binding at the physiological pH of the latex will be the negatively charged $[Ni\ cit]^-$. Minor concentrations of $[Ni\ cit_2]^{4-}$ will only be important when the total citrate will be in excess. Calculating the binding capacity of citric acid at pH 6.6, 0.6 M citric acid is only able to bind a maximum of 0.9 molar equivalents of Ni^{2+}, while a total of 1.2 M nickel ions were found in the plant sap. Since further organic acids do not seem to participate in nickel complexation [19] another ligand must be present as a counter ion for Ni^{2+}.

Possible partners for complexation could be inorganic anions but analysis of the latex proved that neither chloride, sulphate nor phosphate is present in appreciable quantities and, therefore, can be excluded as counter ions for Ni^{2+}. The presence of nitrate in the latex could be demonstrated using brucine reagent and subsequent photometrical determination. An exact quantitation of the nitrate content, however, was not possible due to interference of the assay used with Ni ions. Nevertheless, the presence of nitrate in a concentration of about 0.6 M would make this anion a possible partner in the complexation of nickel in *S. acuminata* latex.

Localization of nickel in the tissue

The nickel analysis of *S. acuminata* tissue had already indicated that the metal is mainly localized in the phloem with its laticifers and the latex showing the highest concentration of nickel present in this plant (see Fig. 2 and Table 1). Microscopic investigations of the plant material should prove these results. In preliminary studies, shoot tissue of *S. acuminata* was examined by light microscopy. To visualize nickel inside the cells, the tissue was stained with dimethylglyoximine (DMG), a dye which sequesters nickel by forming scarlet complexes. DMG was already successfully used by Vergnano Gambi [26] to locate nickel in the stems of the serpentinophyte *Alyssum bertolonii*.

As expected, the red staining of nickel-DMG-complexes was exclusively seen in the phloem cells. The localization of nickel in the phloem was, therefore, also confirmed by microscopic observation.

For detailed studies avoiding ion migration, plant

material was cryofixed and examined in a scanning electron microscope on a cryo stage. From structural observations, the laticifers can be identified by a characteristic feature: due to their lipophilic content the fracture plane of laticifers was characteristically structured, compared to the cells of the phloemic and cortical tissue. After stimulation of X-ray emission at various kV the characteristic spectra were used for identification of element composition. After EDX-mapping of stem cross fractures of *S. acuminata* it was striking that nickel was located predominantly in the laticifers of the phloemic tissue (Fig. 5). The high resolution EDX-point analysis of a laticifer is depicted in Fig. 6. Three characteristic peaks in the spectrum of elements clearly confirmed the presence of nickel in high quantities in *Sebertia* laticifers. In addition, the significant Ca signal confirms the presence of most likely Ca oxalate crystals because of their crystal structure, which are abundant in the cortical tissue of *S. acuminata* as visualized by light microscopy. These results confirm that Ni^{2+} is taken up selectively and actively from soil by *S. acuminata*. The heavy metal ion is then stored as a citrate complex, possibly also with nitrate as a counter ion, mainly in the latex of the laticiferous cells which are part of the phloem of that plant. One of the pressing questions remains, what is the function of Ni^{2+} hyperaccumulation in that plant species?

Why nickel hyperaccumulation?

One reason for the hyperaccumulation of metals is clearly the adaption mechanism which permits growth on heavily metal-contaminated soils [27]. An additional or even primary reason may be the function of the metal as a "cheap" toxin that deters predation

