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To cite this version:

Aida Bani, Dolja Pavlova, E Benizri, Seit Shallari, Liri Miho, et al.. Relationship between the Ni hyperaccumulator Alyssum murale and the parasitic plant Orobanche nowackiana from serpentines in Albania. Ecological Research, 2018, 33 (3), pp.549-559. 10.1007/s11284-018-1593-1 . hal-02008737

HAL Id: hal-02008737

https://hal.science/hal-02008737v1

Submitted on 5 Feb 2019

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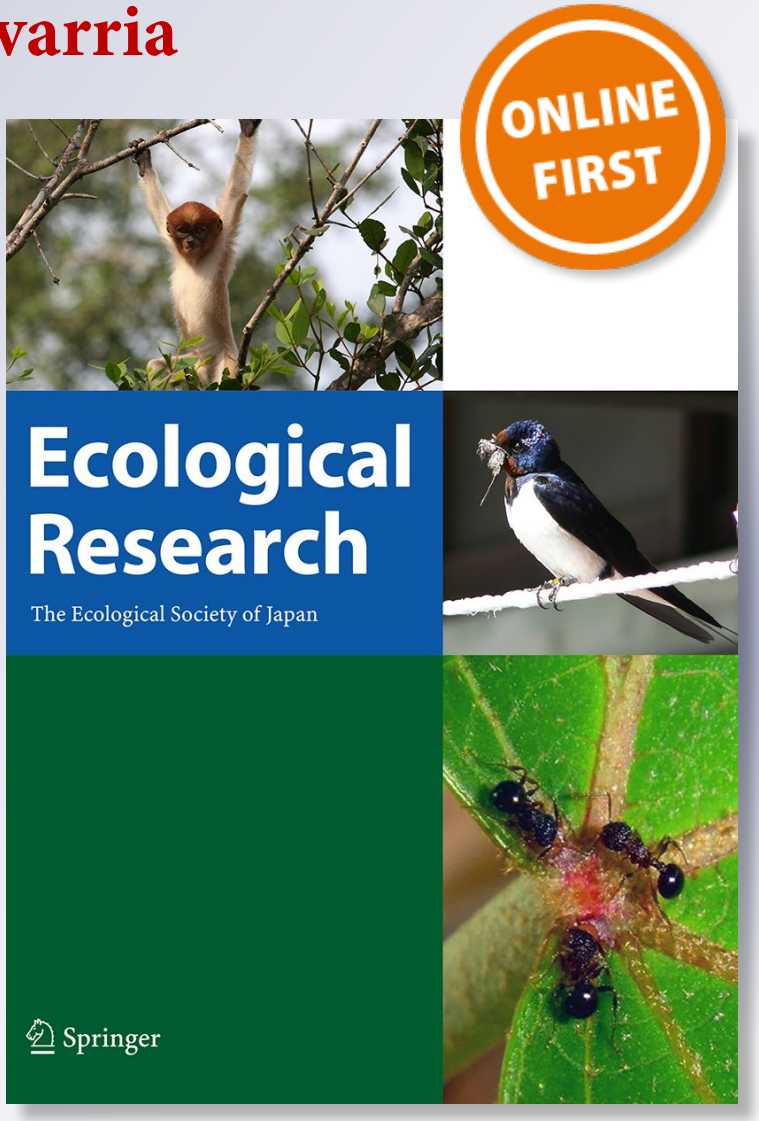
Ecological Research

ISSN 0912-3814

Ecol Res
DOI 10.1007/s11284-018-1593-1



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Ecol Res
DOI 10.1007/s11284-018-1593-1

SPECIAL FEATURE

Ultramafic Ecosystems: Proceedings of the 9th International Conference on Serpentine Ecology

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Seit Shallari · Liri Miho · Marjol Meco
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Relationship between the Ni hyperaccumulator *Alyssum murale* and the parasitic plant *Orobanche nowackiana* from serpentines in Albania

