Alyssum lesbiacum: a new Ni-hyperaccumulator from Lesbos

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Introduction

Ultramafic rocks cover a little less than 1% of the earth's surface and owe their origin to surface processes (Coleman & Jove, 1992). 'Ultramafic' is a broad term applied to rocks such as *serpentinite*, with the general requirement that they contain >70% mafic (also called ferromagnesian) materials (Kruckeberg, 2002).

Serpentine's physical conditions are often inhospitable for most plants. They are frequently rocky with granular texture, easily weathered at the earth's surface (vulnerable to erosion), and characterized by a lack of organic material. Soil moisture-holding capacity is generally low and soil profile development is generally slow and poor (Kruckeberg, 2002). Although the physical properties of the soil and the restricted water supply are undoubtedly important factors in limiting plant growth, the peculiarities of serpentine soils are usually explained in terms of their chemical composition.

Three are the most popular explanations of why edaphic factors may control serpentine floras, known as the 'serpentine syndrome' (Kruckeberg, 1984): (a) Low availability of calcium relative to magnesium; (b) Deficiency of essential macronutrients (P, N, K), and (c) High levels of phytotoxic heavy metals (Ni, Cr, Co, Mn).

High concentrations of potentially phytotoxic heavy metals contained in serpentinitic parent materials and soils, such as nickel (Ni), chromium (Cr), cobalt (Co) and manganese (Mn), can present an additional stress that serpentine plants must tolerate. Baker (1981) proposed two different strategies that metal-tolerant plants can employ to cope with high levels of metals in the soils: exclusion (from the shoots) and accumulation (in the shoot). Based on these strategies, Baker (1981) suggested three types of plant-soil relationships: excluders, accumulators, indicators and hyperaccumulators. *Excluders* are defined as plants that restrict transport of metals to the shoot, and maintain relatively low metal concentrations in the shoot over a wide range of metal concentrations in the soil. The *accumulators* show a tendency or ability to translocate and accumulate high levels of metal in the above-ground plant parts, from both low and high soil metal concentration levels, without any associated toxicity symptoms, i.e. their leaf/root metal concentration quotient is >1.0 (Baker, 1981; Baker & Brooks, 1989; Terry & Bañuelos, 2000). For *indicator* plants, an intermediate response to high metal concentration in the soil is shown, and the metal concentration in the plants reflects a measure of the concentration in the soil, i.e. the plant/soil metal concentration quotient is relatively constant (Baker, 1981).

Hyperaccumulators are defined as plants that accumulate >100-fold more metal in their shoots than normal plants (Brooks, 1998, Reeves & Baker, 2000). For Ni, Co, Cr, the concentration is >1000 mg kg^{-1} (Baker & Brooks, 1989). Hyperaccumulators can simply be viewed as accumulator plants that show an extreme behaviour in metal uptake and translocation to the shoots. This unique characteristic of hyperaccumulators has sparked great research interest, especially for their possible use in the remediation of heavy metal contaminated soils and phytomining (Chaney *et al.*, 2005; 2007). The metal-rich biomass of hyperaccumulators can be disposed in a landfill or burned to generate biomass energy and the ash disposed or recycled by metal smelters as a new kind of metal ore.

The genus Alyssum (Brassicaceae) contains the greatest number of hyperaccumulators of Ni, about 50 taxa (Baker and Brooks, 1989). This genus comprises about 170 species (Baker and Brooks, 1989) The distribution of hyperaccumulating Alyssum species is mostly on serpentine soils in southern Europe and Asia Minor stretching from Portugal in the west to the Iraq/Turkey/Iran border areas in the east (Brooks, 1998).

Alyssum lesbiacum is an endemic species of the Lesbos Island (Strid and Tan, 2002). It is perennial with stems up to 40 cm and is restricted to serpentine soils. The aim of the present study is: (a) to confirm the distribution of *Alyssum lesbiacum* based on literature references and extensive field surveys across the island, (b) to identify the effects of *Alyssum* on community structure and ecosystem properties (biomass production), and (c) to assess the concentrations of Ni and other 'serpentine elements' in *Alyssum lesbiacum* from serpentines areas of the island.

