

## SERPENTINE : A MODEL HABITAT FOR BOTANICAL RESEARCH IN SRI LANKA.

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### ABSTRACT

Serpentine (ultramafic) outcrops are found in most parts of the world and for many years have attracted the attention of soil scientists and plant biologists alike due to the unusual nature of both the substrate and the life forms they harbour. In this paper, we introduce serpentine outcrops as a model habitat for botanical studies and review preliminary findings of a recently conducted soil and floristic survey of four of the five known serpentine outcrops in Sri Lanka. We hope that this paper will stimulate much-needed studies, both exploratory and experimental, and point to the immediate need for conservation of this unique habitat.

### INTRODUCTION

The study of plants growing under unusual soil conditions has a long and fascinating history (Kruckeberg, 2002). Early botanists and explorers often observed unique soil-plant associations and such 'soil-indicator plants' became useful in prospecting for natural deposits of valuable metals and minerals (Brooks, 1972; Baker and Brooks, 1988; Brooks and Johannes, 1990; Brooks *et al.*, 1995; Cannon, 1960, 1971). In the last decades there has been a growing interest in the use of edaphically restricted plants for the study of plant physiology, ecology, and evolution (Mason, 1946; Antonovics *et al.*, 1971; Proctor and Woodell, 1975; Brooks, 1987; Baker *et al.*, 1992; Macnair and Gardner, 1998; Balkwill, 2001). More recently, academic and industrial scientists have come together to explore how such plants can be used for both the reclamation of vast areas of contaminated soils (*i.e.*, phytoremediation) and the extraction of toxic metals (*i.e.*, phytomining) from metal-rich sites such as abandoned mine tailings and waste sites (Brooks, 1998; Brooks *et al.*, 1998; Whiting *et al.*, 2002, 2004; Reeves, 2003). All-in-all, the study of plants growing under extreme soil conditions holds much promise, providing both a model for botanical studies as well a means for an alternative to environmental rehabilitation.

In this regard, plants that grow on serpentine soils have attracted much attention from botanists in many parts of the world (Brooks, 1987; Baker *et al.*, 1992; Roberts and

Proctor, 1992; Balkwill, 2001). Serpentine soils are derived from serpentinite and other such ultramafic rocks and are located along continental margins and on offshore islands, often associated with obducted oceanic crust (ophiolite suites) and plate tectonics (Coleman and Jove, 1992). Ultramafic rocks occupy important positions in the geology of both Cuba and New Caledonia covering areas of 5000 km<sup>2</sup> and 5500 km<sup>2</sup>, respectively. In New Caledonia, approximately one third of the landmass is occupied by rocks of ultramafic origin (Brooks, 1987). The Great Dyke of Zimbabwe is an extensive formation of serpentinite and related rocks about 500 km long and averaging 8 km wide (Wild and Bradshaw, 1977). In western North America, both local and massive occurrences of ultramafics are found; 2860 km<sup>2</sup> in California, 1170 km<sup>2</sup> in Oregon, and 520 km<sup>2</sup> in Washington (Kruckeberg, 1984). There are equally large areas of ultramafics in central Brazil (Brooks *et al.*, 1990). An extensive ultramafic rock cover of 8000 km<sup>2</sup> is found in Sulawesi, Indonesia (Baker *et al.*, 1992), however, the ultramafic regions of Asia are generally the least explored and poorly described of such regions in the world (Baker and Brooks, 1988; Proctor, 2003). Until recently, it was not known whether there were any occurrences of ultramafic rocks in Sri Lanka. Geological exploration work conducted in the 1970s revealed rocks of this type (Dissanayake and Van Riel, 1978; Munasinghe and Dissanayake, 1979, 1980; Dissanayake, 1982) located along a Precambrian suture zone between two plate boundaries, the



Vijayan and Highland Series. Since the deposits were northerly spread close to the boundary of these two Series and were fault-associated, it was believed that the serpentinites of Sri Lanka have a deep-seated origin. The work reported on the composition of the rocks suggests that the mineral sequences favour a primarily pyroxene-derived serpentinite (Dissanayake and Van Riel, 1978; Ranasinghe, 1987).

### **The nature of serpentine soils**

Soils that weather from serpentinite and other ultramafic rocks are generally shallow and rocky. They have extreme physical and chemical properties that strongly reflect the elemental composition of the parental rock. Iron (Fe), magnesium (Mg), and silicon (Si) are chief elements of the ferromagnesian minerals found in serpentine soils, with nickel (Ni), cobalt (Co), chromium (Cr) and manganese (Mn) often occurring in exceptionally high concentrations. Serpentine soils have high values of exchangeable Mg and exceptionally low values of exchangeable calcium (Ca) and are usually deficient in nutrients such as nitrogen (N), phosphorus (P), potassium (K), boron (B), and molybdenum (Mo). Cation exchange capacities (CEC) range from 5.2 – 43 Cmol<sup>(+)</sup> / kg dry soil; pH values are often high and can range from 6.1 – 8.8 (Brooks, 1987; Kruckeberg, 1992). The low nutrient status, cation imbalances and high metal concentrations, along with high temperature effects, moisture stress, soil instability, and resulting biotic conditions, limit plant growth and survival in serpentine habitats (Tadros, 1957; Proctor and Woodell, 1975; Arianoutsou *et al.*, 1993).

