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Mineral composition and seed germination of *Alyssum murale* populations under Ni and Mn stress

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Measurements of Ni, Mn, Mg, Ca, K and Fe concentrations in the soils that correspond to nine populations of *Alyssum murale* growing in different metalliferous regions of North Greece (eight from Vavdos, Chalkidiki area – rich in nickel and one from Grammeni, Drama – rich in manganese), revealed high amounts of Ni-Mg, and Mn-Ca in the soils of Vavdos and Grammeni, respectively. Ca/Mg ratio was very low (<0.24) in Vavdos, but very high (16.8) in Grammeni. All soil samples contained high amounts of Fe, but very low amounts of K. *Alyssum murale* natural plants from Vavdos were capable of taking up Ni from the soil and accumulating it in the leaves, whereas those from Grammeni did not have the ability to accumulate Mn. A positive correlation between Ni or Mn concentration in the plants and the soil where they grow, was also observed.

The populations of *A. murale* from Vavdos exhibited both higher seed germination and index of tolerance to Ni, while the population from Grammeni showed higher seed germination and index of tolerance to Mn.

Key words: *Alyssum murale*, germination, manganese, nickel, nickel-hyperaccumulator, phytotoxicity, tolerance index.

INTRODUCTION

Serpentine soils are extreme habitats for plants. These soils contain high concentrations of heavy metals (nickel in particular), which in general have an adverse effect on plant growth. Another factor that may reduce plant growth on serpentine soils is the low concentration of calcium in relation to magnesium (Proctor, 1971; Konstantinou & Babalonas, 1996). Moreover, serpentine soils are also dry and exposed because of the granular texture and lack of organic material (Karataglis *et al.*, 1982; Babalonas *et al.*, 1984; Brooks, 1987).

Although Ni is an essential micronutrient for higher plants (Brown *et al.*, 1987; Welch, 1995), at relatively high concentrations, it has strong phytotoxic effects on plant growth (Moya *et al.*, 1993; Palacios *et al.*, 1998; Parida *et al.*, 2003).

Manganese is also an essential micronutrient for higher plants implicating in photosynthetic water oxidation and in manganese redox enzymes as well (Yachandra *et al.*, 1996). Elevated concentrations, however, reduce plant growth by affecting significant physiological functions (Foy *et al.*, 1978; Kitao *et al.*, 1997; Sinha *et al.*, 2002).

Despite the phytotoxic effect of heavy metals, a number of plant species have the ability to overcome the adverse environmental factors and a distinctive vegetation can evolve on these soils (Antonovics *et al.*, 1971).

Extremely high uptake (hyperaccumulation) of potentially phytotoxic metallic elements is a phenomenon, which has been found in a wide range of plant species of different families (Baker & Brooks, 1989). Hyperaccumulators are plants native to metalliferous soils that take up large quantities of metallic elements compared to co-occuring species. Plants with tissue concentrations of > 1000 $\mu g\ g^{-1}$ for Ni or

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>10000 μg g^{-1} for Mn (dry weight) have been classified as hyperaccumulators. Nickel is the metal most frequently hyperaccumulated by plants in serpentine soils. In the family Brassicaceae, 48 *Alyssum* species hyperaccumulate Ni in their leaves, a fact which was recognized as a response to the elevated Ni concentrations generally found in serpentine soils (Baker & Brooks, 1989; Reeves *et al.*, 1996).

The aim of this paper was to study the metal concentration of different metalliferous soils and the ability of native *Alyssum murale* plants grown in the above soils to accumulate different metals. Seed germination and index of plant tolerance to the presence of different Ni or Mn concentrations in the nutrient solution, were also examined.

MATERIALS AND METHODS

Plant material and soil collections

Soils, *Alyssum murale* Waldst. & Kit. whole plants and *A. murale* seeds were collected from nine sampling stations (eight stations from a wider area of Vavdos, Chalkidiki {Gioldaki (1), Griva (2), Loukovitis (3), Siladi (4, 5), Tsournara (6), Fotirachi (7, 8)}, latitude 38° 27′ 36″ and longitude 24° 01′ 15″ and one station as control from the area of Grammeni, Drama (9), latitude 39° 13′ 48″ and longitude 24° 26′ 24″.