Received: 3 October 2017 / Accepted: 27 February 2018
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Abstract *Orobanche nowackiana* Markgr. is the only parasitic flowering plant in Europe which is an obligate serpentinophyte. This plant parasitizes the Ni hyperaccumulator *Alyssum murale* Waldst. & Kit. which grows in many areas including serpentine slopes of Mt. Komjan (Albania). The aims of the study were to investigate this host/parasite relationship from a biogeochemical point of view in order to evaluate: (1) the metal accumulation in different parts of the parasite and host plants and (2) the effect of *Orobanche* infection on host growth. Soil and plant samples of *A. murale* and *O. nowackiana* were analyzed for elemental concentrations after acid digestion using inductively coupled plasma atomic emission spectrometry (Ca, Mg, Fe, Ni, Cr, Co, Zn, Cu, Mn, Na, Al), colorimetry (for N and P), and flame photometry (for K). Individuals of *A. murale,* both infected and healthy, showed slight differences in their composition of essential elements (N, P, K) and of some micronutrients. The parasite had higher P, K, Na, and lower N, Ca, Mg, Ni, Zn, Co, Mn and Al concentrations than the host. *Orobanche nowackiana* is a Ni accumulator with 299 mg kg^{-1} in the leaves. Hyperaccumulation of Ni by *A. murale* did not prevent attack by *O. nowackiana*. After infection there was a decline in Ni concentration and the dry weight of all organs of the host, thus reducing the biomass yield and consequently Ni phytoextraction yield of *A. murale*. This infection could be a potential threat to the use of *A. murale* for Ni agromining.

Keywords *Alyssum murale* · Biomass · Metal accumulation · Parasitic plant · Phytoextraction yields

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Published online: 30 March 2018

Introduction

The genus *Orobanche* L. (Orobanchaceae) comprises approximately 170 species associated with various host plants throughout the world (Uhlich et al. 1995). The genus is considered Mediterranean in its distribution and can parasitize a wide range of hosts in members of the Apiaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae and Solanaceae (Barker et al. 1996) which grow on different soil types.

Parasitic plants can have an important influence on host survival and reproductive success and can cause heavy economic losses (Kuijt 1969; Press et al. 1990; Boyd et al. 1999). They specifically interact with the host at different levels: (1) germination of parasite seed; (2) initiation and development of haustorium; (3) transfer of water and solutes; (4) host responses to infection, and cause irreversible damage to host physiology, reducing its biomass and fertility (Kuijt 1969; Delavault 2015). For instance, several *Orobanche* and *Phelipanche* species parasitize important crops and are of major importance

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in Europe, where about 70% of farm land is infected with seeds of broomrapes (Gevezova et al. 2012). The effects of *Orobanche* infection on the growth and mineral composition of the host have been studied in parallel observations of host and parasite (Singh et al. 1971; Barker et al. 1996; Labrousse et al. 2010; Fernández-Aparicio et al. 2016). In all cases, reduction in host biomass was mentioned for both vegetative and reproductive organs. Also, both parasite and host compete directly for resources within a host plant (Fernández-Aparicio et al. 2016).