The stressed and highly selective nature of serpentine habitats is undoubtedly a consequence of the interplay of various physical, chemical, and biotic factors. The combination of these factors, with their particular intensities, forms the feedback loop that is known as the 'serpentine syndrome' (Jenny, 1980). The causes of the 'serpentine syndrome' have been traced to imbalance of Ca and Mg (Vlamiš and Jenny, 1948; Walker, 1954; Walker *et al.*, 1955; Madhok and Walker, 1969; Proctor and Woodell, 1975; Nagy and Proctor, 1997), Mg toxicity (Novak, 1928; Proctor, 1970), heavy metal toxicity (Antonovics *et al.*, 1971; Proctor and McGowan, 1976; Brooks, 1987), or low levels of essential nutrients such as N and P (Gordon and Lipman, 1926; Proctor and Woodell, 1975; Brooks, 1987; Proctor, 2003). A vast amount of research has

been conducted world-wide on various aspects of plant life on ultramafic substrates (Whittaker *et al.*, 1954; Krause, 1958; Proctor and Woodell, 1975; Kruckeberg, 1984, 1992; Brooks, 1987; Roberts and Proctor, 1992; Balkwill, 2001; Proctor, 2003) allowing us to make some informed generalizations on plants found in these 'harsh' environments.

### **The nature of serpentine plants**

Plants growing on serpentine soils are often morphologically and physiologically adapted to deal with the physical and chemical characteristics of this extreme edaphic environment. They are frequently dwarf and xeromorphic, with chlorotic, narrow, and glaucous leaves. They show strong sclerenchymatic development and possess enlarged root systems (Kruckeberg, 1984). These observations imply that the plants are perhaps well-equipped to deal with both water stress and nutrient stresses prevalent in these habitats. In fact, an ongoing comparative study of serpentine shrubs and their closest relatives growing off of the serpentine substrate clearly show that there are multiple traits relating to both functional morphology and physiology that differ between such species pairs, sometimes even between populations of the same species (Rajakaruna and Ackerly, unpublished data). An important aspect of plants growing in serpentine soils is their capacity to tolerate and even hyperaccumulate (Brooks, 1998; Reeves, 2003) toxic levels of heavy metals, notably nickel. Plants found in these environments have both external and internal mechanisms to tolerate high metal concentrations (Baker, 1987; Baker and Walker, 1990; Verkleij and Schat, 1990; Pollard *et al.*, 2002; Baker *et al.*, 2000). By avoiding excessive uptake of metal ions, adopting excretory mechanisms, and developing storage devices, metal-tolerant plants are able to survive in soils toxic to most vegetation. Studies have shown that mechanisms of metal tolerance may be different in different species and for different metals (Shaw, 1990; Tilstone *et al.*, 1997; Llugany *et al.*, 2003). The evidence to date suggests that cell-wall binding, active storage in vacuoles, complexation by organic acids (Lee *et al.*, 1977, 1978; Mathys, 1977) and metal binding proteins and peptides, all play their part (Bradshaw *et al.*, 1990; Strange and Macnair, 1991; Shaw, 1999). There is also evidence that novel low-molecular weight compounds, the so-called phytochelatins (Salt *et al.*, 1989; Clemens *et al.*, 1999; Cobbett,

2000) and metallothioneins (Robinson, 1990; Robinson *et al.*, 1993; Zhou and Goldsbrough, 1994; Rauser, 1995; Zenk, 1996), may be involved in metal tolerance.

Although less than 1% of the Earth's land surface is covered by ultramafic rocks, the areas harbour a disproportionately higher percentage of endemic species. New Caledonia provides an exceptional case with 60% of the island's flora restricted to the ultramafics. Here, two monotypic families (Oncothecaceae and Strasburgeraceae), 38 genera (mostly monotypic!) and over 900 species are found on ultramafic soils (Kruckeberg and Rabinowitz, 1985; Jaffré *et al.*, 1987, 1997; Macnair and Gardner, 1998). Continental areas within South Africa, Zimbabwe, Brazil, Cuba (Brooks, 1987; Brooks *et al.*, 1990; Borhidi, 1995; Reeves *et al.*, 1999; Balkwill, 2001), and California also host a wide variety of species adapted to these extreme soils. In Cuba, 14.6% of the total flora (~920 taxa) is endemic, exclusively, to serpentine (Borhidi, 1995). This is especially remarkable given only 7% of the land surface of Cuba is of serpentinitic origin. In Zimbabwe, there are at least 20 species endemic to serpentine outcrops (Kruckeberg and Rabinowitz, 1985), while in California, 10% (215 species) of the flora endemic to that state is either wholly or largely restricted to serpentine soils (Kruckeberg, 1984).

### **The serpentine habitats of Sri Lanka: the background**

The serpentine habitats in Sri Lanka have not been extensively studied; the authors are aware of only one published report by Brooks (1987). Brooks lists only three plant species (*Evolvulus alsinoides*, *Cymbopogon flexuosus*, *Morinda tinctoria*) in his brief account of the serpentines of the island indicating that the serpentine flora of Sri Lanka (as well as of India) is somewhat impoverished with regard to numbers of species as well as the percentage endemism. Brooks mentions that the floras do not hold the same potential for future botanical research as other Asian serpentine floras, particularly those of the offshore islands of Southeast Asia (the Malay Archipelago), where large numbers of endemic species have been recorded (Brooks, 1987; Proctor, 2003). Currently, there are no published records of any aspects of the fauna or specialized plant-insect or –microbial relationships (Hopkins, 1987; Boyd *et al.*, 1994; Boyd, 1998; Ghaderian *et al.*, 2000; Wall and Boyd, 2002; Héry *et al.*, 2003) of these sites, making the Sri Lankan sites some of the better documented geologically (Dissanayake and Van Riel, 1978; Munasinghe and Dissanayake, 1979, 1980; Dissanayake, 1982) yet natural historically least known serpentinized areas in Asia.

