Plant and Soil **138**: 195–205, 1991.

PLSO 9113

Comparative studies of nickel, cobalt, and copper uptake by some nickel hyperaccumulators of the genus Alyssum

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Received 4 March 1991. Revised July 1991

Key words: Alyssum, cobalt uptake, germination tests, hyperaccumulators, copper uptake, nickel uptake, plants, toxicity

Abstract

The uptake of Ni, Co, and Cu by the nickel hyperaccumulator *Alyssum troodii* Boiss and the non-accumulator *Aurinia saxatilis* (L.) Desv. were studied in pot trials using artificial rooting media with varying concentrations of the metals added as soluble salts, singly and in combination. The ability of five other Ni hyperaccumulating species of *Alyssum* to hyperaccumulate Co was also investigated.

Leaves and stems of *A. troodii* accumulated Ni to almost the same extent (8000–10 000 $\mu g\,g^{-1}$). In roots, the highest Ni concentration was 2000 $\mu g\,g^{-1}$. In leaves of *Au. saxatilis*, the maximum Ni concentration was only 380 $\mu g\,g^{-1}$ and the level in roots was even lower.

In media containing Co, the maximum concentration of this element in *A. troodii* (2325 $\mu g\,g^{-1}$) was ten times higher than in the non-accumulator species. Slightly less Co was found in stems and roots of both species. Among the other Ni hyperaccumulators, the maximum concentration of Co in leaves ranged from about 1000–8000 $\mu g\,g^{-1}$.

Copper concentrations were the same in all organs of both species when they were grown in copper-rich media and were in the range 40–80 $\mu g\,g^{-1}$, showing that neither plant was capable of taking up Cu at levels comparable to those of Ni and Co.

When both plants were grown in media containing equal amounts of both Co and Ni, the Co concentrations in plant organs were the same as for specimens grown in media containing Co only. However, the Ni levels were lower in both species. Uptake of Co therefore appeared to suppress Ni uptake.

Pot trials showed that the order of tolerance was $Ni > Cu > Co$ for *A. troodii* and $Ni > Co \approx Cu$ for *Au. saxatilis*, whereas the seedling tests showed the order to be $Co > Ni > Cu$. At metal concentrations $\geqslant 10\,000\ \mu g\,g^{-1}$, the overall tolerance of *A. troodii* was greater than that of *Au. saxatilis* which exhibited equally low tolerance to Ni and Cu.

We conclude that in *A. troodii*, *A. corsicum* Duby, *A. heldreichii* Hausskn., *A. murale* Waldstein & Kitaibel, *A. pintodasilvae* T.R. Dudley, and *A. tenium* Hálácsy, Ni tolerance and hyperaccumulation conveys the same character towards Co. This behaviour should be investigated in other hyperaccumulators of Ni and/or Co.

Introduction

Plants that have an inordinately high concentration of Ni have been termed *hyperaccumulators* (Brooks et al., 1977a), and are defined by a metal content of $> 1000\ \mu g\,g^{-1}$ (0.1%) in dried tissue. This concentration is at least a factor of 10–20 higher than for 'normal' plants growing in the same nickel-rich environment. The first hyperaccumulator of Ni was discovered by Minguzzi and Vergnano (1948) who found over 1% of this element in dried leaves of *Alyssum bertolonii* Desvaux growing in nickel-rich serpentine soils from Tuscany, Italy. Subsequently, nearly 50 additional species of *Alyssum* were found to possess hyperaccumulator status (Brooks and Radford, 1978; Brooks et al., 1979).

The discovery of hyperaccumulators of Ni has led to an upsurge of interest in these plants because of the interesting questions that arise when a normally phytotoxic element is accumulated by plants to such an extraordinary degree. Phytochemical studies have also been encouraged because it is now possible to isolate milligram quantities of metal complexes for further study instead of the microgram quantities to be expected with non-accumulating species.

The results of the above studies have been reported for Ni (Homer et al., 1991; Lee et al., 1977; Pancaro et al., 1978), and for Cu and Co (Morrison, 1980). Several pot trial studies have also been carried out in an attempt to gain some insight into the nature of the metal uptake. In this respect, the investigations of Brooks et al. (1979), Morrison (1980b) and Baker (1983) are noteworthy.

The question as to whether tolerance of one metal by a selected species endows the plant with the ability to exhibit tolerance towards another metal, has been addressed by Reeves and Baker (1984). They concluded that a constitutional metal tolerance may exist within the hyperaccumulator *Thlaspi goesingense* Hálácsy after observing the performance of serpentine and non-serpentine populations in media containing elevated levels of Ni, Co, and Zn. Fiedler (1985) extended this view to the genus *Calochortus* (Liliaceae) based on her studies of Ni, Co, and Cu uptake. The ability of a plant to take up unusually high levels of more than one metal (co-accumulation) was also observed in *T. goesingense* by Reeves and Baker (1984). They attributed this behaviour to the existence of a non-specific metal detoxification system. From soil culture experiments, Hajar (1987) showed that *Thlaspi alpestre* L., a coloniser of lead mine wastes, was tolerant to Ni, Co, Mn, Cd, Al, Cu, Fe, Mo and Mn. Specimens accumulated metal over the range 1000–50 000 $\mu g\,g^{-1}$ in either roots or shoots or both.