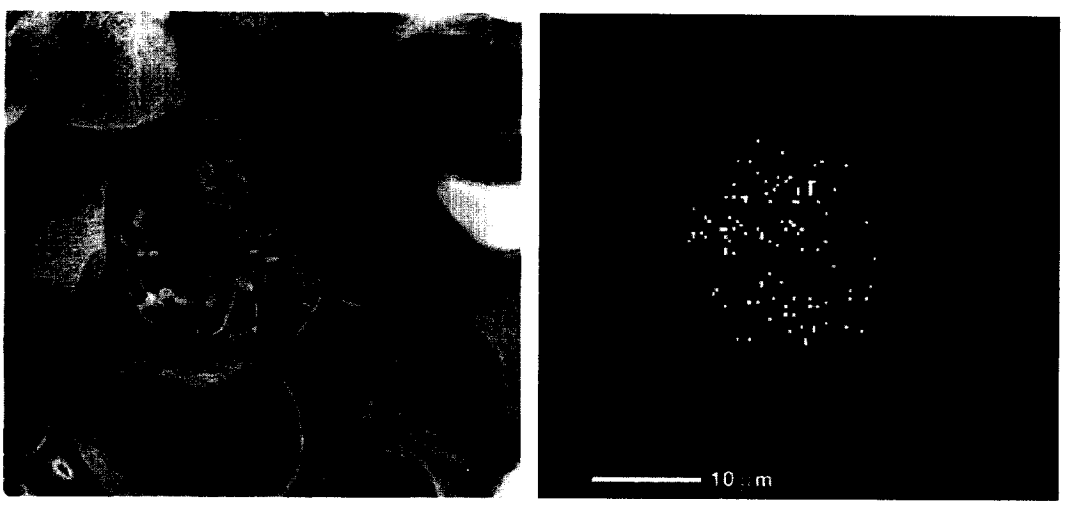

Fig. 5. Scanning electron micrographs of a laticifer of *Sebertia acuminata* (cryo preparation; frozen hydrated; left) and corresponding EDX mapping of nickel K_α signal (right). Nickel is highly concentrated within the laticifer.

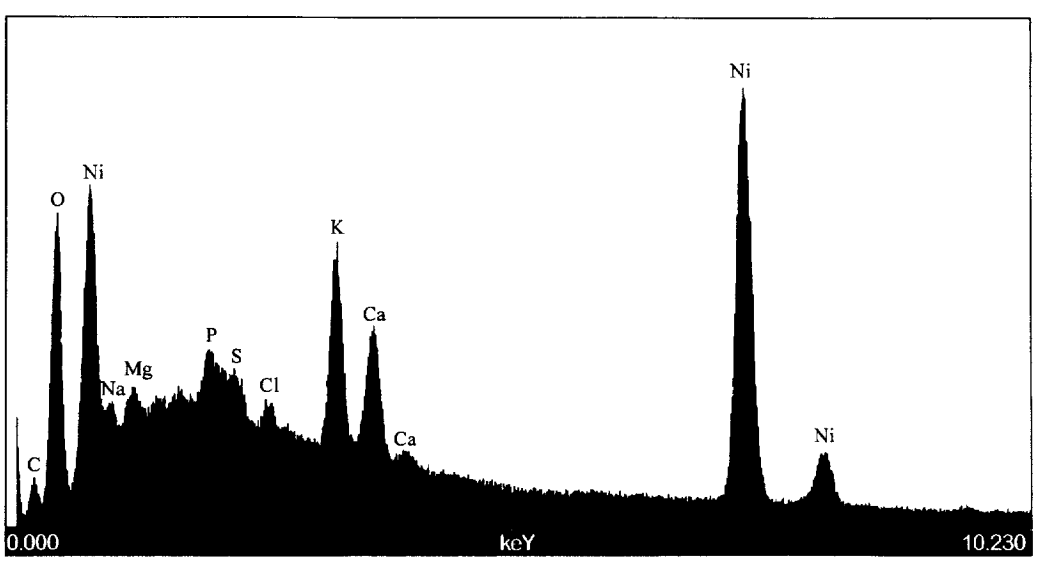

Fig. 6. Spectrum of elements in a laticifer of *Sebertia acuminata*. The elements were directly determined in a *Sebertia* laticifer using an EDX system.