The observations made by Brooks (1987; personal communication, 1999) on Sri Lanka's serpentine flora during his brief visit to the island in 1981 are intriguing given the fact that Sri Lanka has greater biodiversity per unit area than any other country in Asia (Baldwin, 1991). Approximately 30% of the angiosperms (total 3100), 18% of the ferns (total 314), 35% of lichens (total 110), and 22% of the vertebrates (total 568) are endemic to the island. This high level of biological diversity and endemism, combined with the vulnerability of the habitats in which these species are often found, has attracted the attention of local and international conservation authorities. Sri Lanka, together with the Western Ghats of India, has now been declared one of 25 biodiversity hotspots in need of immediate protection (Myers *et al.*, 2000). Most of these conservation efforts and scientific studies are duly directed at the highly diverse lowland rainforests of the southwest where, for example, approximately 50% of the endemic angiosperms are locally restricted. The Sinharaja lowland rainforest, now a World Heritage Site with an area of 8,800 hectares or 89 km<sup>2</sup>, is the home for 75, 95, 58, and 51% of Sri Lanka's endemic tree, bird, mammal, and butterfly species, respectively (Myers, 1990). While this level of diversity and endemism provides the need for special attention (De Zoysa and Raheem, 1990; Gunatilleke and Gunatilleke, 1985; Gunatilleke and Gunatilleke, 1991), it is also important that other habitat types, such as serpentine habitats, are also identified, characterized, and conserved. The lack of studies on the serpentine habitats of Sri Lanka indicates that there is generally a low awareness of the presence of this unusual habitat, with immense potential for harbouring unique assemblages of plants and animals.

In 1999, the first preliminary survey of four of the five known serpentine outcrops in Sri Lanka was conducted (Rajakaruna and Bohm,

2002). The survey was directed at describing the nature of the serpentine soils and exploring the floristic diversity found in these sites. To our knowledge, there have been no systematic studies previously done on the soils or flora of these sites. Thus, the paper added to the very limited knowledge on the serpentine soils and vegetation of the Indian subcontinent (Banerjee, 1972; Roy, 1974; Brooks, 1987) as well as that of Asia (Proctor, 2003). In the rest of this paper, we summarize the key findings reported in Rajakaruna and Bohm (2002) and a resulting pharmacological study using Sri Lanka's serpentine plants (Rajakaruna *et al.*, 2002b) in order to make our preliminary observations known to the larger scientific community in Sri Lanka. We hope that this paper will make regional scientists and conservationists aware of the unique nature of serpentine habitats and stimulate research as well as much-needed efforts to conserve these sites from any future destruction (Whiting *et al.*, 2002, 2004).

## RESULTS AND DISCUSSION

### Preliminary survey of Sri Lanka's serpentine habitats: a summary

There are at least five major serpentine outcrops in Sri Lanka (Figure 1). The following is a description of four of the sites explored during a brief visit to the island in 1999 (Rajakaruna and Bohm, 2002).

#### Site 1: Ussangoda

The Ussangoda (Welipatanwila) outcrop is located near Tangalla en route to Hambanthota on a coastal bluff overlooking the Indian Ocean. The area of the serpentine outcrop is approximately 3 km<sup>2</sup>. The soil is characterized as hematite-rich clayey sand and is approximately 40 cm deep. The top 10 cm is overlain with very finely grained (loess-like) particles giving this heavily laterized site a reddish hue. This coloration has led to the common belief among local people that this is the site where betel, *Piper betel* L. (Piperaceae), was first brought to Sri Lanka - the red color of the soil resulting from the deposition of masticated waste of betel. Previous geochemical studies (Ranasinghe, 1987) have documented a total Ni content of 1000 ppm ( $\mu\text{g/g}$

soil) and a Cr content of 7700 ppm ( $\mu\text{g/g}$  soil) for these soils. The site is almost devoid of vegetation except for scattering of small herbaceous plants. The boundary of the serpentine is clearly marked by low thorn shrubs interspersed with a yet to be identified species of *Opuntia* (Cactaceae).

#### Site 2, 3: Indikolapelessa and Ginigalpelessa

The Indikolapelessa and Ginigalpelessa (Udawalawe) outcrops are located in the south-central part of the island. Each of these outcrops measures *c.* 1 km<sup>2</sup> in area. The soil is dark brown and poorly-developed. The total Ni content in the Ginigalpelessa site averages around 2100 $\mu\text{g/g}$ , while the Cr content averages around 10,000  $\mu\text{g/g}$  (Ranasinghe, 1987). There is no geochemical information presently available for the site at Indikolapelessa. Dissanayake and Van Riel (1978, 1979) recorded a Ni content of 0.8-1.9% for the Udawalawe serpentinite.

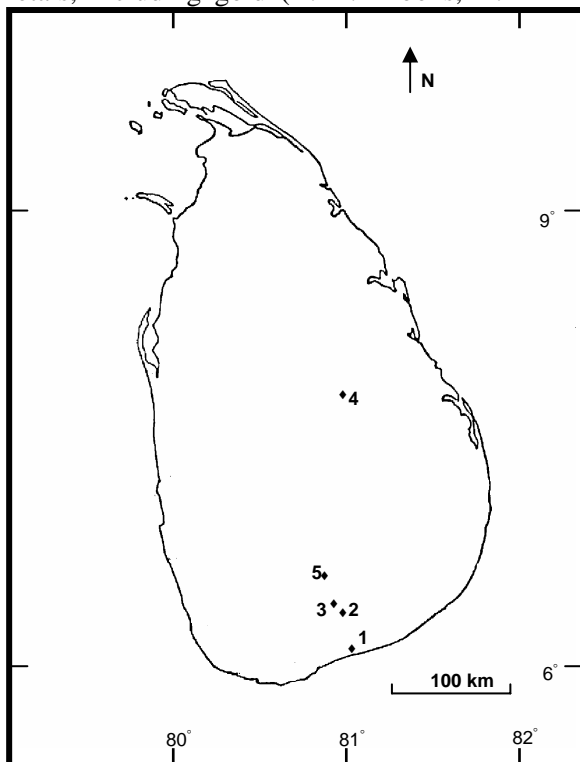
#### Site 4: Yodhagannawa

The Yodhagannawa (Yudhaganawa) deposit is located in the north-central part of the island. There is presently no geochemical information available for the site. Interestingly, the reddish hue of the soil here has led to another folk belief that the red colour resulted from blood spilled during a battle between locals and Europeans during the colonial periods.