Determination of metal concentration

Nickel, manganese, calcium, magnesium, potassium and iron contents were determined in soil, root, stem and leaves by wet digestion of dried material (80°C to constant weight) in a mixture (4:1) (v/v) of nitric acid and perchloric acid on a hot plate (130°C) up to complete digestion. Metal concentrations were measured by an Atomic Absorption Spectrophotometer (Perkin Elmer 2380).

Seed germination

A. murale seeds (30) from each sampling station of Vavdos and Grammeni were sterilized with 0.1% $HgCl_2$ for 10 min, rinsed excessively with running water and germinated in a thermostat at 21 ± 1°C on moist filter paper, impregnated with a modified Hoagland nutrient solution, pH 5.5 (1:10 and 1:1 for macronutrients and micronutrients, respectively). Nickel or manganese were supplied as $NiSO_4.6H_2O$ or $MnSO_4.H_2O$ at eight levels (0, 0.16, 0.5, 1, 1.5, 2, 2.5 and 3 $mmoll^{-1}$) at the first moment of the experiment. Firstly, 8 ml of Hoagland nutrient solution with different nickel or manganese concentrations were added to each Petri dish, while the solution quantity was daily checked. The germination percentage was marked every day (from the first until the seventh day).

Measurement of metal tolerance

Metal tolerance of *A. murale* seedlings from Vavdos (mixed population) and Grammeni to Ni or Mn was estimated by using root growth inhibition by different Ni or Mn concentrations applied and expressed as index of tolerance (I.T.)

$$\frac{\text{root length + metal}}{\text{root length without metal}} \times 100$$

Statistical analysis

All experiments were repeated three times. Statistical analysis was performed on the data using an ANOVA model (completely randomized block) and Duncan's criterion ($p < 0.05$).

RESULTS

Soil samples (1-8) from Vavdos, Chalkidiki (Table 1) showed high concentrations of Ni and Mg, and a very low Ca:Mg (0.09-0.24) ratio. In contrast, soil samples from station 9 from Grammeni, Drama showed high concentrations of Mn and Ca and a very high Ca:Mg (16.8) ratio. Furthermore, high concentrations of Fe were observed in soil samples of Vavdos and lower in Grammeni, but K concentrations were at the lower level of normal soils in Grammeni and much lower in Vavdos.

The pH level of the soil samples studied was almost neutral (Grammeni) or slightly alkaline (Vavdos) (Table 1).

According to Table 2, Ni concentrations in the root and stem of *A. murale* populations of Vavdos were low in comparison to Ni concentrations in the soil and higher in the leaves. In all cases, the difference of Ni concentrations among roots, stems and leaves was statistically significant ($p < 0.05$). Nickel concentrations in the plant parts of the Grammeni population were low.

Manganese concentrations of *A. murale* roots, stems and leaves were very low (Table 2) in relation to Mn concentrations in the soil of Vavdos stations and especially of Grammeni station.

TABLE 1. Mean concentrations and standard deviation of elements Ni, Mn, Ca, Mg, K, and Fe in μg^{-1}, Ca/Mg ratio and pH of soils at the sampling stations of Vavdos (1–8) and Grammeni (9), for the n number of samples

Sampling stations	n	Ni	Mn	Ca	Mg	K	Fe	Ca/Mg	pH
1.	12	1729±160	970±146	13567±1712	122472±10846	287±35	75657±5500	0.11	8.4±0.16
2.	9	1187±320	690±79	23563±2452	120233±8564	377±38	53503±4572	0.20	8.3±0.21
3.	9	2064±76	717±65	21111±3114	115134±10205	283±45	69613±3899	0.18	7.92±0.17
4.	9	1535±257	528±56	25383±3323	106699±7163	312±72	56227±5519	0.24	7.93±0.18
5.	12	2244±259	782±112	15715±1483	124319±7998	370±72	56853±4961	0.13	8.08±0.16
6.	12	1929±175	752±73	20120±2547	126603±8555	363±50	69052±5180	0.16	7.98±0.13
7.	9	1963±241	843±103	13608±1504	100743±10438	480±34	69012±4594	0.14	8.1±0.19
8.	9	1169±168	611±65	10842±1806	111625±9014	297±46	55600±3782	0.09	8.1±0.13
9.	6	67±12	10633±802	130833±9902	7766±1232	1470±161	32300±4164	16.8	7.12± 0.35