Metal uptake patterns for accumulators and non-accumulators have been described by Baker (1981). He proposed that in the former, there is a linear increase in the elemental content of the tissue with increasing concentrations of the element in the substrate until a plateau is reached at a relatively high metal content in the soil. For non-accumulators, there is restricted entry of the metal at low concentrations of the element in the soil until a point is reached at which the restricting mechanism breaks down and there is unlimited absorption over quite a small concentration range in the soil, until the plant succumbs. A third type of uptake is completely linear in which the elemental content of the plant is directly related to the metal concentration in the soil. Similar uptake patterns have been proposed by Berry (1986) from laboratory and field studies. Barry and Clark (1978) have also reported that the range of substrate metal concentrations over which measurements are made, dictates the plant/substrate relationship. Thus a plant may exhibit all of the above uptake patterns over different concentration ranges of the element in the substrate. Edaphic factors, such as pH and availability of the metal to the plant, also warrant consideration.

It is interesting to note that in water-culture experiments, Gabbrielli et al. (1990) observed that addition of Ca reversed nickel-induced restriction of root growth in the nickel-excluding serpentine plant *Silene italica* L., but reduced root growth in the Ni hyperaccumulator *Alyssum bertolonii*. This represented a confirmation of the adaptation of the species to a calcium-deficient soil in the presence of metals like Ni. It is important to note that the addition of lime to serpentine soils has been shown to improve their fertility for crops (Crooke and Inkson, 1955; Proctor, 1971).

In the present work, we have used pot trials to study the mode and extent of Ni, Co, and Cu uptake by *Alyssum troodii* (a hyperaccumulator of Ni from Cyprus) and have compared it with *Aurinia saxatilis*, formerly classified under *Alys-*

sum (Dudley, 1966), a native of Turkey. We have also carried out germination trials on seeds of both species. Pot trials were also used to study the uptake of Co by five other Ni hyperaccumulators of the genus Alyssum.

Materials and methods

Pot trials

Peat/pumice mixtures containing 10% (w/w) of Ni, Co, and Cu were prepared from the corresponding reagent-grade nitrate salts. Serial dilutions were then carried out with a 1:1 peat/pumice mixture containing Osmocote slow-release fertilizer (N, P, K), lime, dolomite, and micronutrients (Cu, B, Mo, Mn, Zn, Fe) to yield media with 47, 78, 130, 216, 360, 1000, 3333, and 10 000 (1%) $\mu g\ g^{-1}$ of each element on a dry-weight basis. Composite soils containing Ni and Co together at equal concentrations of 24, 39, 65, 108, 180, 300, and 500 $\mu g\ g^{-1}$ were also prepared. Constant nitrate levels were maintained by the addition of appropriate amounts of 10% calcium nitrate (agricultural grade) in pumice powder.

Seeds were germinated in the 1:1 peat/pumice diluent containing added fertilizer. After 2–3 weeks, seedlings were transplanted into plastic pots containing experimental soils (ca 200 g). Each pot contained one seedling and there were ten replicates for each separate metal concentration. Controls consisted of the peat/pumice mixture containing fertilizer, micronutrients and calcium nitrate, but none of the three metals. All pot trials were carried out in a glasshouse maintained at an average temp of 22°C. Plants were watered twice a day by capillary action and harvested at the end of 3 months.

In addition to trials on *A. troodii*, Co uptake was also studied in the following hyperaccumulators of Ni: *A. corsicum*, *A. heldreichii*, *A. murale*, *A. pintodasilvae*, and *A. tenium*. Seedlings of each species were grown in vials containing about 2 g of cobalt-augmented rooting medium. Five replicates were prepared for each concentration and each vial contained one seedling. Plants were harvested after 6 weeks.

Sample preparation and analysis

Plant samples were rinsed with distilled deionised water and together with rhizosphere soil samples were air-dried. The dry weight of each plant was recorded. *Alyssum troodii* samples were sorted into leaves, stems and roots, and *Au. saxatilis* was divided into leaves and roots as the stems are extremely short and difficult to separate.

Plant samples (ca 0.2 g) were ashed at 500°C overnight and the resulting ash dissolved in 2–5 cm^3 of 2 *M* HCl prepared from redistilled constant-boiling 6 *M* acid. The mixtures were then shaken and centrifuged for 10 min, after which the supernatants were analysed for Ni, Fe, Cu, Mn, Co, Zn, Ca, Mg, Na, and K by atomic absorption spectrometry (AAS) using an IL457 instrument. Blank determinations were also performed. Leaves of the other species were analysed for Co only.

Ground (<60 mesh) soil samples (1 g) were digested with 10 cm^3 of the 2 *M* HCl in a water bath for 20–25 min. Digests were filtered and adjusted to 10 cm^3 with deionized water. The metals were determined as before.