Both Ussangoda and Yodagannawa sites are protected. Ussangoda has been declared as a 'biologically sensitive area' while Yodagannawa is part of a nature reserve (Wasgamuwa National Park). While the latter may receive a higher level of protection, the site at Ussangoda is regularly used as a playground by local people and visitors. Flat portions of the outcrop are used as a cricket playing ground inflicting much damage to the soil as well as the limited number of herbaceous species that are present. Extensive use of this site will undoubtedly alter the soil properties and have a negative impact on the abundance of species present. The sites at Indikolapelessa and Ginigalpelessa are also somewhat protected owing to the fact that they are found within the boundaries of a state-run sugar cane plantation (Sevanagala Sugar Plantation). The staff indicated

that they have attempted to cultivate sugar cane in these sites but that the soil is not favourable for growth. While their lack of knowledge concerning the nature of serpentine substrate was apparent from these comments, it was also disturbing to realize that these sites may be used for future development, once it is realized that the sites are not suitable for cultivation. Both Ussangoda and Ginigalpelessa sites have been explored for geochemical prospects of various heavy metals, including gold (R. R. Brooks, R.

Ranasinghe, personal communications, 1999). Although it is known that high concentrations of both Ni and Cr have been found in these sites, the nature of the explorations or the decisions made regarding any future prospecting is unknown to the authors.



**Figure 1. A map of Sri Lanka showing the approximate locations of the five serpentinite outcrops. Sites 1: Ussangoda; 2: Indikolapelessa; 3: Ginigalpelessa; 4: Yodhagannawa; 5: Katupotha (unexplored).**

### Serpentine soils of Sri Lanka

Since details of soil collection, sample preparation, and analyses are provided in Rajakaruna and Bohm (2002), we summarize the main findings for the purposes of this paper. The chemical and physical features of the soil samples collected from the serpentine sites in Sri Lanka are typical of such substrates found in other parts of the world (Brooks, 1987), with concentrations of DTPA-extractable (Lindsay and Norvell, 1978) heavy metals such as Ni and Cr uniformly high and Ca/Mg quotients consistently low. High heavy metal and low Ca concentrations make the soils unfavourable for normal plant growth. The

soils of Ussangoda differed the most from a typical serpentine soil. Here, pH and cation exchange capacity (CEC) were low and the Ca/Mg quotient was high. Further, Ussangoda soils are distinctive in having much higher concentrations of extractable Al, Co, and Zn and much lower concentrations of Fe, Mg, and Si. This may have resulted from heavy laterization of soils of this site, which results in loss of the divalent cations such as Fe, Mn, Co, and Ni through solubilization and leaching (Brooks, 1987). The unusually low CEC and low pH may also have contributed to loss of these ions as well as the greater availability of Al. Table 1 lists the

means for the various soil characteristics determined for these Sri Lankan sites.

### Serpentine plants of Sri Lanka

The floristic survey documented a significantly larger number of species for the flora of Sri Lanka's serpentine habitats than previously recorded by Brooks (1987). For practical details on the methodology for plant collection, identification, and tissue analysis refer to Rajakaruna and Bohm (2002). The three dominant families represented are Poaceae, Asteraceae and Fabaceae (Table 2). The floristic composition was typical of serpentine habitats in other parts of the tropics with Asteraceae, Euphorbiaceae, Fabaceae, Poaceae and Rubiaceae well represented. All 21 families identified in the survey were known to occur in serpentine habitats elsewhere in the world. Further, at least 17 of the 40 genera identified are known from serpentine sites elsewhere (Table 3; Brooks, 1987, 1998; Baker *et al.*, 1992), suggesting that the survey has added perhaps 23 new genera to the list of plants that are known to occur on serpentine soils. Many of the genera (e.g., *Hybanthus*, *Evolvulus*), as well as the same species in some cases, occur in an arc-like distribution that runs between eastern Africa and Western Australia presenting an intriguing phytogeographic pattern. The total number of taxa collected per site were, Ginigalpelassa (25), Indikolapelassa (12), Ussangoda (9), and Yodhagannawa (3). Since the collections were made within a limited time span not all species growing in each site could be obtained. The most commonly found species, occurring in three of the four sites, were *Leucas zeylanica*, *Cymbopogon flexuosus*, *Eupatorium odoratum*, and *Phoenix farinifera* all found in two sites each. Surprisingly, none of the species identified was endemic to the island. However, the precise taxonomy of several species is questionable. These include: *Hybanthus enneaspermus*, *Evolvulus alsinoides*, *Crotalaria biflora*, *Desmodium triflorum*, *Epaltes divaricata*, *Geniosporum tenuiflorum*, *Fimbristylis falcata*, *Cymbopogon flexuosus* as well as an unidentified species each of *Canthium* and *Phyllanthus*. One of the more interesting observations made was in the colour polymorphism of *Evolvulus alsinoides*. Whilst it is known that the species has two colour morphs (Austin in Dassanayake and Fosberg, 1980), it is unknown whether there is a correlation between colour and any edaphic