Table 3. The influence of the diet composition on the development of *Drosophila melanogaster* larvae. Adults of *D. melanogaster* were grown on apple juice agar complemented with yeast suspension under the addition of different combinations of sucrose (32 mM), citrate (581 mM) and $NiCl_2$ (1188 mM) as well as latex containing citrate (581 mM) and Ni^{2+} (1188 mM)

	Sucrose	Sucrose/ Citrate	Addition of Sucrose/ Citrate/Nickel	Latex
Larvae after 6 days	83 (3–4 mm)	75 (3–4 mm)	5 (< 1 mm)	2 (< 1 mm)
Cocoons after 8 days	70	43	0	1 (Degenerated)

thus protecting the plant against herbivores [27]. In an elegant set of experiments it was shown that larvae of *Pieris rapae* were not able to cocoon and so died when they were fed with leaves of the nickel hyperaccumulator *Strepthanthus polygaloides* grown on nickel-rich soil [28]. The larvae survived, however, and developed into adults when they were fed with *Strepthanthus* plant parts grown on soil with normal Ni^{2+} concentrations.

In order to get an insight into the possible function of the nickel containing latex of *S. acuminata*, *Drosophila melanogaster* flies were exposed to different diets with and without the addition of nickel. The egg deposition and the development of larvae were monitored. The results of these investigations are outlined in Table 3.

Drosophila adults living on their usual diet with the addition of sucrose showed normal development with many larvae (83) after 6 days which cocooned as usual. The addition of citrate barely influenced the larvae formation (75), however, the cocoonation was reduced to about 60%. The addition of citrate together with nickel, or the addition of *Sebertia* latex drastically reduced the numbers of larvae (5 and 2, respectively) which, in addition, were not able to cocoon. However, the toxicity of the nutrient may not be the reason for this result. It was observed that the flies avoided the food which contained nickel, thus they died of starvation. A truly toxic effect of nickel could not, therefore, be confirmed in this study. The latex as well as the composition of an artificial latex was avoided by the *Drosophila* flies thus demonstrating the repellent effect of the plant sap. This is, without doubt, an indication that nickel functions as a means to prevent predation and attack by insects.

DISCUSSION

Although nickel is advantageous for plants in minor concentrations [29, 30], the same metal causes immediate cell death when incorporated in critical phytotoxic amounts. In contrast, there exists a number of plants which are able to accumulate nickel in unphysiological and normally fatal concentrations without showing symptoms for intoxication [3, 27]. The most impressive example for this ability of hyperaccumulation are the nickel concentrations found in the tissue of *S. acuminata*.

Plants that are able to incorporate certain metals in high concentrations must have evolved mechanisms for detoxification of these substances [2, 31]. In general, heavy metals are detoxified inside the cells through complexation by phytochelatins [4, 5]. In nickel hyperaccumulators, however, these metal-sequestering peptides are not functional because the enzyme phytochelatin synthase responsible for the synthesis of $(\gamma\text{-glu–cys})_n$–gly is not activated by nickel. Moreover, HPLC analysis of *Sebertia* latex samples now prove that phytochelatins are not present in the plants. These findings confirmed that phytochelatins are not involved in the detoxification as well as in the hyperaccumulation of nickel.

A further mechanism for avoiding toxic effects of accumulated metals is their sequestration as insoluble salts or chelates. For instance, in some lichens copper is bound as oxalate thus allowing their growth on contaminated soils [32]. Moreover, the amino acid histidine was shown to be a ligand for the complexation of nickel. In nickel-resistant strains of *Saccharomyces cerevisiae*, histidine is responsible for the binding of the metal inside the yeast vacuole [22]. In nickel hyperaccumulating *Alyssum* species, histidine is not only involved in the nickel tolerance, but also plays an essential role in the high rates of nickel transport into the xylem, which is required for hyperaccumulation in the shoot of this plant [9]. The possible involvement of amino acids in nickel chelation is discussed by Homer *et al.* [33] in detail, who found proline as the dominant amino acid in the nickel accumulating plant *Alyssum troodii*. In *S. acuminata* latex, Lee *et al.* found, by high voltage paper electrophoresis, nickel bound as negatively charged Ni(II)–citrato-complexes and $Ni(H_2O)_6^{2+}$ cations [18]. This was the first indication that a tricarboxylic acid can function as a ligand for metals in hyperaccumulating species.