In order to obtain a measure of the availability of the metals to the plants, soil samples (0.8 g) were extracted with 5 cm^3 of 0.05 *M* NH_4-EDTA (pH 7) by shaking for one hour at 20°C (MAFF, 1981). After filtration and adjustment of the volume to 5 cm^3 with the extractant, the resulting extracts were analysed for Ni, Co, and Cu by AAS.

Soil pH

The pH of the soils was determined by use of a combination type Orion Research Digital Ionanalyzer after 10 g of sample had been equilibrated overnight with 25 cm^3 of distilled deionized water. The instrument was calibrated with phosphate and acetate buffers at pH 6.89 and 4.05, respectively.

Germination tests

Single element aqueous solutions of Ni, Co, and Cu at concentrations of 50, 100, 500, 1000, 5000, 10 000, 15 000, 20 000, 25 000, and 30 000 μg cm^{-3} were prepared from 10% aqueous metal nitrate solutions. Composite solutions were similarly prepared and contained Ni and Co together at concentrations of 10, 15, 25, 50, 100, 250, 500, 1000, 2500, 5000, and 7500 μg cm^{-3}. Forty seeds of each of the two plant species were placed on filter papers soaked in each of the above solutions and kept in Petri dishes. The filter papers were moistened daily with deionized water to prevent dehydration. Controls consisted of filter papers moistened with deionised water only. Following an initial interval of 7 days, counts of germinated seedlings were made every three days for a total period of 25 days.

Observations of seed appearance and seedling vigour were made and the position of the dishes was rearranged regularly to minimise differences in illumination.

Analysis of seeds

To determine the metal content of seeds of *A. troodii* and *Au. saxatilis*, 124 seeds of the former and 62 of the latter were weighed and ashed at 500°C overnight. After addition of 5.5 cm^3 of 2 *M* HCl, analyses were performed exactly as for the plant tissue.

Results

Metal uptake

The nickel contents of leaves, roots, and stems of *A. troodii* and *Au. saxatilis*, are shown in Figure 1. The values for the metal content of the rooting medium refer to the final concentration after the 90-day pot trials and are slightly lower than the initial nominal concentrations, owing to leaching effects and plant uptake. It will be observed that the curves for leaves and stems of *A. troodii* are almost completely coincident and greatly exceed the Ni content of roots despite the latter having been in intimate contact with interstitial Ni solutions in the artificial media. In the case of *Au. saxatilis*, Ni levels in leaves and roots were fairly similar. Overall, the metal contents of organs of the hyperaccumulator were about 10 times higher than in *Au. saxatilis*. About 40% of the *A. troodii* plants survived a Ni content of 3000 μg g^{-1} in the rooting medium, but the content of this element remained about the same as for specimens grown in media containing 825 μg g^{-1} (nominally 1000 μg g^{-1}). *Aurinia saxatilis* would not grow in mixtures containing >245 μg g^{-1} Ni. Maximum Ni contents were 10 084, 8540, and 1959 μg g^{-1} in leaves, stems, and roots of *A. troodii*, and were 383 and 66 μg g^{-1} in leaves and roots of *Au. saxatilis*.

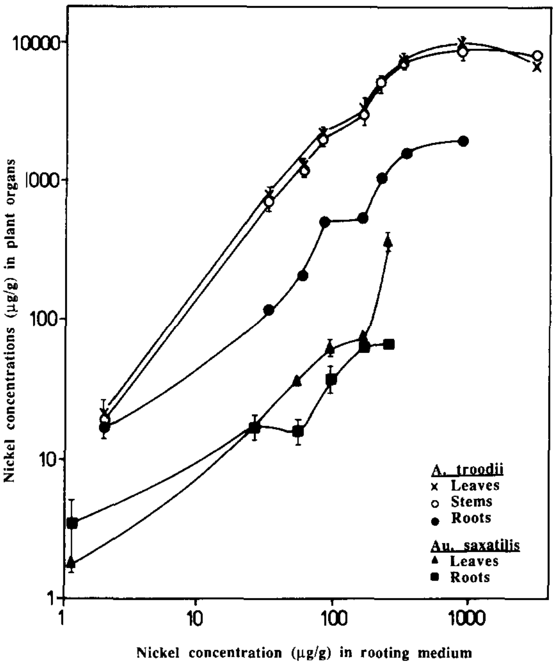

Fig. 1. Concentrations of nickel in plant organs after 90-day pot trials in rooting media containing various concentrations of nickel.

Corresponding data for uptake of cobalt by several plant species are shown in Figure 2. *A. troodii* accumulated a maximum of 2325 μg g^{-1} Co in leaves, and 880 and 1322 μg g^{-1} in stems and roots, respectively. Leaves and roots of *Au. saxatilis* had maximum concentrations of 117 and 37 μg g^{-1} Co. None of the Ni hyperaccumulators tolerated Co concentrations of >800 μg g^{-1} in the substrate. *Aurinia saxatilis* would not survive in substrates with more than 58 μg g^{-1} Co.