differences in their microhabitats. Such edaphically-correlated pigment differences are known in species that inhabit distinct soils (Rajakaruna and Bohm, 1999; Schemske and Bierzychudek, 2001). In comparison to serpentine floras of the islands of southeast Asia, the Sri Lankan serpentine flora appears to be impoverished with regard to both number of species as well as degree of endemism. It is unclear why an island as rich in endemics as Sri Lanka should harbour such an apparently impoverished serpentine flora. A factor that may have contributed to this apparent anomaly may be that sampling in this survey was limited to those species that could be identified using floral or other reproductive structures. Rare or endemic taxa could have been easily overlooked if they were not in flowering condition. Further, sampling was carried out over only a day or even hours depending on the site, seriously limiting opportunities for a thorough survey of the flora. Limited sampling also prevented observation of any minor morphological features that might indicate taxonomically recognizable differences especially in those taxa mentioned earlier where there were obvious differences in plant size and growth habit relative to populations that are found off the serpentine substrate (personal observations). For example, at least 17 varieties of *Evolvulus alsinoides* have been described globally (Austin in Dassanayake and Fosberg, 1980) and the small-leaved, prostrate growth habit plants found at Ussangoda may represent an edaphic race or even subspecies restricted to the serpentine substrate. Brooks (1987) states that *Cymbopogon flexuosus* found in the Sri Lankan serpentine sites is a form endemic to serpentine. This observation made by Brooks (1987) is worthy of further investigation.

Tissue analyses indicate that elemental concentrations found in the Sri Lankan serpentine plants (Rajakaruna and Bohm, 2002) are typical of those found in serpentine plants elsewhere (Brooks, 1987, 1998; Roberts and Proctor, 1992; Baker *et al.*, 1992). Most of the plants collected accumulated higher than 'normal' concentrations of several elements: Al, As, Cd, Co, Cu, Cr, Na, Ni, Pb, and Zn. Concentrations of all these elements were greater than those that are normally considered to be toxic to most plants (Kabata-Pendias and Pendias, 1984; Mehta and Farago, 1994). Of particular interest was the discovery of

three Ni and five Cu hyperaccumulators (Table 4). Hyperaccumulators demonstrate extreme metal tolerance and are able to accumulate over 100 times the concentration found in non-hyperaccumulator plants growing on the same substrate (Brooks, 1998). For most metals, hyperaccumulation is defined as more than 1000 µg metal per g dry leaf tissue (0.1%) however for Zn and Mn, 1% is regarded as exceptional (Baker and Brooks, 1989). All three Ni hyperaccumulators were discovered at Ussangoda. Two of the three Ni hyperaccumulators discovered, *Evolvulus alsinoides* and *Hybanthus enneaspermus*, also occur in serpentine sites in Queensland, Australia (R. D. Reeves, Massey University, New Zealand, personal communication), but neither of these accumulates sufficient Ni to qualify as a hyperaccumulator. Since *E. alsinoides* is known to comprise numerous varieties, it is tempting to speculate that the population found at Ussangoda may represent a physiologically distinct form, perhaps an edaphic race. At least seven taxa from the genus *Hybanthus* are known to hyperaccumulate Ni (Brooks, 1998; Reeves, 1992, 2003; Reeves and Baker, 2000). Given the predisposition of taxa from this genus to hyperaccumulate Ni, it is possible that the population found at Ussangoda may possess this unique capacity. Both species, however, warrant further detailed investigation to confirm these preliminary findings. The third Ni hyperaccumulator revealed by the survey, *Crotalaria biflora*, has not been previously recorded to hyperaccumulate this metal in other parts of its range. However, *C. cobalticola* Duvign. & Plancke from the Democratic Republic of Congo (formerly Zaïre), is a known Co hyperaccumulator (Brooks *et al.*, 1995). It would be of interest to find out if the tendency to hyperaccumulate metals is more widespread in this large genus.

*Rinorea bengalensis* (Wall.) O.K. (Violaceae to which *Hybanthus* also belongs), a species that was not encountered in the current survey, also hyperaccumulates Ni (Brooks and Wither 1977a, b; Jopony and Baker, 2000).

*Rinorea bengalensis* is found in a broad arc-like distribution encompassing Sri Lanka, the Malay Archipelago, New Guinea, the Solomon Islands, and Queensland, Australia. Brooks and Wither (1977a, b) conducted a survey of herbarium specimens from the entire range of this species and found that Ni hyperaccumulation is widespread in this plant. The level of Ni found in the herbarium specimen of this species from Sri Lanka was reported to be in excess of 10,000 ppm (10-fold the minimum required to be a hyperaccumulator!). This high level indicates that the specimen tested was obviously collected from a serpentine site, the locality indicated on a map presented by Brooks and Wither (1977a) suggesting a collection in the central part of the island. This species was not encountered in our field exploration and is worthy of intense exploration, especially in the Udawalawe sites or at the unexplored site near Katupotha.