Concentrations of Ca in the leaves of *A. murale* populations from Vavdos were higher, about 15 and 8 times in comparison with concentrations of the root and stem respectively and with those of the soil as well ($p < 0.05$). Calcium concentrations of roots, stems and leaves of *A. murale* populations from Grammeni were much higher compared to those from Vavdos populations, respectively ($p < 0.05$) (Table 2).

Values of Mg concentrations of *A. murale* roots, stems and leaves from Vavdos sampling stations lay at normal levels (Table 2), yet much lower than those of the soil samples where the plants came from. However, Mg concentrations in the leaves were higher ($p < 0.05$) than the ones in the stems and roots. On the other hand, Mg concentrations in the leaves of *A. murale* from Grammeni were at normal levels and those in the stem and especially the root were very low (Table 2).

One can see (Table 2) that the leaf K concentration in *A. murale* populations was higher than those of the root and stem, and much higher than the ones of the soil samples ($p < 0.05$).

Leaf Fe concentrations of *A. murale* populations from Vavdos, although higher than those of the root and particularly of the stem ($p < 0.05$), are much lower than the ones measured in the soils they were grown in. In contrast, Fe concentrations in the root of *A. murale* from Grammeni were higher than those in the leaves and stems ($p < 0.05$), but much lower in the respective soil (Table 2).

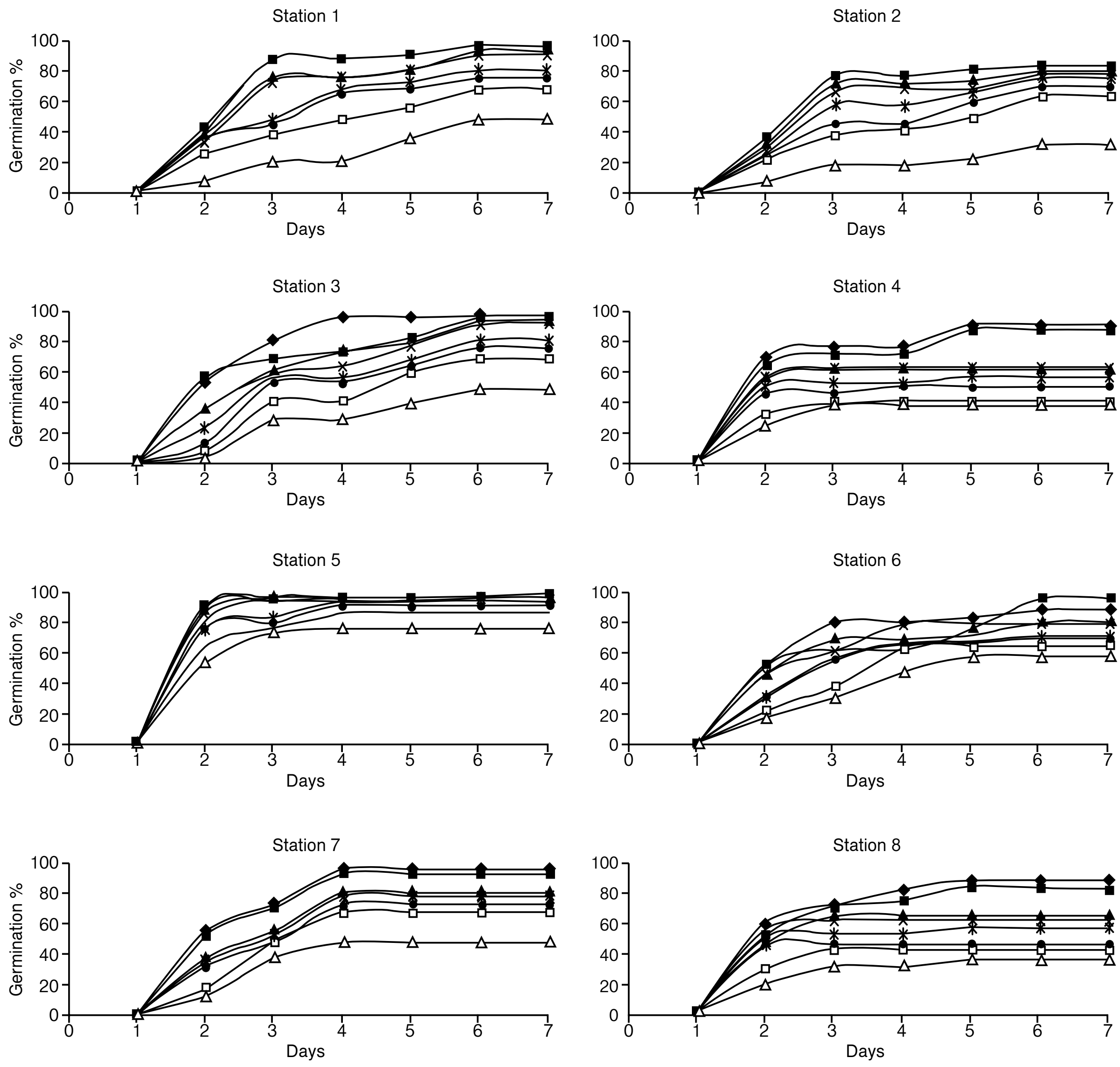
Figure 1 shows the percentage variation in the germination of seeds coming from different *A. murale* populations affected by Ni. Seed germination was initiated on the 2nd day in all populations and in all concentrations (0-3000 μM Ni), except for the Grammeni population where germination started on the 3rd day. In almost all populations from Vavdos, seed germination in low concentrations (160 μM Ni) was equal or higher than that of the control. In most cases there was an increase of seed germination with respect to time (till the 6th day), while there was a decrease of seed germination with respect to Ni concentration in the nutrient solution (Fig. 1).