Methods

Study sites

The distribution of *Alyssum lesbiacum* populations in serpentine soils of the island is depicted in Figure 1. Four populations were selected in the following sites: Loutra, Ampeliko, Olympos, and Vatera (Table 1; Figure 1). For two of these sites (Ampeliko and Olympos), one non-serpentine site was also selected, in order to serve as a control for comparisons with serpentine sites. Non-serpentine sites were selected as close as possible to serpentine ones (Table 1) to minimize environmental variability between them.

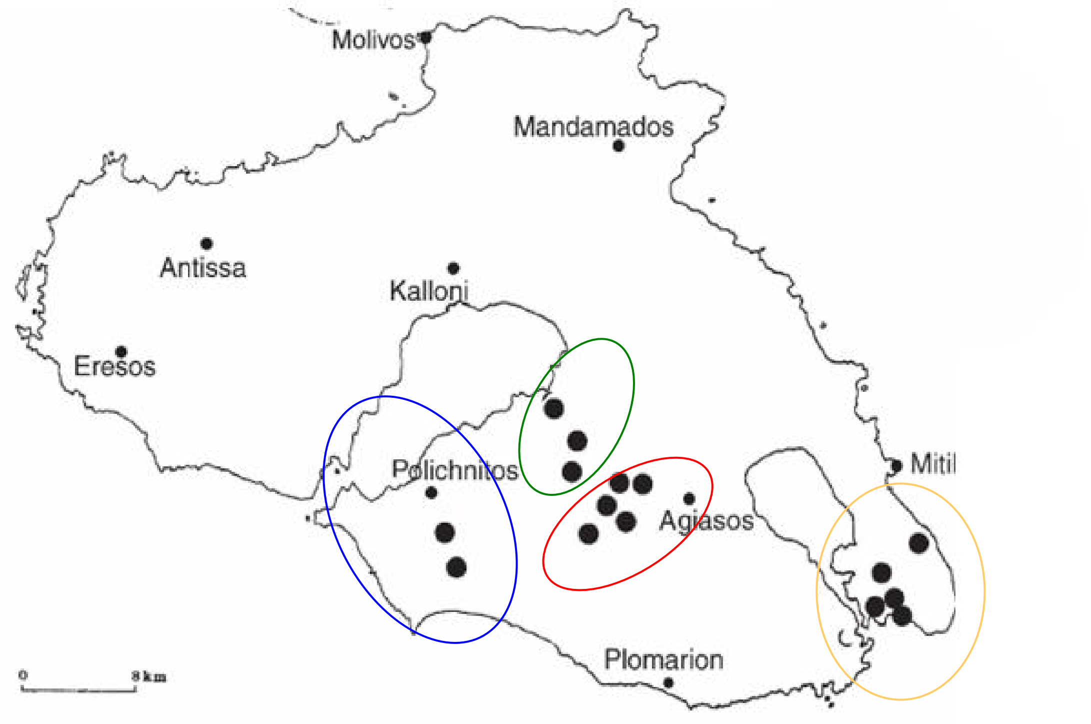

Figure 1. *Alyssum lesbiacum* populations (Bazos &Yannitsaros, 2004; Present study, 2007). Selected sites are indicated with a different-color circle: Loutra (yellow); Olympos (red); Ampeliko (green); Vatera (blue).

Table 1. Study sites on serpentine and non-serpentine soils in Lesbos Island.

Name	**Co-ordinates**		**Altitude (m)**	**Orientation**	**Slope**
	Latitude (N)	**Longitude (E)**			
Olumpos					
Serpentine site	39° 04' 33.3"	026° 20' 16.3"	759	NW	15%
Non-serpentine site	39° 04' 33.3"	026° 20' 16.3"	770	NW	15%
Loutra					
Serpentine site	39° 02' 36.8"	026° 32' 55.0"	94	NW	13%
Vatera					
Serpentine site	39° 01' 57.1"	026° 15' 52.5"	37	NE	6%
Ampeliko					
Serpentine site	39° 05' 46.4"	026° 19' 59.9"	361	SW	20%
Non-serpentine site	39° 08' 42.6"	026° 16' 31.9"	25	NE	24%

Soil sampling

Five soil samples were generally taken from a depth of 0–10 cm after removing any loose organic matter on the surface; this can be taken to represent the rooting zone of the herbs and small shrubs. The main purposes of the soil sampling have been (1) to confirm that the plant collections were being made from areas of essentially ultramafic composition, and (2) to show the concentration ranges of the elements that are of most relevance and special interest in the ultramafic soils. Soil sample were also taken from non-serpentine sites at the end of May 2007.