A surprising finding of the tissue analyses was the discovery of five Cu hyperaccumulators: *Geniosporum tenuiflorum*, *Clerodendrum infortunatum*, *Croton bonplandianus*, *Waltheria indica*, and *Tephrosia villosa* all hyperaccumulated this metal. To date, almost all Cu hyperaccumulators discovered have been from the copper mineralized soils of Democratic Republic of Congo (Brooks *et al.*, 1992; Reeves and Baker, 2000). Hyperaccumulation of Cu by serpentine plants appears to be a very rare phenomenon. However, tolerance to potentially toxic levels of Cu has been observed in *Mimulus guttatus* Fischer ex DC (Scrophulariaceae), a species that occurs in serpentine soils in California (Tilstone and Macnair, 1997a, b). Although none of the Cu hyperaccumulators discovered in Sri Lanka has been previously known to have this capacity, both Fabaceae and Lamiaceae (three of the five taxa belong to these two families) have genera with the capacity to hyperaccumulate Cu (Reeves and Baker, 2000). The discovery of these new Cu hyperaccumulators from the ultramafics of Sri Lanka is even more surprising since Brooks in a biogeochemical and geobotanical survey of the Seruwila copper-magnetite prospect near



**Table 1.**

**The species collected from the four serpentine sites in Sri Lanka. Site abbreviations are GGP=Ginigalpelassa, IP=Indikolapelassa, U=Ussangoda, Y=Yodhagannawa.**

Family	Species	Site
<i>Asteraceae</i>	<i>Epaltes divaricata</i> (L.) Cass.	U
Convolvulaceae	<i>Evolvulus alsinoides</i> (L.) L.	U
<i>Cyperaceae</i>	<i>Fimbristylis falcata</i> (Vahl.) Kunth	U
Fabaceae	<i>Cassia auriculata</i> L.	U
Fabaceae	<i>Crotalaria biflora</i> (L.) L.	U
Fabaceae	<i>Desmodium triflorum</i> (L.) DC.	U
Rubiaceae	<i>Tarenna asiatica</i> (L.) Alston	U
Rutaceae	<i>Toddalia asiatica</i> Lamk.	U
Violaceae	<i>Hybanthus enneaspermus</i> (L.) F. Muell.	U
Fabaceae	<i>Tephrosia purpurea</i> Pers.	Y
Lamiaceae	<i>Geniosporum tenuiflorum</i> (L.) Merr.	Y
<i>Lamiaceae</i>	<i>Leucas zeylanica</i> (L.) R. Br.	Y
Arecaceae	<i>Phoenix farinifera</i> Roxb.	IP
Asteraceae	<i>Eclipta prostrata</i> (L.) L.	IP
Asteraceae	<i>Eupatorium odoratum</i> L.	IP
Asteraceae	<i>Spilanthes calva</i> DC.	IP
Asteraceae	<i>Vernonia cinerea</i> (L.) Less.	IP
Euphorbiaceae	<i>Phyllanthus myrtifolius</i> Moon	IP
Fabaceae	<i>Tephrosia villosa</i> Pers.	IP
Lamiaceae	<i>Leucas zeylanica</i> (L.) R. Br.	IP
Malvaceae	<i>Abutilon indicum</i> Sweet	IP
Malvaceae	<i>Sida acuta</i> Burm.	IP
Poaceae	<i>Cymbopogon flexuosus</i> (Steudel) W. Watson.	IP
Solanaceae	<i>Physalis minima</i> L.	IP
Amaranthaceae	<i>Aerva lanata</i> (L.) Juss. Ex Schult.	GGP
Apocynaceae	<i>Carissa spinarum</i> L.	GGP
Arecaceae	<i>Phoenix farinifera</i> Roxb.	GGP
Asclepiadaceae	<i>Calotropis gigantea</i> (L.) R. Br.	GGP
Asteraceae	<i>Eupatorium odoratum</i> L.	GGP
Euphorbiaceae	<i>Croton bonplandianus</i> Baill.	GGP
Euphorbiaceae	<i>Croton hirtus</i> L'Her.	GGP
Euphorbiaceae	<i>Euphorbia rubicunda</i> Blume	GGP
Euphorbiaceae	<i>Phyllanthus</i> L. sp.	GGP
Fabaceae	<i>Cassia fistula</i> L.	GGP
Lamiaceae	<i>Leucas zeylanica</i> (L.) R. Br.	GGP
Lamiaceae	<i>Ocimum sanctum</i> L.	GGP
Liliaceae	<i>Asparagus zeylanicus</i> Hook. f.	GGP
Myrtaceae	<i>Syzygium cumini</i> Skeels	GGP
Poaceae	<i>Apluda mutica</i> L.	GGP
Poaceae	<i>Aristida setacea</i> Retz.	GGP
Poaceae	<i>Eragrostis ciliaris</i> (L.) R. Br.	GGP
Poaceae	<i>Cymbopogon flexuosus</i> (Steudel) W. Watson	GGP
Rhamnaceae	<i>Zizyphus oenoplia</i> Mill.	GGP
Rubiaceae	<i>Canthium puberulum</i> Thw. ex. Hook. f.	GGP
Rubiaceae	<i>Canthium</i> Lam. sp.	GGP
Rubiaceae	<i>Morinda tinctoria</i> Roxb.	GGP
Sterculiaceae	<i>Waltheria indica</i> L.	GGP
Verbenaceae	<i>Clerodendrum infortunatum</i> L.	GGP
Verbenaceae	<i>Lantana camara</i> L.	GGP



Trincomalee did not find any plant with a copper concentration  $> 21 \mu\text{g/g}$  in leaf dry matter.

The occurrence of hyperaccumulators in specific families and within certain genera in various parts of the world raises the question as to whether some families as well as genera possess a constitutive or biochemical factor that predisposes them to serpentine tolerance accompanied by the accumulation of large amounts of metal (Bradshaw *et al.*, 1990). So far  $>320$  (and growing!) hyperaccumulators of Ni have been discovered (Reeves and Baker, 2000).

Brassicaceae and Euphorbiaceae include 82 species each while Asteraceae, Flacourtiaceae, Buxaceae, and Rubiaceae include 26, 19, 17, and 12 species, respectively (Reeves and Baker, 2000; Borhidi, 2001). Of the 24 or so known Cu hyperaccumulators, 14 belong to Cyperaceae, Lamiaceae, Poaceae, Scrophulariaceae, Fabaceae and Asteraceae (Baker and Brooks, 1989; Reeves and Baker, 2000). The hyperaccumulators of Ni and Cu discovered in Sri Lanka add to these lists further confirming the observation that certain families are apparently predisposed to having species with the capacity to accumulate metals.