There was very little difference in the toler-

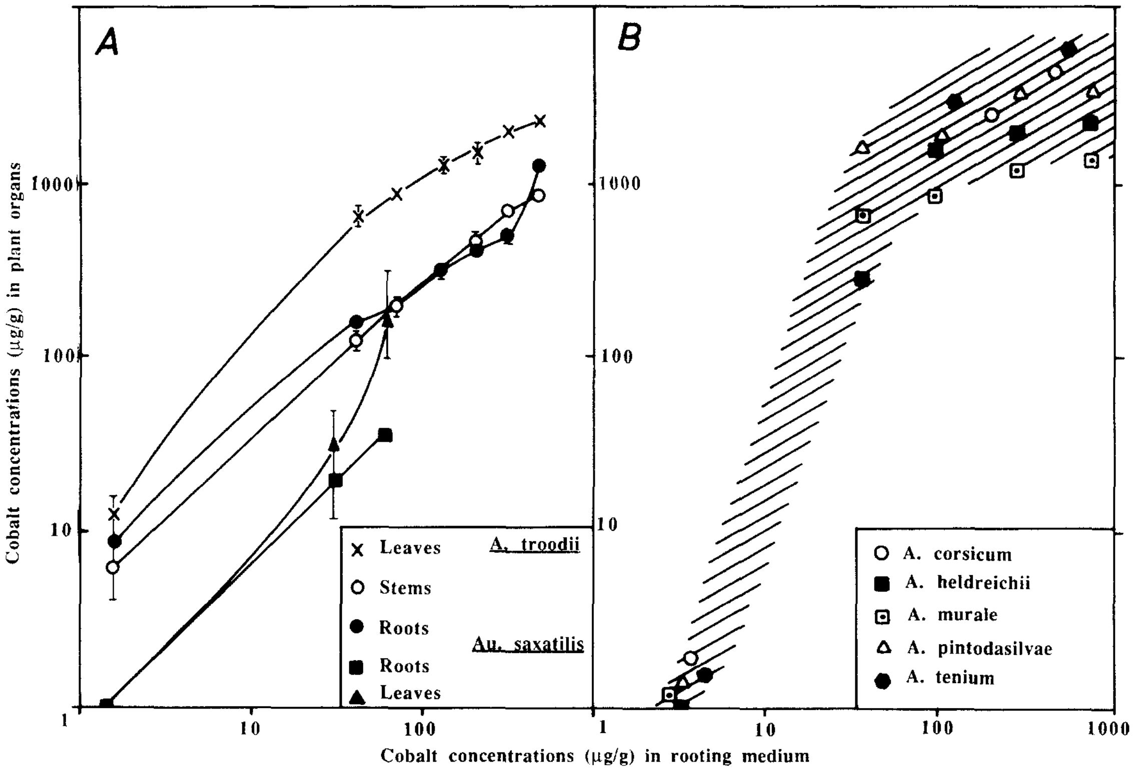

Fig. 2. (**A**) Concentrations of cobalt in plant organs after 90-day pot trials in rooting media containing various concentrations of cobalt. (**B**) Concentrations of cobalt in leaves of *Alyssum* spp. after 72-day pot trials in rooting media containing various concentrations of cobalt.

ance to, and uptake of, Cu in both test species (Fig. 3). Maximum tissue concentrations of this element were below 100 $\mu g\,g^{-1}$ in all cases, though *A. troodii* had a slightly higher Cu content in leaves (71 $\mu g\,g^{-1}$) than had *Au. saxatilis* (45 $\mu g\,g^{-1}$).

When plants were grown in media containing equal amounts of Co and Ni (Fig. 4), uptake of Ni was reduced appreciably, though that of Co was only slightly lower. Maximum values for Ni and Co in leaves, stems and roots of *A. troodii* were 752, 528, and 179 $\mu g\,g^{-1}$, and 2202, 1109, and 752 $\mu g\,g^{-1}$, respectively, whereas for leaves and roots of *Au. saxatilis*, the corresponding values were 46 and 22 $\mu g\,g^{-1}$, and 144 and 38 $\mu g\,g^{-1}$.

Plant-available metal concentrations at time of harvesting

For all rooting media, concentrations were de-

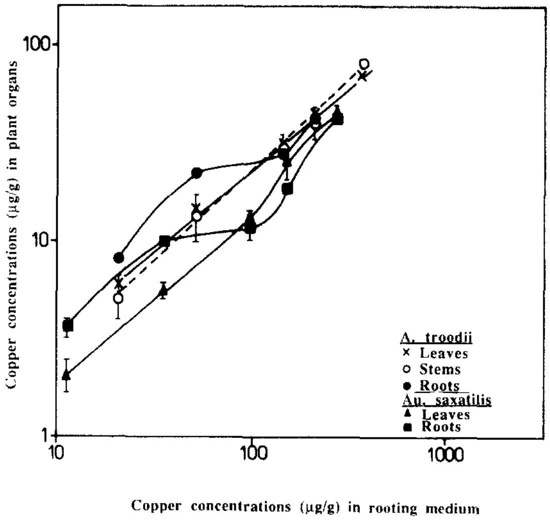

Fig. 3. Concentrations of copper in plant organs after 90-day pot trials in rooting media containing various concentrations of copper.