In our own study, we determined a citrate concentration of 0.6 M in the latex of the mature *Sebertia* tree under investigation. ^{13}C NMR spectroscopy proved unequivocally the identity of this tricarboxylic

acid. Gel filtration of latex samples confirmed that nickel was, indeed, sequestered by citric acid to about 37%, while another 50% of the metal still eluted as free ion. At the physiological pH of the latex, the negatively charged $[Ni\ cit]^-$ is the main anion for nickel binding [25]. Calculating the binding capacity of citric acid found in the latex it was noticed, however, that nickel cannot completely be bound by citric acid. Additional counter ions must, therefore, exist in the latex. Although an exact quantification was not achieved, nitrate was found in the latex in amounts which would make nitrate a possible partner for the charge counter balance of nickel in *S. acuminata*.

While nickel was measured in all parts of the *Sebertia* tree, the latex showed by far the highest metal concentration. The localization of nickel complexes inside the laticifers of *S. acuminata* was, for the first time, confirmed by light microscopy after staining with dimethylglyoximine, a dye which was already used by Vergnano Gambi [26] to locate nickel in *Alyssum bertolonii*. Scanning electron microscopy (SEM) proved our finding in qualitative and quantitative detail. Application of an EDX system now offered the possibility to analyse the spectrum of elements directly in frozen hydrated laticifer tissue of *S. acuminata*. Although small amounts of nickel were found in surrounding structures, the EDX investigation confirmed the presence of that metal inside the laticifers. Moreover, the elemental composition as well as their quantitative differences could be analysed thus confirming that nickel was specifically accumulated in the latex to an unusual extent of 15–30% of dry weight.

The soil on which the *Sebertia* plant was growing contained further heavy metals in high amounts, but nickel was the only metal incorporated in high concentrations. Therefore, the uptake of nickel must follow a highly selective mechanism and an effective translocation from root to shoot seems to be essential for generating the hyperaccumulator phenotype [9]. As proposed by Still and Williams [34], the nickel diluted in the soil may be complexed by membrane-bound 'selectors' (translocators) at the outer surface of the roots. Then, the metal will be passed onto so-called 'transport ligands', for instance citrate or malate that are able to form nickel complexes. This hypothesis was later extended by Morrison (cited in Ref. [15]), who proposed that the nickel–selector-complex and the transport ligand form a triple complex. Subsequently, the nickel–transport complex may be released to the xylem and the selector returns to the outer root membrane. This mechanism could exclude the occurrence of free phytotoxic nickel ion levels thus allowing nickel to be immediately detoxified by complexation when entering the plant. Nevertheless, further studies are necessary to elucidate the exact mechanism for the uptake and transport of nickel complexes inside the plant as well as for their discharge into the laticifers where these complexes accumulate in molar concentration.

The benefit of hyperaccumulating nickel seems to be the protection against predators. The nickel-rich leaves of *Strephanthus polygaloides* prevented the development of the herbivorous larvae of *Pieris rapae* as well as the growth of the phytopathogenic bacterium *Xanthomonas campestris* [28]. Similar observations were made in our own experiments with *Drosophila melanogaster*. Natural *Sebertia* latex as well as artificial nickel containing diets had a repellent effect on the flies, which avoided the contact with that specific food and rather died of starvation.

It is interesting to note that in the habitat of the ultramafic soils of the Rivière Bleue in the vicinity of *S. acuminata* only a few species are nickel hyperaccumulators. In contrast, most plant species show no unusual concentration of Ni^{2+} in their organs above ground. In these plants the specific uptake and transport of Ni^{2+} as well as tolerance against nickel or its complexes is not present. Ecologically, therefore, there is no reason to develop heavy metal hyperaccumulation to cope with the soil condition. We assume now that heavy metal hyperaccumulation is an extremely easy and 'cheap' way for the plant to build up an inorganic defence system against predators and potential phytopathogenic microorganisms. Thus, toxic heavy metal hyperaccumulating species may have by-passed during evolution the creation of low or high molecular weight defence compounds, their underlying genes and enzymes just simply using for that purpose what was available as inorganic metal in the soil in higher than normal concentrations. The cost of adaptation to this metal has to be cheaper if the above consideration is valid, if compared to the elaboration of complex organic molecules.