More specifically, seed germination (6th day) of *A. murale* populations (1-8) ranged from 31% to 75% in the presence of 3000 μM Ni in the nutrient solution, while that of population 9 was about 13% in the presence of 1000 μM Ni in the nutrient solution. No germination was observed in higher concentrations (1500-3000 μM Ni in nutrient solution)

TABLE 2. Mean concentrations and standard deviation of Ni, Mn, Ca, Mg, K and Fe in μg^{-1} of root, stem and leaves of 30 *Alyssum murale* plants at the sampling stations of Vavdos (1–8) and Grammeni (9)

Sampling stations	Ni			Mn			Ca		
	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
1.	654±132	797±108	5170±335	39±5	28±7	95±19	1615±287	3390±438	32957±1383
2.	508±56	685±66	3652±290	21±6	16±4	94±15	1677±473	3480±437	24753±1970
3.	317±96	583±60	3500±406	16±3	24±7	99±28	1858±288	3390±519	26767±2868
4.	607±74	927±74	9697±571	25±3	17±3	139±20	2057±291	3083±409	29538±2091
5.	735±111	896±49	8181±318	23±5	18±3	117±13	2350±518	2520±432	36353±2486
6.	589±179	824±111	7182±510	22±4	25±6	90±15	1746±199	2662±338	25413±2361
7.	718±106	985±161	8010±301	41±5	34±7	96±20	1779±467	3535±526	35467±2859
8.	655±123	792±89	9432±339	23±4	20±4	93±19	1545±362	2639±448	25265±1899
9.	19±3	66±5	132±18	43±8	56±6	148±21	32900±2488	36763±3655	49750 ±4150

