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STUDIES ON PLANTS THAT HYPERACCUMULATE COPPER, COBALT AND NICKEL: THEIR POTENTIAL FOR USE IN PHYTOMINING AND PHYTOREMEDIATION.

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ABSTRACT

This thesis reviews three lines of research on hyperaccumulators to examine their potential for phytoremediation and phytomining. The first line of research was to test the affect of nutrient addition on biomass and nickel uptake by two nickel hyperaccumulators, *Alyssum bertolonii* and *Streptanthus polygaloides*. Addition of fertiliser increased the biomass although the maximum amount added was found to be suboptimal. Nutrient addition did not affect the rate of nickel uptake. Larger plants contained a more dilute nickel content but still had an overall larger amount than smaller plants.

The second line of research was to test the affect of chelates on metal uptake by copper and cobalt flora of the Democratic Republic of Congo (formally Zaïre) and a copper tolerant plant from Spain, *Erica andevalensis*. EDTA and Citric Acid increased uptake of copper in these plants but had no effect on the uptake of cobalt and nickel. EDTA increased the uptake of lead by *Alyssum bertolonii* but did not affect the uptake of zinc and cadmium.

The third line of research was to examine the reality of hyperaccumulators of copper and cobalt. Copper and cobalt hyperaccumulation does in fact exist but not to the extent reported previously. There is a good possibility that the previously reported values for copper and cobalt hyperaccumulation are in some cases erroneous due to high iron levels indicating contamination of plant samples by soil.

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INTRODUCTION

HYPERACCUMULATORS

Natural mineral deposits containing particularly large quantities of heavy metals are present in many regions of the globe. These areas often support very characteristic plant assemblages and species that thrive in these metal-enriched environments. Examples of such distinct plant communities include the serpentine flora (growing on Ni, Cr, Mn, Mg and Co rich soils), the seleniferous flora (growing on Se rich soil), the uraniferous flora (growing on U rich soils), the calamine flora (growing on Zn and Cd rich soils), and Copper/Cobalt floras. As a result of their association with specific ore deposits, many metallophyte plants are used as so-called indicator species in prospecting for mineral deposits. For example, the copper flower (*Haumaniastrum katangense*) is a classical indicator of Cu and Co ores in central Africa.

There are three basic categories of plant that grow on metalliferous soils. Metal excluders effectively prevent metal from entering their aerial parts when growing in a broad range of metal concentrations in the soil. Metal non-excluders actively accumulate metals in their above-ground tissues and can be roughly divided into two groups: indicators and hyperaccumulators. Metal levels in the tissues of indicator species generally reflect metal levels in the soil. Hyperaccumulators can concentrate metals in their above-ground tissues to levels far exceeding those present in the soil.

Baumann (1885) recorded the first hyperaccumulator of any metal when he reported over 1% zinc in the dried leaves of *Thlaspi calaminare* and *Viola calaminaria* growing over zinc deposits near Aachen, Germany. Minguzzi and Vergnano (1948) were the first to discover high nickel levels in plants. They reported over 1% nickel in leaves of *Alyssum bertolonii* growing over ultramafic rocks in Tuscany.

By far the greatest volume of work and general interest in hyperaccumulators has been centered on the genus *Alyssum*. This followed the original discovery of over 1% Ni in *Alyssum bertolonii* by Minguzzi and Vergnano in 1948. Doksopulo (1961) reported the discovery of a second hyperaccumulator of nickel, *Alyssum murale*, in 1961. In the early

1970's, Severne and Brooks (1972) discovered a third nickel plant, *Hybanthus floribundus*, in Western Australia. Following this early work, over 200 hyperaccumulators of Ni have now been reported, largely as the result of chemical tests on herbarium specimens.

The term *hyperaccumulator* was first used by Brooks *et al.* (1977a) and was originally used to define plants containing more than 1000 μg/g nickel in dry tissue. This threshold was based on histograms showing discrete plots of two different populations of the same plant genus with no overlap of hyperaccumulators and non-accumulators at the 1000 μg/g Ni boundary (see Figure 1). By 1983, Brooks using mainly herbarium specimens had identified 44 species of the *Alyssum* genus as hyperaccumulators of Ni.