The soils were air dried and sieved initially to <2 mm to remove any stony material. Sub-samples of 2–3 g were ground to 70-mesh (<215 µm) and oven-dried at 60°C. A further sub-sample of 0.15–0.20 g was weighed to ±0.0001 g and transferred to a polypropylene beaker on a water bath at 100°C for digestion with 10 ml of a 1:1 HF/HNO3 mixture. After the solution had been taken to dryness the residue was dissolved in 10 ml of conc. HCl, taken to dryness again, and the residue was dissolved in 10.0 ml of warm 2M HCl. Further dilutions by a factor of 5–15 were necessary to give solutions with sufficiently low Fe concentration (<1,000 mg/l) for multielement analysis by inductively coupled plasma (ICP) emission spectroscopy.

Plant sampling

Specimens from *Alyssum lesbiacum* individuals of each site were collected during May 2007. Samples of leaf material were washed for several minutes in deionised water. After drying at 60°C, ~0.2 g of leaf material was set aside in paper packets for analysis; 0.05–0.15 g of the dried plant tissue was then weighed to ±0.0001 g and ashed in a muffle furnace over a period of 5 h, with the temperature being raised in stages to 500°C for the last 2 h. After cooling, the ash was dissolved in 5.0 ml of warm 2 M HCl for analysis for 14–18 elements by ICP (ARL 34000 and upgraded versions). Plant analyses were taken place at the School of Botany of the University of Melbourne.

Community biomass production

In each site, total aboveground community biomass was sampled from five plots of 50x50 cm each at the time of peak standing crop (i.e. May 2007). The plant material was sorted to species and it was dried (60 °C, 24 h) and weighed.

Results

The concentrations of Ni, Ca, Cr, Mg, Al, Fe, K, Zn, Mg, Pb, P, Cu, Mn, and Co in the soils of both serpentine and non-serpentine sites are shown in Table 2. The non-serpentine soil from Ambeliko shows clearly the typical differences in composition between serpentine and non-serpentine soils. The same can be said for Olympus, although some of the 'ultramafic' elements such as Cr and Ni are slightly higher here in non-serp soils than in the Ambeliko non-serpentine soils. For Vatera, the serpentine soil is lower in elements such as Cr and Ni anyway, and may indicate only partial ultramafic origin. According to these results, serpentine soils contain large amounts of Ni (more than 1000 mg/kg).

Table 2. Typical soil analyses of serpentine and non-serpentine soils of Lesbos (total concentrations in mg/kg, or % where indicated). Data are means of the five soil samples per site and soil type.

	Ni		**Ca%**		**Cr**		**Mg**		**Al**		**Fe**		**K**	
Site	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**
Ambeliko	3325.668	45.293	0.602	0.336	4863.068	33.931	3.948	0.725	2.422	2.692	10.021	2.064	0.288	0.242
Vatera	336.598	-	0.849	-	262.742	-	2.672	-	2.346	-	4.749	-	0.368	-
Loutra	1196.951	-	1.202	-	1118.000	-	4.136	-	2.656	-	8.114	-	0.329	-
Olymbos	1948.463	123.540	0.895	0.901	2040.272	129.832	8.406	1.542	2.530	3.402	9.580	7.181	0.267	0.564

Table 2. (continued)

	Zn		**Pb**		**P**		**Cu**		**Mn**		**Co**	
Site	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**	**Serp**	**NonSerp**
Ambeliko	132.083	34.080	25.071	25.942	917.566	247.522	36.951	8.930	1913.464	272.630	99.167	2.722
Vatera	61.416	-	25.303	-	448.539	-	21.427	-	804.735	-	8.534	-
Loutra	66.445	-	30.857	-	581.767	-	38.132	-	924.539	-	23.616	-
Olymbos	78.455	88.782	22.640	28.250	1033.361	530.873	23.067	40.545	1419.197	1218.492	76.161	6.694

The results of Ca, Mg, K, Na, Co, Cu, Fe, Mn, Ni, P, Zn analyses of *Alyssum lesbiacum* leaves collected in four serpentine sites of the island are shown in Table 3. Concentrations of Ni are very high across all sites, ranging between 8,000 and 14,000 mg/kg (Figure 2; Table 3). This features are along with the remarkably high Ca in this plant also (especially considering the low soil Ca).