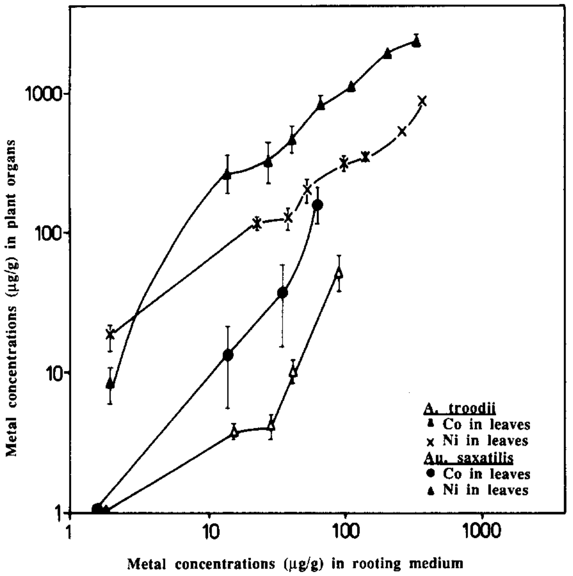

Fig. 4. Concentrations of nickel and cobalt in plant organs after 90-day pot trials in rooting media containing variable concentrations of both elements.

termined in both HCl (2 *M*) and NH_4-EDTA (0.05 *M*) extracts. The data shown in Table 1 indicate that for single-element media, metal extractability was highest for Cu and lowest for Ni. In mixtures of two elements, Co was retained by the soil to a greater extent than was Ni. Extractions with NH_4-EDTA gave percentage recoveries that were invariably lower than extraction with 2 *M* HCl.

Relative biomass yields and accumulatory capacity

Relative biomass yields for both plant species grown in artificial mixtures containing varying amounts of the three metals are shown in Figure 5. These were calculated by determining the average yield per plant in a given group and expressing this as a percentage of the highest individual yield within the group. Mean maximum yields for *A. troodii* were achieved in mixtures containing 212 $\mu g\, g^{-1}$ Ni, 65 $\mu g\, g^{-1}$ Co, and 142 $\mu g\, g^{-1}$ Cu. These values correspond to average metal burdens of 22 121, 1584, and 53 μg of metal per plant. For *Au. saxatilis*, the corresponding maximum yields were at 94, 29, and 36 $\mu g\, g^{-1}$ for Ni, Co and Cu, respectively, and the average weights of metal were 473, 214, and 86 μg per plant.

When the two plants were grown in soils containing both Ni and Co, the weights of Co were similar to those obtained when the metal was administered separately, while the Ni burdens were lower. Maximum yields were achieved at lower soil metal concentrations (42–58 $\mu g\, g^{-1}$).

Table 1. Extractable heavy metal contents at the end of 90-day pot trials

Metal		Metal concentrations ($\mu g\, g^{-1}$) or % extraction						
Nickel	Nominal concentration	47	78	130	216	360	600	1000
	Extracted by 2 *M* HCl	68%	73%	62%	54%	59%	53%	82%
	Extracted by NH_4-EDTA	44%	56%	44%	39%	37%	37%	52%
Cobalt	Nominal concentration	47	78	130	216	360	600	
	Extracted by 2 *M* HCl	80%	80%	91%	87%	79%	73%	
	Extracted by NH_4-EDTA	62%	58%	63%	57%	73%	66%	
Copper	Nominal concentration	68	99	151	237	380*		
	Extracted by 2 *M* HCl	76%	96%	94%	86%	93%		
	Extracted by NH_4-EDTA	65%	86%	82%	69%	80%		
Nickel (with cobalt)	Nominal concentration	24	39	65	108	180	300	500
	Extracted by 2 *M* HCl	97%	97%	82%	94%	81%	89%	75%
	Extracted by NH_4-EDTA	68%	75%	68%	73%	66%	84%	70%
Cobalt (with nickel)	Nominal concentration	24	39	65	108	180	300	500
	Extracted by 2 *M* HCl	57%	73%	65%	63%	64%	84%	68%
	Extracted by NH_4-EDTA	51%	73%	65%	63%	64%	84%	68%

* Higher concentrations due to copper present in soil additives.