For hundreds of years, heavy metal accumulating plants have been used as indicators for metallic deposits [7, 10]. Nowadays, the ability of metal hyperaccumulation is the object of intensive investigations, especially with regard to the ecological exploitation of such plants. A further understanding of the underlying mechanisms will possibly improve the use of hyperaccumulating plants for the detoxification and phytoremedation of contaminated soils [11, 35].

EXPERIMENTAL

Plant material. Plant tissue from a *Sebertia acuminata* tree was collected at the Rivière Bleue in New Caledonia in March/April 1991. After removing the bark, latex was collected and deep frozen until use. Branches of 0.5–1 cm dia. were dried prior to analysis. Cortical tissue (phloem) was sepd by peeling of the inner xylem. Seeds were germinated on the original soil, irrigated with d.d. H_2O and the seedlings kept under normal greenhouse conditions at 25°. Mature fruits were collected in December.

Analytical methods. Nickel was quantified at 232 nm by AAS operated in flame mode using a Perkin–Elmer 1100B instrument. Prior to analysis, the plant

and soil material was dried for 2 days at 60°. The dry material (0.2 g) was mixed with 1 ml HCl and HNO_3 (SUPRAPUR, Merck) as well as 1 ml H_2O and the mixt. incubated for 4 hr at 180° using a Parr-apparatus. After dilution with H_2O to 20–50 ml, 1 ml of the sample (metal concn about 1–10 ppm) was analysed by AAS.

Chloride, sulphate and nitrate were measured according to Ref. [36]. For the determination of phosphate the method of Fiske/Subbarow as modified by Ref. [37] was used. Citric acid was enzymically measured using a test-kit of Boehringer (Mannheim) which applies the enzyme citrate lyase for determination. For the determination of phytochelatins, plant material was prepd according to Ref. [6]. Phytochelatins were assayed by HPLC with post column derivatization as described by Ref. [24].

Gel filtration of latex samples was performed on a Sephadex G10-column, 50 × 3 cm (Pharmacia), pre-equilibrated with H_2O at pH 6.6. Elution was achieved with H_2O as solvent at a flow rate of 40 ml hr^{-1} and frs of 2 ml were collected. Free nickel ions were subsequently eluted with 20 mM citric acid at a volume of 96 ml after separate calibration with 1 mg nickel as $Ni(NO_3)_2$.

Ion exchange chromatography on DOWEX 1-X8 (200–400 mesh, Serva; column: Pharmacia, 3 × 1 cm) was performed using 20 ml 4 M HCO_2H and subsequently 15 ml 8 M HCO_2H as eluent at a flow rate of 40 ml hr^{-1}. Frs of 1 ml were collected for analysis; citric acid appeared in the 4 M HCO_2H fr. ^{13}C NMR spectra were taken at 90.6 MHz on a Bruker AM 360 spectrometer. Dioxane was used as an int. stand.

Investigation of repellent effects. Drosophila melanogaster mutant *rosy* flies were provided by Dr Sommer, Zoological Institute, University of Munich. About 25 adult flies were kept in cages on apple juice agar (90 g agar, 3 l H_2O, 100 g sucrose, 6 g Nipagin, 1 l apple juice) and fed with yeast suspension under the addition of various combinations of sucrose (32 mM), citrate (581 mM) and $NiCl_2$ (1.188 M). The agar dishes were collected every 1–2 days and observed for larvae development.

Microscopic methods. Light microscopy was done on a photomicroscope Axiophot (Zeiss, Oberkochel). Nickel complexes in the tissue were visualized by staining with 0.1 M aq. dimethylglyoximine yielding a scarlet colour.

For *in situ* studies, the plant tissues were cryofixed with nitrogen slush at −194°. The frozen specimen were transferred *in vacuo* to a cryo prepn chamber, fractured at −190°, etched at about −90° and sputter coated with 2 nm gold palladium. The specimen were transferred to the cryo stage of the scanning electron microscope and kept at a temp. range from −170° to −190°. EDX analysis was performed at an acceleration voltage from 10–25 kV either in spot or mapping mode.

All pictures were taken with a field emission scanning electron microscope (Hitachi S-4100) equipped with an Oxford cryo transfer system. Element analysis was performed with an EDX system (Tracor Voyager, 10 mm^2 Lithium-drifted Silicon-Detector).

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