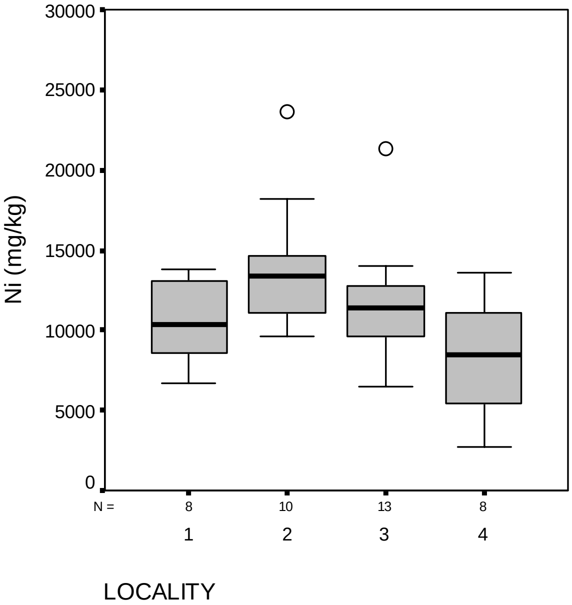

Figure 3. Concentration of Ni in the leaves of *Alyssum lesbiacum* individuals in four serpentine sites in Lesbos (1: Vatera, 2: Ambeliko, 3: Olympos, 4: Loutra). Box plots show the range of the data, the quartiles, the median value as the black midline, and extreme values as dots, in each site.

Table 3. The range of elemental concentrations (mg/kg) in leaves of *Alyssum lesbiacum* individuals collected from different locations in Lesbos. Mean concentration and standard error of mean (SE) are shown.

Site	**Ca**		**Mg**		**K**		**Na**		**Co**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Vatera	50338.98	2533.291	6186.865	615.3419	14481.22	2047.648	2138.452	82.5872	13.54347	2.695644
Ambeliko	51002.89	2392.294	6206.747	924.9976	13823.66	1154.858	1770.024	117.6053	19.89075	2.411824
Olympus	39442.46	2583.375	3614.668	324.93	13186.54	841.5704	1738.234	71.70727	12.30866	1.888419
Loutra	46670.35	7996.473	5187.581	560.0484	8625.294	887.247	2032.492	229.7191	18.46244	2.920637

Site	**Cu**		**Fe**		**Mn**		**Ni**		**P**		**Zn**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Vatera	2.904025	0.210292	410.6227	69.14384	62.81485	4.523757	10577.32	955.0887	1633.804	455.5331	37.7682	7.503213
Ambeliko	2.982929	0.259598	221.8247	25.67463	40.95429	5.077129	14090.66	1322.88	1383.579	120.4571	127.959	33.79929
Olympus	3.173355	0.317532	216.0447	23.11787	41.74779	6.21721	11655.88	1010.866	1350.259	155.8896	119.409	13.02656
Loutra	3.383339	0.580345	350.5077	68.9154	61.38481	4.727027	8295.394	1288.285	1530.262	244.5753	83.547	22.21281

Table 4 presents the amounts of phytoextracted nickel (in kg/ha) for each site (see also Figure 5). We have estimated that the leaf biomass of Alyssum equals to half of the quantity of its total aboveground biomass (= leaves plus stems plus reproductive organs).

Table 4. Mean *Alyssum lesbiacum* yields and mean phytoextracted nickel amounts across the four serpentine sites in Lesbos.

Site	**Alyssum shoot yield (g/m^2)**	**Alyssum leaf yield (g/m^2)**	**Leaf Ni Concentration (mg/kg)**	**Phytoextracted Ni (kg/ha)**
Vatera	51.72	25.86	10577.32	2.735
Ambeliko	147.96	73.98	14090.66	10.424
Olympos	180.216	90.108	11655.88	10.503
Loutra	80.76	40.38	8295.394	3.350