**Table 2.**

**Mean ( $\pm$  SE) for various soil features in soil samples collected from the four ultramafic sites in Sri Lanka. Element concentrations are in  $\mu\text{g/g}$  dry soil and CEC is in  $\text{Cmol}^{(+)}/\text{kg}$  dry soil. The mean concentrations for the four sites (L-R) are a result of soil analysis of 5, 6, 6, and 3 samples, respectively.**

Soil Feature	Ginigalpelessa	Indikolapelessa	Ussangoda	Yodhagannawa
Aluminum	0.2 ( $\pm$ 0.07)	0.5 ( $\pm$ 0.2)	6.6 ( $\pm$ 1)	0.13 ( $\pm$ .02)
Barium	1( $\pm$ 0.52)	0.97 ( $\pm$ 0.21)	0.3 ( $\pm$ 0.08)	0.2 ( $\pm$ 0.01)
Boron	0.01 ( $\pm$ 0.006)	0.01 ( $\pm$ 0.006)	0.04 ( $\pm$ 0.002)	0.01 ( $\pm$ 0.01)
Cadmium	0.0002 ( $\pm$ 0.0002)	0.02 ( $\pm$ 0.01)	0.002 ( $\pm$ 0.002)	0
Calcium	921 ( $\pm$ 294)	1030 ( $\pm$ 239)	166 ( $\pm$ 13.9)	128 ( $\pm$ 4.6)
Ca/Mg	0.35 ( $\pm$ 0.1)	1.04 ( $\pm$ 0.5)	1.83 ( $\pm$ 0.2)	0.14 (0.01)
CEC	42.2 ( $\pm$ 3.6)	34.3 ( $\pm$ 3.6)	8.5 ( $\pm$ 0.8)	15.4 ( $\pm$ 0.7)
Chromium	0.07 ( $\pm$ 0.03)	0.05 ( $\pm$ 0.01)	0.05 ( $\pm$ 0.01)	0.05 ( $\pm$ 0.01)
Cobalt	1.94 ( $\pm$ 0.56)	2.56 ( $\pm$ 0.94)	5.3 ( $\pm$ 0.98)	1.4 ( $\pm$ 0.14)
Copper	0.75 ( $\pm$ 0.15)	1.4 ( $\pm$ 0.6)	0.62 ( $\pm$ 0.05)	0.22 ( $\pm$ 0.02)
Iron	58.2 ( $\pm$ 10.6)	39.9 ( $\pm$ 4.5)	16.5 ( $\pm$ 0.6)	46.8 ( $\pm$ 6.6)
Lead	0.15 ( $\pm$ 0.03)	0.13 ( $\pm$ 0.04)	0.25 ( $\pm$ 0.06)	0.05 ( $\pm$ 0.01)
Magnesium	2810 ( $\pm$ 164.1)	1752.1( $\pm$ 357.8)	94.6 ( $\pm$ 10.4)	937.5 ( $\pm$ 50.5)
Manganese	32.8 ( $\pm$ 10.3)	76.1( $\pm$ 18.3)	63.8 ( $\pm$ 8.2)	25.7 ( $\pm$ 2.4)
Molybdenum	0.001( $\pm$ 0.0006)	0.002 ( $\pm$ 0.0017)	0	0
Nickel	104.2 ( $\pm$ 37.1)	59.2 ( $\pm$ 21.2)	45.6 ( $\pm$ 5.9)	66.9 ( $\pm$ 9.9)
pH (water)	6.3 ( $\pm$ 0.3)	5.6 ( $\pm$ 0.2)	4.6 ( $\pm$ 0.1)	5.4 ( $\pm$ 0.2)
pH (CaCl <sub>2</sub> )	5.7 ( $\pm$ 0.4)	5.3 ( $\pm$ 0.2)	4.3 ( $\pm$ 0.1)	5 ( $\pm$ 0.2)
Potassium	144 ( $\pm$ 29.5)	419.6 ( $\pm$ 232.4)	224.2 ( $\pm$ 27.9)	67.5 ( $\pm$ 7.5)
K/Na	12.8 ( $\pm$ 5)	39.1( $\pm$ 15)	12.5 ( $\pm$ 2.9)	8.3 ( $\pm$ 0.8)
Silicon	13.1( $\pm$ 1.6)	19.6 ( $\pm$ 5.4)	0.4 ( $\pm$ 0.05)	18 ( $\pm$ 0.9)
Sodium	26.7 (11.4)	8.9 ( $\pm$ 1.1)	23.2 ( $\pm$ 6.7)	8.1 ( $\pm$ 0.5)
Zinc	0.63 ( $\pm$ 0.2)	0.92 ( $\pm$ 0.2)	2.1( $\pm$ 1.4)	0.7 ( $\pm$ 0.08)

Table 3.

The phytogeography and previously documented metal hyperaccumulating behaviors of 17 genera found on the serpentine substrate in Sri Lanka. Information from various sources listed in Brooks, 1987, 1998; Baker *et al.*, 1992; Reeves and Baker, 2000 and Reeves, personal communication, 2000.