Sampling stations	Mg			K			Fe		
	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
1.	3265±421	1292±289	3967±367	1712±374	1437±340	3599±409	767±310	232±38	1309±240
2.	2897±469	1683±271	6037±747	2253±461	1747±371	7250±740	956±115	563±15	2576±441
3.	2106±277	920±155	4273±387	2190±416	1363±308	6250±567	917±214	590±46	2300±469
4.	3787±405	1748±381	6083±563	2407±286	1905±472	7655±718	777±132	348±45	1759±421
5.	3110±564	1840±524	4797±383	2530±411	1967±422	7127±721	933±259	353±51	1263±284
6.	3337±484	1404±270	4191±292	2004±313	1810±355	5373±529	1037±277	306±40	1786±442
7.	3714±570	1757±274	4815±384	2003±456	1305±198	6225±704	898±85	224±97	1895±465
8.	3982±501	1550±224	4945±381	2290±512	1781±409	6007±623	947±84	248±38	2328±422
9.	383±51	703±76	2114±214	2253±458	2110±507	8450±810	4510±530	256±42	841±124



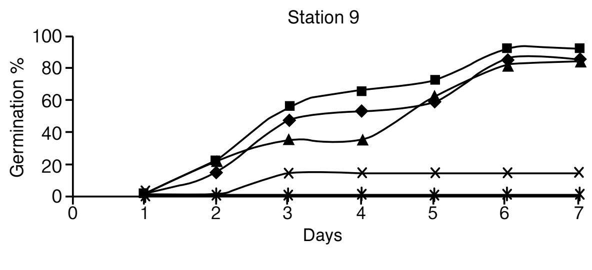


FIG. 1. Germination of *A. murale* seeds from the sampling stations of Vavdos (1-8) and Grammeni (9) in different concentrations of Ni [0 (♦), 160 (■), 500 (▲), 1000 (×), 1500 (*), 2000 (●), 2500 (□) and 3000 μM(△)] in nutrient solution.

(Fig. 1).

In all Mn concentrations in the nutrient solution, seed germination of the two populations (1-8 mixed populations and population 9) began on the 2nd day (Fig. 2). Contrary to the population of Grammeni, that from Vavdos did not display any difference in the percentage of seed germination with respect to time, whereas in both populations, a negative correlation of seed germination in connection with the increase of Mn concentration in the nutrient solution was observed. The percentage of seed germination (6th day) in the presence of 3000 μM Mn in the nutrient solution was 24.3% and 30.5% in the populations from Vavdos and Grammeni, respectively.

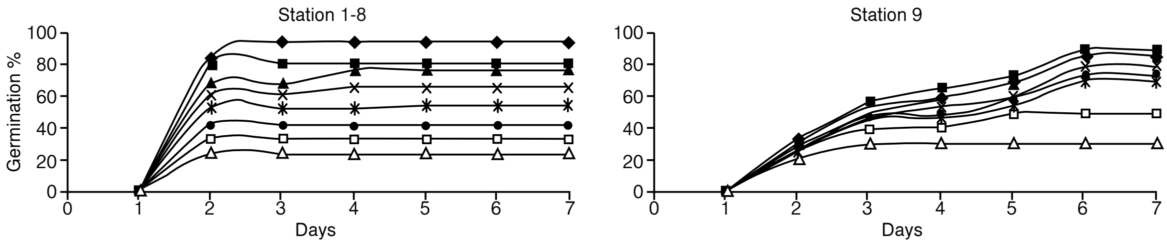


FIG. 2. Germination of *A. murale* seeds from the sampling stations of Vavdos (1–8, mixed population) and Grammeni (9) in different concentrations of Mn [0 (♦), 160 (■), 500 (▲), 1000 (×), 1500 (*), 2000 (●), 2500 (□) and 3000 μM(△)] in the nutrient solution.