The threshold of hyperaccumulation will vary considerably for different trace elements. Reeves *et al* (1995) have suggested the following concentrations (in the plant dry matter) as thresholds to define accumulation: 10000 μg/g for Zn and Mn; 1000 μg/g for Ni, Co, Cu, Cr and Pb; and 100 μg/g for Cd and Se. These values represent a concentration about 100 times greater than the normal elemental concentration ranges found in plants.

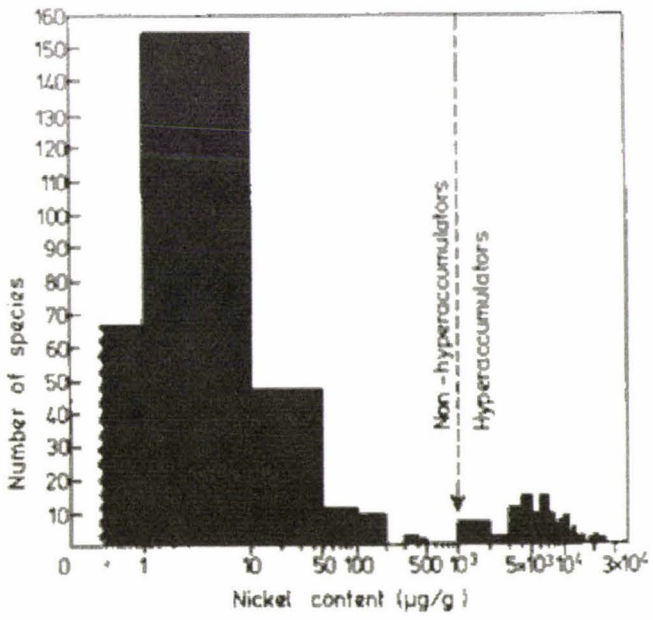

Figure 1: Histograms showing the abundance of nickel in *Alyssum* species. All values > 1000 μg/g (0.1%) are from section Odontarrhena of the genus. Source: Brooks (1987).

Almost all metal-hyperaccumulating species known today were discovered on metalliferous soils, either natural or man-made, and almost all hyperaccumulators are endemic to these soils. This suggests that hyperaccumulation is an important ecophysiological adaptation to heavy-metal stress and one of the manifestations of heavy-metal resistance.

For a long time the ability of plants to accumulate metals was considered a detrimental trait. Being at the bottom of many natural food chains, metal-accumulating plants are directly or indirectly responsible for a large proportion of the dietary uptake of toxic heavy metals by humans and other animals. Although some heavy metals are required for life, their excessive accumulation in living organisms is always toxic.

There are three fields in which hyperaccumulators have made significant impacts: mineral prospecting, phytoarchaeology, and 'green remediation' of toxic soils.

Use of Hyperaccumulators in Mineral Prospecting

Hyperaccumulators can be used for geobotanical exploration whereby the type of vegetation can be used to determine and map areas of mineralisation (Nicks *et al*, 1995). Biological methods of prospecting involve mapping the distribution of plant species confined to certain types of mineralisation. Although there are over 400 hyperaccumulators of nickel, there are only about 20 species that can tolerate and accumulate copper and cobalt. Until recently, these plants were believed to be confined solely to the Shaban Copper Arc in Shaba Province, Democratic Republic of Congo (Zaïre) which has the world's richest and most diverse copper flora. However, there have been reports that the Chinese plant, *Elsholtzia haichowensis*, a member of the Lamiaceae (mint family) and confined to copper mines in the Daye region of Hubei province, Central China, is also a hyperaccumulator of copper.

Two species of *Haumaniastrum*, *H. katangense* and *H. robertii* were once known as copper flowers and were used by early prospectors to delineate mineralised ground since they will not grow in soils containing less than 100 μg/g copper and/or cobalt. Brooks *et al.* (1980) have now identified 26 hyperaccumulators of cobalt and 24 of copper. A further 9 taxa were found to be able to hyperaccumulate both metals. All of these 38 plant

species can be used, or have been used, to prospect for copper and/or cobalt in Democratic Republic of Congo (Zaïre).