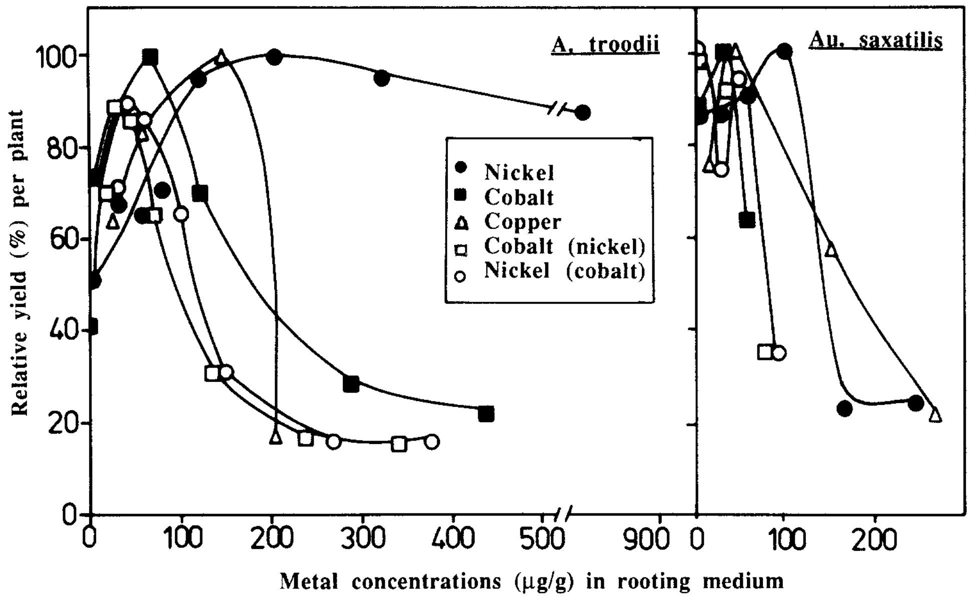

Fig. 5. Biomass yield curves for 90-day pot trials of plants grown in metal-enriched rooting media.

The effect of nickel, cobalt and copper on seed germination

In the controls, 75–85% of the *A. troodii* seeds and 75% of the *Au. saxatilis* seeds germinated after 22 days. Seedling mortality was evident beyond this period. The data are shown in Figure 6.

In the case of *A. troodii*, and in substrates with Ni concentrations in the range 50–500 $\mu g\,cm^{-3}$ there was 80–90% germination. The germination in the presence of Co was 70–90% over a wider concentration range (50–5000 $\mu g\,cm^{-3}$). The maximum percentage germination in the presence of Cu was 60–80% for a concentration of 50 $\mu g\,cm^{-3}$. Seedlings showed a greater tolerance to Co than to Ni and/or Cu. At lower concentrations, Cu was more detrimental than were Ni or Co and produced seedlings of poor vigour that had an unusual blue-green colour. Both growth and development were seriously retarded. For seeds exposed to all three elements at concentrations above 10 000 $\mu g\,cm^{-3}$, mainly radicles were observed, and the few shoots that developed, were chlorotic.

In experiments with seed of *Au. saxatilis*, the highest germination in the presence of Ni (68–

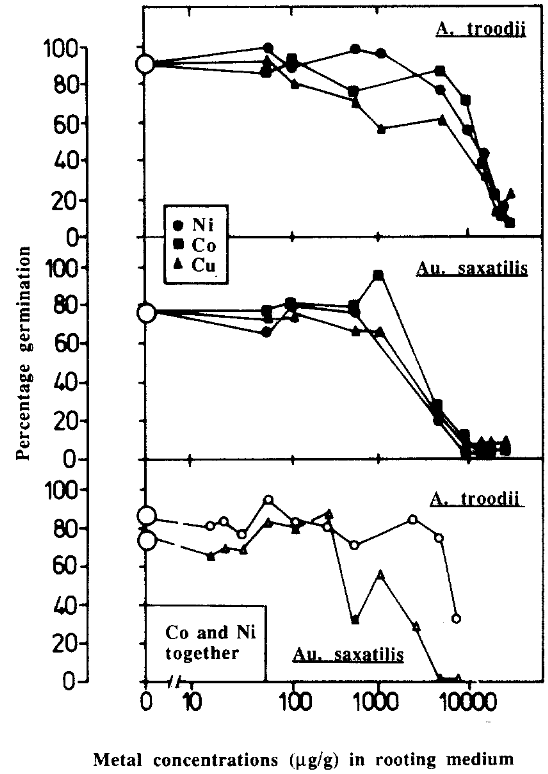

Fig. 6. Percentage germination after 22 days of seeds of two species as a function of metal content of the rooting medium.

75%) was observed with solutions containing 100 μg cm^{-3} Ni. Some 70–90% of seeds germinated in the presence of 1000 μg cm^{-3} Co. In the presence of Cu, maximum germination (70%) was observed at 100 μg cm^{-3}. Compared with the response of seeds to Ni and Cu over the range 50–1000 μg cm^{-3}, more seeds germinated in media containing Co. Germination was generally severely affected at metal concentrations in excess of 15 000 μg cm^{-3} and in this respect *Au. saxatilis* resembled *A. troodii.*

When seeds of both plants were exposed simultaneously to Ni and Co, the highest percentage germination of *A. troodii* seeds (63–93%) occurred at concentrations of 50 μg cm^{-3}. Some 78–85% of *Au. saxatilis* seeds germinated in the concentration range of 50–250 μg cm^{-3}. Germination of both seed types declined beyond this level. Substantial fungal growth was observed in the case of *Au. saxatilis*, and none was observed with the hyperaccumulating species, perhaps because of the antifungal nature of its Ni burden.