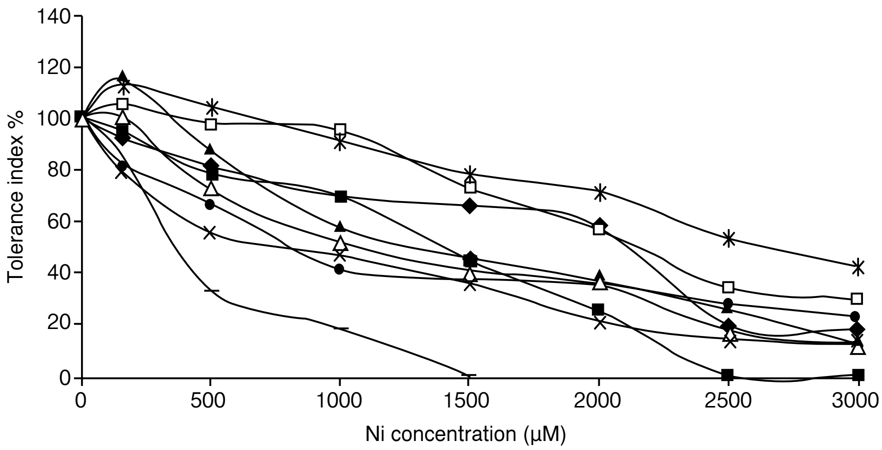


FIG. 3. Effects of different Ni concentrations in the nutrient solution on the tolerance index of *A. murale* seedlings from the sampling stations 1 (♦), 2 (■), 3 (▲), 4 (×), 5 (*), 6 (●), 7 (□), 8 (△) and 9 (–).

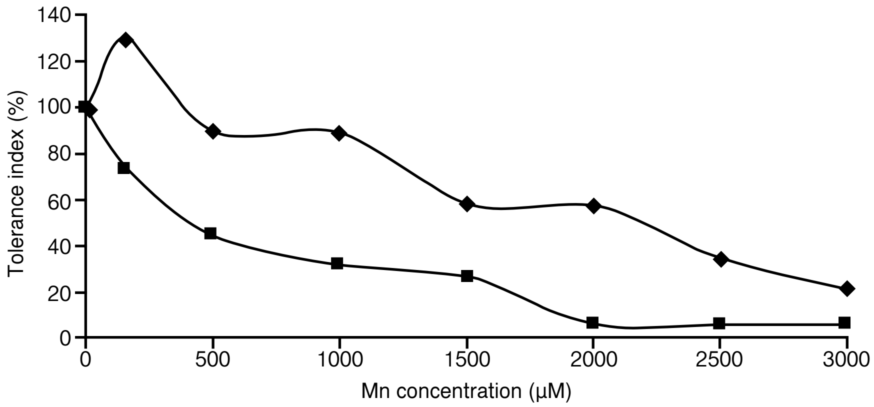


FIG. 4. Effects of different Mn concentrations in the nutrient solution on the tolerance index of *A. murale* seedlings from the sampling stations 1-8 (mixed population) (■) and 9 (♦).

Figure 3 depicts the effects of various Ni concentrations on the tolerance index of *A. murale* populations. In all cases, a decrease of the index was evident with increasing Ni concentrations in the nutrient solution. There was a remarkable decrease in the index of tolerance of *A. murale* population from Grammeni, whose index of tolerance zeroed in the concentration of 1500 μM Ni.

The effects of increasing Mn concentrations on the index of tolerance is shown in Fig. 4. It is deduced that the index of tolerance in the mixed population from Vavdos significantly decreases (almost by 90%) in the presence of 2000 μM Mn, whereas that in the Grammeni population decreases by 70% at a Mn concentration of 3000 μM.

DISCUSSION

Our results showed (Table 1) that the metalliferous soil of Vavdos, Chalkidiki is a typical serpentine soil with an unfavourable calcium/magnesium ratio (Proctor, 1971; Babalonas *et al.*, 1984; Abou Auda *et al.*, 2002) and high concentrations of nickel and magnesium. It has been reported that elevated amounts of Ni in the soil in combination with a low Ca/Mg ratio are responsible for the reduced productivity of serpentine soils (Babalonas *et al.*, 1984; Chiarucci *et al.*, 1998, 1999). Metalliferous soils of Grammeni, Drama contained high amounts of manganese and especially of calcium. It is known that increased concentration of Mn in combination with low pH result in plant toxicity (Kinzel, 1982; Kabata-Pendias & Pendias, 2001). The physiological role of the soil pH is of importance, since it influences the availability to plants of nutrient elements (Schroeder, 1984). According to Sutchiffe & Baker (1981), the availability of soil macronutrients is optimal at pH 7, while for most micronutrients it ranges between pH 5.5 and 6.