Many hyperaccumulators of nickel have been, or could be used for mineral prospecting. For example, all of the 48 *Alyssum* species with this characteristic indicate the presence of ultramafic substrates and by inference the presence of Ni, Cr and Co that are associated with this rock type. Most hyperaccumulators of nickel are endemic to nickel-rich ultramafic substrates and as such can serve as indicators of this rock type.

The association of the hyperaccumulators *Viola calaminaria* and *Thlaspi calaminare* with calamine zinc deposits in Western Europe has been known for over 100 years. As in the case of nickel, virtually all of the "zinc plants" are geobotanical indicators of zinc and/or other sulphide deposits.

There is only one clearly documented case of a lead hyperaccumulator. *Thlaspi rotundifolium subsp. cepaeifolium* from Raibl on the Austrian/Italian border has been found to contain 7000 μg/g of this element in dried tissue (Reeves *et al.*, 1983). This species has not yet been used for mineral exploration but does have this potential.

Cannon (1959, 1960, 1964) has reported the use of species of *Astragalus* for geobotanical prospecting for uranium in the western United States. Some *Astragalus* species such as *A. pattersoni* and *A. preussi* are confined to selenium-rich soils and indirectly indicate the presence of uranium in the substrate (selenium and uranium are associated geochemically in the mineral carnotite).

Phytoarchaeology and Hyperaccumulators

The hyperaccumulator *Alyssum corsicum* was first discovered growing over a few hectares of ultramafic rock in the suburbs of Bastia in Corsica. Later however it was found to have its origin in Anatolia (Turkey) on the eastern shores of the Mediterranean. It was concluded that the seed had been brought to Bastia by the Venetians as weed seed in shipments of corn and had colonised this small part of Bastia since it would only grow over ultramafic soils. This small colony of hyperaccumulating *Alyssum* traces an ancient trade route.

The Zaïrean copper flower *Haumaniastrum katangense* can also be used to indicate the presence of archaeological remains beneath the soil. Artisans of the 14th Century Kabambian culture of what is now Shaba Province, Democratic Republic of Congo (Zaïre) used to smelt copper to produce copper crosses that were used for currency. The smelters were fashioned from abandoned termite mounds and copper ores were brought to the site from elsewhere. After several years, the termite hills weathered to ground level and the residual copper ore formed a toxic ring around the original smelters. This toxic soil became colonised by *H. katangense* and the sites were later recognisable as bare open areas in the savanna. Archaeologists digging below the carpet of this hyperaccumulator of copper have been able to uncover numerous artifacts from the Kabambian and later cultures (Figure 2).

Figure 2: Abandoned native copper smelter fashioned from a termite hill at Luisha colonised by *Haumaniastrum katangense* (not in flower) and the fern *Nephrolepis undulata*. Source: Brooks *et al.* (1992).

Hyperaccumulators and Green Remediation of Soils

Only recently has the value of metal-accumulating plants for environmental remediation been fully realised. At present, remediation technologies consist primarily of removal and replacement, or simply isolation of contaminated sites. Water treatment facilities also do a relatively poor job of removing toxic metals from residential and industrial effluents. Hyperaccumulators can also be used to colonise polluted areas, such as old mine dumps and further to remove pollutants from the soil (Baker *et al*, 1995). This is termed phytoremediation (removal of pollutants from the soil by use of plants) and is based on the principle that a crop of hyperaccumulating plants may be grown on the heavy metal enriched soil and harvesting of these plants would lower the heavy metal concentrations. Phytomining is a process where these plants may be used to extract heavy metals from low grade ores that would otherwise not be economic (Chaney, 1983; Robinson *et al.*, 1997). Burning the crop produces a 'bio-ore' which can then be sold to small smelting companies as a sulphur-free, non-polluting raw material (Nicks and Chambers, 1994; 1995).

The application of plants in environmental cleanup is an emerging technology that has been called phytoremediation (I Raskin, Grant Proposal #R81869, 1991). Three subsets of this technology are being developed. First, phytoextraction (Salt *et al.*, 1995), in which metal-accumulating plants are used to transport and concentrate metals from the soil into the harvestable parts of roots and aboveground shoots. Second, rhizofiltration (Dushenkov *et al.*, 1995), which uses plant roots to absorb, concentrate and precipitate toxic metal from polluted effluents. Finally, phytostabilisation, which involves the use of plants to eliminate the bioavailability of toxic metals in soils.