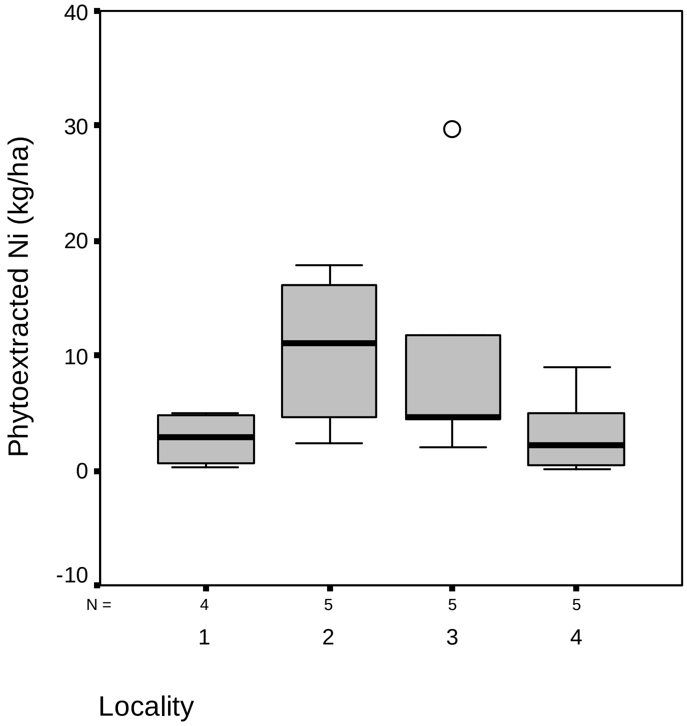

Figure 4. Phytoextracted nickel amounts across the four serpentine sites in Lesbos (1: Vatera, 2: Ambeliko, 3: Olympos, 4: Loutra).

Conclusions

- We provide the first evidence that *Alyssum lesbiacum* is a nickel hyperaccumulator.
- *Alyssum lesbiacum* can easily accumulate from 8000 - 14000 mg Ni kg^{-1} (0.8 – 1.4%) dry weight with no yield reduction. This amount is comparative with those of other Ni hyperaccumulators. For example, the dried leaves of *Alyssum bertolonii* — a nickel hyperaccumulator from Tuscany, Italy - contain on average about 1% (10,000 mg/kg) nickel.
- The amount of phytoextracted nicked is low compared with other species, either *Alyssum* species either species from other genera (e.g. 200kg/ha; Li et al. 2003). The amount of phytoextracted nicked is dependent on the biomass production achieved by species in each year. In our study, we observed that the species did not reach at its maximum yield in one year. Therefore, we have to focus on the study of *Alyssum* biomass at the time of peak standing crop (there is not scientific evidence if *Alyssum lesbiacum* achieves its maximum biomass at the second or third year of its growth).
- The increased biomass levels of other *Alyssum* species used for phytoextraction purposes are coming from field or greenhouse experimental studies. Therefore, effective phytoextraction requires, except from plant genetic ability, the development of optimal agronomic management practices including (a) soil management practices to improve the efficiency of phytoextraction; and (b) crop management practices to develop a commercial cropping system (Li et al. 2003). For example, modification of soil pH or soil fertility may affect the efficiency of phytoextraction of heavy metals such as Ni (Robinson et al. 1997). Fertilizer addition (N, P, K) significantly increased shoot biomass yield, but did not affect shoot Ni concentration, and therefore total amount of phytoextacted Ni was increased (e.g. Robinson et al. 1997; Li et al. 2003).

List of Publications

Journal Paper

E. Kazakou, P.G. Dimitrakopoulos, A.J.M. Baker, R.D. Reeves, A.Y. Troumbis. Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. *Biological Reviews* (in review).

E. Kazakou, G. Adamidis, P.G. Dimitrakopoulos, M. Godino, A.J.M. Baker, R.D. Reeves, A.Y. Troumbis. Comparisons in heavy metals concentrations of soils and plant tissues between serpentines and non-serpentines sites in Lesbos. (*In preparation*).

Master Thesis

Adamidis G. (2007). From functional leaf traits to community productivity: comparing serpentine with non-serpentine sites in Lesbos. Post-graduate Master Program on Environmental and Ecological Engineering, Dept. of Environment, University of the Aegean (Coordinated by P. Dimitrakopoulos and El. Kazakou)

First Degree Dissertation

Koufaki Th. (2007). Differences in community structure and productivity between serpentine and non-serpentine soils. Dept. of Environment, University of the Aegean (Coordinated by P. Dimitrakopoulos and El. Kazakou)

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