Plants of *A. murale* from Vavdos area accumulated Ni in their organs, particularly in the leaves, indicating that *A. murale* is a Ni hyperaccumulator (Babalonas *et al.*, 1984; Baker & Brooks, 1989; Abou Auda *et al.*, 2002). Reeves & Baker (1984) have concluded that hyperaccumulation is not only a microevolutionary response to metalliferous soils, but it has a wider ecological significance.

On the contrary, spontaneous plants of *A. murale* from Grammeni, grown in a soil containing high manganese concentrations were unable to uptake Mn, probably because Ca uptake acted antagonistically or because the chemical forms of manganese (oxides containing Mn II, III and IV predominant forms, Giller *et al.*, 1992), were not easily taken up by plants.

The increased Ca concentration of the leaves, in comparison to the other plant organs (root and stem) can be explained by the fact that plants accumulate Ca because of the antagonistic functions of Ca not only to Ni, but also to the other heavy toxic metals (Proctor & Woodell, 1975; Karataglis *et al.*, 1982; Babalonas *et al.*, 1984; Gabbrielli & Pandolfini, 1984; Chiarucci *et al.*, 1998, 1999). It has been suggested that in Ni hyperaccumulators there is a positive relationship between Ca and Ni uptake (Babalonas *et al.*, 1984).

Although Mg amounts in the soil of Vavdos are very high, the ones taken up by the plants are rather not enhancing Ca amount which compensates for the toxic action of various toxic metals including Mg (Johnston & Proctor, 1981; Gabbrielli & Pandolfini, 1984). High soil Mg concentrations are widely accepted as a cause of ultramatic soil toxicity, particularly when they are combined with low soil Ca (Walker, 1954; Proctor, 1971). Magnesium toxicity is probably attributed to its antagonistic action to other macronutrients and micronutrients (Mengel, 1968; Brooks, 1987; Proctor & Baker, 1994; Chiarucci *et al.*, 1998, 1999; Kabata-Pendias & Pendias, 2001).

The low potassium concentration in the soils of Grammeni and Vavdos (lower than those of normal soils) may be one of the factors limiting plant growth. Because of its significance, a good amount of K is taken up by *A. murale* plants. Adequate amounts of potassium are considered to be necessary for the normal growth of plants, since K is implicated in physiological functions, such as activation of enzymes, regulation of the osmotic potential and the pH, etc. (Mengel, 1968; Epstein, 1972; Taiz & Zeiger, 2002).

The amount of Fe taken up by plants although high enough, especially in the leaves, is much lower than that in the soils where plants grow. This low Fe uptake is probably the outcome of the antagonistic function of Ca in combination with the high soil pH, which reduces Fe availability to plants. The lower concentration of Fe in the leaves with respect to Fe concentration in the root of *A.murale* plants from Grammeni, could probably arise from the fact that Fe transport within plant parts is highly affected by Ca concentration (Kabata-Pendias & Pendias, 2001).

Ni and Mn are also known to be antagonistically functioning towards Fe, thus limiting its uptake (Mengel, 1968; Epstein, 1972; Bergmann, 1988; Kabata-Pendias & Pendias, 2001).

The observed negative correlations of seed germination and tolerance index, in relation to increasing Ni and Mn concentrations in the nutrient solution (Figs 1-4) are probably attributed to the toxic function of the above metals. It is known that increased Ni concentrations inhibit DNA, RNA and protein synthesis, seed germination and seedling growth (Woolhouse, 1983; Espen *et al.*, 1997; Abou Auda *et al.*, 2002; Parida *et al.*, 2003), while increased Mn concentrations cause inhibition of plant growth (Sumner *et al.*, 1991; Kitao *et al.*, 1999; Abou Auda *et al.*, 2002). However, the observed differences in seed germination percentage and in plant tolerance index of various *A. murale* populations, grown in various Ni or Mn concentrations in the nutrient solution probably indicate the adaptation of populations to increased Ni or Mn concentrations in the soil where they come from (Baker & Brooks, 1989; Rout *et al.*, 1999; Monni *et al.*, 2000; Kitao *et al.*, 2001; Abou Auda *et al.*, 2002).

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