Metalliferous soils such as ultramafic (i.e. serpentine) soils, known to have abnormally high concentrations of potentially phytotoxic trace elements, including nickel (Ni), cobalt (Co) and chromium (Cr), low calcium/magnesium (Ca/Mg) ratio and general nutrient deficiencies (Brooks 1987; Harrison and Rajakaruna 2011) are of great importance in the study of plant physiology, ecology, evolution, metal accumulation used in phytoremediation, and phytomining practice (van der Ent et al. 2015). Nickel agromining operations use cultivated hyperaccumulator plants ('metal crops') on Ni-rich (ultramafic) soils, followed by harvesting and incineration of the biomass, to produce a high-grade 'bio-ore' from which Ni metal or pure Ni salts are recovered (Nkrumah et al. 2016). Such technology could be part of a modern agricultural activity in Albania, where the most efficient Ni-accumulating wild species, *Alyssum murale* Waldst. & Kit., can be cropped on the large suitable ultramafic surfaces (Bani et al. 2013, 2014, 2015a, 2018). This species occurs widely in Albania as a native weed in agricultural lands on ultramafic Vertisols and Cambisols and it is considered suitable for phytoextraction of heavy metals (Bani et al. 2015a, b), for agromining (Nkrumah et al. 2016), and for the restoration of metal-enriched sites degraded by mining activities (O'Dell and Claassen 2011). *Alyssum murale* is the host plant of the parasitic species *Orobanche nowackiana* Markgr. across ultramafic areas of the Balkans and Turkey. As *O. nowackiana* is an obligate root holoparasite completely lacking chlorophyll, it depends on its host for carbon, water and nutrients. Although *A. murale* is not yet fully developed as a crop species, the host/parasite relation is of interest for agromining practice in Albania.

Orobanche nowackiana is the only known serpentine-endemic among the parasitic flowering plants in Europe (Edmondson 1992). This species is recorded in Central and Northern Greece, Albania, and SW Anatolia (Hartvig 1991; Foley 2000; Mullaj et al. 2010). According to Foley (2000), this species was only known from three localities in Albania, but later two more localities from southern Albania were reported (Barina and Pifko 2008; Mullaj et al. 2010).

Orobanche nowackiana in Greece can parasitize various serpentine plants and other Ni hyperaccumulators of the Brassicaceae family, such as *Bornmuellera baldaccii* (Degen) Heywood, *B. tymphaea* (Hausskn.) Hausskn., *Alyssum heldreichii* Hausskn., *A. murale* and *A. smolikanum* Nyár. (Hartvig (1991). However, the only known host for *O. nowackiana* in Albania is *A. murale*. The endemic Ni hyperaccumulator *A. lesbiacum* (Candargy) Rech. F. is also reported as a host of *O. nowackiana* (Foley 2000). Most of the reported hosts of *O. nowackiana* are serpentine endemics except *A. murale*.

The parasite/host relationship is also of interest for the adaptation of *O. nowackiana* to high Ni concentrations in the tissues of the host. The parasite/Ni hyperaccumulating host relationship was only studied up to now for the American species *Cuscuta californica* Hook. & Arn. and the Ni hyperaccumulator *Streptanthus polygaloides* A. Gray in order to estimate metal accumulation by the parasite and defense of metal hyperaccumulator plants from herbivore/pathogen attack (Boyd et al. 1999). The present study is based on the hypothesis that attack by the parasitic plant *O. nowackiana* on *A. murale* may affect its mineral composition and Ni phytoextraction yield. As *O. nowackiana* is a holoparasite we suggest that higher Ni concentrations are accumulated in the tubercle. The work presented in this paper aims to evaluate: i) metal accumulation in different parts of healthy and infected host plants (*A. murale*) and in plant organs of the parasite, and ii) the effect of *Orobanche* infection on the biomass production and elemental concentration of the host plant.

Methods

Study site

The study site is located in Southeastern Albania on Komjan Mountain, on the eastern shore of Dushku Lake at 1200 m asl. This ultramafic area belongs to the southeastern Albanian ophiolitic massif, one of the main ultramafic outcrops in the Balkans (Stevanović et al. 2003). Dushku Lake is surrounded by a forest belt, which is dominated by *Pinus nigra* Arnold. In the southwestern part of the lake, the forest is dominated by *Fagus sylvatica* L. whereas in the eastern part of Dushku Lake a large serpentine area is deforested and the medicinal plant *Salvia officinalis* L. and plum trees are cultivated. On open terrain the natural herbaceous vegetation features *Alyssum murale*, *Sanguisorba minor* Scop., *Thymus longicaulis* C. Presl and *Herniaria glabra* L.. *Orobanche nowackiana* was found in a meadow on the eastern shore of Dushku Lake (40°47′57.74″N, 20°19′58.25″E) parasitizing several plants of *A. murale*. The study area covers about 300 m^2 with a relatively dense cover of both species and a remarkable homogeneity in species composition.