Nickel content of seeds

The Ni content of *A. troodii* seeds was 1906 μg g^{-1} and the concentration of Co was 1.7 μg g^{-1} this being similar to the Cu concentration. Calculations revealed that the average weight of Ni per seed was 0.98 μg and that of Co (and Cu) was 10^{-4} μg. In soils containing the lowest concentration of added metal, the weights of these three metals in a single plant (2264, 1044, and 25 μg for Ni, Co, and Cu) based on the average biomass, greatly exceeded these values. A similar relationship was observed in *Au. saxatilis* with respect to Cu (the Cu content was 2.91 μg g^{-1}). No Ni and Co were detected in seeds of this plant.

Discussion

Our data have clearly shown that *A. troodii* and *Au. saxatilis* can be regarded as hyperaccumulator and non-accumulator of Ni, respectively. Whereas both plants will accumulate Co and Ni to some degree, uptake of Cu is restricted.

Eskew et al. (1983, 1984) demonstrated that Ni is essential for soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.) while Brown et al. (1987) concluded the same for barley (*Hordeum vulgare* L.). In Ni-deficient nutrient solutions containing added NO_3^- or NH_4^+, Eskew et al. (1983, 1984) observed leaflet tip necrosis caused by a toxic build-up of urea. This was reduced in beans from seed containing as little as 2.5 ng Ni, and was absent when the seed contained 160 ng Ni. Brown et al. (1987) demonstrated that barley grain containing <30 ng Ni was non-viable. This they attributed to the impairment of the maturation processes. Based on the findings of these workers, Ni has been shown to satisfy two of the three essentiality criteria (Alloway, 1990). Thus a rather strong case exists for Ni to be classified as an essential element for higher plants. The work of Eskew et al. (1983, 1984) and Brown et al. (1987) reduces the credibility of the proposal of Timperley et al. (1970) relating restricted metal uptake to internal control and essentiality. Copper is an essential element for higher plants and is required at a much higher concentration level. Thus its restricted uptake and toxicity and deficiency symptoms are more readily discerned.

An important feature of the work addressing the Ni essentiality to higher plants is the extremely low levels of Ni dealt with. Purification of all reagents was necessary to prevent contamination which could otherwise lead to incorrect conclusions regarding the levels of Ni required by the plant. It is important to note that the background levels of Ni in natural soil and experimental media are almost always adequate for the plant needs. Thus, Ni deficiency symptoms are probably rare. It is at present an open question whether Ni is indeed essential for hyperaccumulators, particularly as they appear to grow quite well in nickel-poor soils. Reeves et al. (1981) suggested that in such soils they may have less resistance to fungal attack and suffer competition from other more vigorous species.

Cobalt has been proven to be essential for *Rhizobium* bacteria that associate symbiotically with legume roots (Reisenauer, 1960), free-living nitrogen-fixing bacteria (e.g. *Azotobacter* spp.), and blue-green algae (Alloway, 1990). No requirement has been demonstrated in higher plants including hyperaccumulators. The uptake of inordinately high levels of Ni (and Co) by the hyperaccumulator may thus not reflect essentiality, but may simply be an adaptation to its natural habitat. However, it has been shown that both elements stimulate vegetative growth in several ways in addition to serving as activators for one or more enzymes (Mishra and Kar, 1974; Shkolnik, 1984).

We suggest on the basis of the chemical and physiological similarities of Co and Ni that one mechanism controls the uptake of both elements. Thus one ligand may bind to Ni and/or Co at the soil water/plant interface, and complexing by another ligand can then effect xylem transport, terminating in detoxification in the leaves. Cognizance should also be taken of changes in membrane permeability, nature of membrane acceptors, and the role of root exudates. The lower uptake of Co by the two plant species relative to Ni in single element pot trials, coupled with the lower metal concentrations in which specimens survived, is indicative of a lower tolerance to Co.

The higher concentrations of Co in leaves relative to stems and roots (Fig. 2a) has also been noted by Gustafson (1956) and Langston (1956). They reported that Co supplied through the root systems tended to accumulate in leaf margins with higher concentrations in young leaves. Hajar (1987) reported that the concentrations of Ni, Co, Mn and Zn in shoots of *Thlaspi alpestre* L. were twice as high as in the roots. We observed a similar partitioning with respect to Ni and Co.

When both Ni and Co were present in the rooting medium, Co levels in the plant material were virtually unchanged from concentrations in single-element pot trials. However, the nickel content was reduced in these combined element trials. When these two metals are present in high concentrations in the rooting medium, we must give consideration to the stability of the complexes formed in uptake mechanisms. Relative to Ni, the greater influx of Co into roots and subsequent higher concentrations in leaves may be the result of root uptake mechanisms that favour complexing of Co relative to Ni. This could result in the suppression of Ni uptake in order to facilitate simultaneous accumulation of high levels of these two elements. Support for this theory comes from work by Cataldo et al. (1978) who noted reduced Ni uptake and transport into stems of soybean plants by the presence of Cu, Zn, Fe, and Co ions. Further evidence for the selectivity of Co over Ni in transport systems has also been demonstrated in fungi and bacteria (Fuhrmann and Rothstein, 1968; Willecke, 1972).