Genus (Family)	Recorded Distribution on Serpentine	Metal Hyperaccumulating Behavior (if documented)
<i>Aristida</i> (Poaceae)	Zimbabwe	
<i>Cassia</i> (Fabaceae)	Australia	
<i>Crotalaria</i> (Fabaceae)	Angola, Zimbabwe, Zaire	Cobalt (1 taxon)
<i>Cymbopogon</i> (Poaceae)	Oman, Zimbabwe	
<i>Desmodium</i> (Fabaceae)	Maryland, U.S.A.	
<i>Epaltes</i> (Asteraceae)	W. Australia	
<i>Eragrostis</i> (Poaceae)	Zimbabwe	
<i>Eupatorium</i> (Asteraceae)	Maryland, U.S.A.	
<i>Euphorbia</i> (Euphorbiaceae)	Anatolia, Brazil, Oman, Zimbabwe, Cuba	Nickel (2 taxa)
<i>Evolvulus</i> (Convolvulaceae)	W. Australia	
<i>Fimbristylis</i> (Cyperaceae)	South Africa, New Caledonia, W. Australia	
<i>Hybanthus</i> (Violaceae)	New Caledonia, W. Australia, Cuba	Nickel (7 taxa)
<i>Leucas</i> (Lamiaceae)	Zimbabwe	
<i>Morinda</i> (Rubiaceae)	Philippines	
<i>Phyllanthus</i> (Euphorbiaceae)	Philippines, Sabah, New Caledonia, Cuba	Nickel (10 taxa)
<i>Syzygium</i> (Myrtaceae)	Philippines	
<i>Tarenna</i> (Rubiaceae)	New Caledonia	

Table 4.

A sample of species showing high metal accumulating behaviour collected from the Sri Lankan serpentine sites (from Rajakaruna and Bohm, 2002). Soil concentrations, when listed, are *extractable* or *exchangeable* values determined for soils collected from 0-10cm depth. Plant tissue concentrations are *total* values for whole plants in herbaceous species and shoots in shrubs. Hyperaccumulators of Ni and Cu are in bold. Site abbreviations are GGP=Ginigalpelassa, IP=Indikolapelassa, U=Ussangoda, Y=Yodhagannawa.

Species	Site	Element	Soil ( $\mu\text{g/g}$ dry)	Plant ( $\mu\text{g/g}$ dry)
<i>Aristida setacea</i>	GGP	Cr	0.1	19
		Ni	15	439
<i>Calotropis gigantea</i>	GGP	Na	66	12017
<i>Canthium sp.</i>	GGP	Cr	-	16
<i>Cassia auriculata</i>	U	Cu	0.5	885
<i>Clerodendrum infortunatum</i>	GGP	Cu	-	2208
<i>Crotalaria biflora</i>	U	Ni	42	1048
<i>Croton bonplandianus</i>	GGP	Cu	-	2206
<i>Croton hirtus</i>	GGP	Cr	-	40
<i>Cymbopogon flexuosus</i>	GGP	Cr	-	26
	IP	Cr	-	18
<i>Epaltes divaricata</i>	U	Ni	52	733
		Cr	0.1	17
<i>Evolvulus alsinoides</i>	U	Ni	55	1297
		Cr	0.1	23
<i>Geniosporum tenuiflorum</i>	Y	Cr	0.1	21
		Cu	0.3	2299.3
<i>Hybanthus enneaspermus</i>	U	Ni	29	1786
		Cr	0.1	21
<i>Leucas zeylanica</i>	GGP	Cr	0.1	28
	IP	Cr	0.1	19
<i>Phyllanthus sp.</i>	GGP	Cu	-	821
<i>Phyllanthus myrtifolius</i>	IP	Cr	-	22
<i>Tephrosia villosa</i>	IP	Cu	0.2	1723
<i>Waltheria indica</i>	GGP	Cu	-	1534

## CONCLUSIONS

The study reported in Rajakaruna and Bohm (2002) and summarized here represents the

first broad-scale floristic survey of the serpentine habitats of Sri Lanka. Owing to time constraints, sampling was limited and was restricted to those

species identifiable using reproductive structures. This type of haphazard sampling may have had an effect on the unexpected paucity of endemic or rare species. Reiterating the preliminary nature of this survey, it should also be pointed out that no attempts were made to determine cellular localizations of any of the accumulated metals or investigate any other physiological aspects of the plant species found at these sites. The findings, however, warrant detailed collections, expanded taxonomic study, and potentially significant ecophysiological investigations of Ni and Cu hyperaccumulation.

Serpentine habitats provide a multitude of opportunities for botanical research. In addition to taxonomic and physiological studies of the nature described in this paper, serpentine habitats also provide unique opportunities to explore specialized plant-microbe interactions (Hopkins, 1987; Ghaderian *et al.*, 2000; Hungate *et al.*, 2000; Jaffré *et al.*, 2001) and plant-herbivore interactions (Boyd, 1998; Mesjasz-Przybylowicz and Przybylowicz, 2001), the process of speciation (Kruckeberg, 1996; Macnair and Baker, 1994; Macnair and Gardner, 1998; Rajakaruna and Whitton, 2004), as well discovery of novel compounds that may be produced in response to edaphic stresses. It is possible such compounds could have secondary and often unforeseen effects on other organisms (Hanawa *et al.*, 2000; Pernas *et al.*, 2000). In fact, a study conducted on the pharmacological properties of the Sri Lankan serpentine plants (Rajakaruna *et al.*, 2002b) document that 29 of the 45 species are active against at least one microorganism tested, suggesting that these plants may produce chemical compounds with important antimicrobial properties.

Serpentine habitats of Sri Lanka are easily accessible and can provide a living laboratory for numerous scientific studies both exploratory and experimental in nature. In many ways, they can provide a counterpart to the highly regarded and carefully studied rainforests of the southwest. The lack of information and awareness of the serpentines of Sri Lanka will only lead to the destruction of these unique habitats that have so far gone unnoticed as potential sites for harbouring a wealth of genetic diversity (Whiting *et al.*, 2002, 2004).

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