The concentration of Co in serpentine and related soils is usually five to ten times lower than that of Ni (Brooks et al., 1977b). However this relationship is not reflected in the Ni/Co ratio in field specimens of *A. troodii* where the Co content is two orders of magnitude lower than that of Ni. Despite the general rule that plants usually accumulate lower amounts of Co than Ni, there are a few exceptions, as for example accumulation of Co by the genus *Nyssa* (Brooks et al., 1977b) where Co concentrations are usually twice those of Ni, and, of course, the Cu/Co-tolerant flora of Southcentral Africa (Brooks and Malaisse, 1985) where the Co content is orders of magnitude higher than that of Ni. The Ni content of such soils is much lower than that in serpentine soils.

The pH of the rooting medium was in the range 4.4–5.3 compared to 6.8 reported by Krause (1958) for serpentine soils. Our lower pH was a result of incorporating peat into the rooting medium because the low absorbent power of pumice would not otherwise have allowed retention of the added heavy metals. In this respect, therefore, our experimental rooting medium represents a departure from that of serpentine soils. We observed that the hyperaccumulator *A. troodii* survived soils with total concentrations of Ni and Co at least five times higher than those found in most serpentine soils. At the lower pH of the media used in this work, the plant-available quantities of Ni and Co are higher than those of serpentine soils by an even larger factor. This may indicate that, in the natural habitat, plants such as *A. troodii* are not close to their limits of metal tolerance. Alternatively, the effects of the increased Ni and Co concentrations in the pot trials may be ameliorated by the better nutrient status of the medium. The tolerance and accumulation of Co by the hyperaccumulator, which is not observed in the field, is most likely attributable to substantially lower Co concentrations found in serpentine soils. It has been reported (Alloway, 1990) that at low pH, Ni and Co become more available to the plant, resulting in increased metal uptake.

From Figures 1–5 it will be noted that the highest metal concentrations in plant organs were found in plants growing in artificial media with metal contents in excess of those in which maximum biomass yields were achieved. There is therefore a concentration lag between maximum yield and maximum metal uptake. Beyond the point of maximum metal uptake, the plants show decreased vigour and biomass. Chlorosis was also evident particularly in specimens of *Au. saxatilis* growing in media containing added Ni and Co. Leaf size in these plants was similar to that of the controls, but root size was drastically reduced.

Crooke and Knight (1955) have demonstrated that chlorosis can be related to changes in the Ni/Fe ratio and Bollard (1983) has shown that cobalt toxicity symptoms are in part linked to Fe deficiency symptoms. Mengel and Kirkby (1987) have noticed a similar inverse relationship between Cu and Fe. We were able to reach similar conclusions in our own work by statistical correlation analysis of the data. There was a very highly significant inverse relationship ($P < 0.001$) between Co in the substrate and Fe in the leaves of *A. troodii*. Similar highly significant inverse relationships were observed between Fe in plants and the Cu and Ni content of the rooting media.

Our results for the germination tests carried out on *A. troodii* are similar to those of Mishra and Kar (1974) and Welch (1981) who reported that Ni at low concentrations has a stimulatory effect on germination. This effect was observed for wheat (*Triticum aestivum* L.), peas (*Pisum sativum* L.) and castor bean (*Ricinus communis* L.). Small increases in germination percentage of *A. troodii* were observed over a wide range of added Ni. In *Au. saxatilis* the toxic effects of Cu, Co and Ni became apparent at lower concentrations than was the case with *A. troodii*. Cu did not appear to stimulate germination of seeds of either species as evidenced by the appreciable decline in percent germination over the range of Cu concentrations used.

Our findings from the germination trials generally reinforce conclusions from the pot trials: i.e. that tolerance to both Ni and Co is greater than to Cu, and that the high levels of Ni in the plants represent genuine uptake of the heavy metals and are not just redistribution of the original Ni in the seeds.

Whereas the metal uptake strategy of *A. troodii* is no doubt an adaptation to its natural environment, it appears that *Au. saxatilis* does not possess the ability to accumulate heavy metals to the same degree. The most significant finding to arise from our work is the observation that among the species of *Alyssum* studied, hyperaccumulation of Ni is associated with the potential to accumulate Co to a similar degree. This potential is realised when the Co is present in a more available form than is normal in serpentine soil. This same pattern may well be found among other taxa comprising more than 150 species of various genera that have the ability to hyperaccumulate nickel. Hyperaccumulators of Co (Brooks and Malaisse, 1985) may have a capacity to take up Ni to a similarly high degree. Research in this field should be continued since it could prove useful in biogeochemical prospecting, geobotany, and in the amelioration of metal-contaminated soils. Multiple and co-accumulation by species that are normally non-accumulators of either Co or Cu should also be studied further.

Acknowledgements

We gratefully acknowledge collection of seed by the late Mme M Conrad of Bastia, Corsica; Mr M Ch Iacovides of Nicosia, Cyprus; Messrs M and Y Foskolou of Tinos, Greece; Dr J L S Keesing of Kew, England; Dr W Greuter of Athens, Greece; and Dr A R Pinto da Silva of Portugal. We would also like to thank Ms L Burton for assistance with the pot trials.

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