Studied species

Alyssum murale is a Ni-hyperaccumulating flowering plant from the family Brassicaceae that occurs widely in

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the eastern Mediterranean region. It is a perennial species which can be found on ultramafic Vertisols and Cambisols of Albania and is a spontaneous weed in other crops. Seven years of field study allowed optimization of the growth of efficient populations of *A. murale* for use in phytomining (Bani et al. 2007, 2009, 2010, 2015a, 2018).

The parasitic species of the genus *Orobanche* (Orobanchaceae), commonly known as broomrape, are well known plants that negatively affect a wide variety of plants, most of them crop species. *Orobanche nowackiana* is a small perennial plant, with branched stems, 5–10 cm tall and yellow flowers combined in dense spikes (Foley 2000). In Albania it is distributed in montane ultramafic localities. The description of *O. nowackiana* is detailed by Markgraf (1926). According to Foley (2000), due to extremely similar morphological features, *O. nowackiana* and *O. rechingeri* Gilli (described in 1966 and collected in Greece) are conspecific and *O. rechingeri* should be considered as a synonym of *O. nowackiana*.

The stem of *O. nowackiana* appears on the surface of the soil in late April or early May and finishes the growth cycle by the end of July. The parasite tubercles grow underground for several weeks to several months and then produce aboveground flowering shoots which bloom in May. In total, the whole life cycle from seed germination to seed production lasts about 5 months (Kroschel 2001). Collected *A. murale*-infected (and *A. murale*- healthy) individuals were about 2 years old at the beginning of the flowering stage. As the life cycle of *Orobanche* is synchronized with that of the host plant (Delavault 2015) collected parasite individuals were also in the flowering stage but younger than their hosts.

Plant and soil samples

The Ni hyperaccumulator species *A. murale* was collected in the flowering stage both in May 2016 and 2017. In 2016, four plots were chosen at a distance of 20–30 m from one another in the studied site to cover all the area. In 2016, in each plot, both infected and healthy *A. murale* and parasite were collected for elemental analyses (one set per plot). In 2017, again four plots were selected but three sets were taken from each plot. All three sets per plot were used for biometrical analysis of plants and one of them per plot was used for elemental analysis. The healthy plants were selected within 1 m of the infected plants. The *Orobanche* samples corresponded to a significant subset of the whole population and it was impossible to sample more individuals without threatening the population of this rare species of Albanian flora.

The dry weight of each sample was measured for the whole plant and separately for root, shoots and leaves. The length of the main shoot, the number of branches, and the length of the main root of each plant were also measured. Based on the average mass of 3 healthy and 3 infected *A. murale* plants harvested in each of the four plots and the fact that optimal plant density for biomass production is 4 plants per m^2 we also calculated biomass yield for 1 ha for healthy and infected *A. murale*.

For both years the same elemental analyses were made for the whole plant and separately for root, shoots and leaves of infected and healthy *A. murale* and parasite. Plants were dried at 70 °C for 72 h in an oven after rinsing. Among the sets, individuals of *O. nowackiana* were collected separately from the host. They were carefully removed from the host root where the tubercle with short adventitious roots was developed. These adventitious roots were not removed because, according to Delavault (2015), they have a rudimentary cap but lack a root hair zone thus making them unable to take up water and mineral compounds from the soil. Plant samples from the parasite were carefully cleaned from soil particles and divided into the following parts: tubercle with adventitious roots, achlorophyllous shoots (stems), leaves (scales), and flowers. All plant parts were analyzed for Ni concentration and other elemental composition.

Soil samples were also collected in order to determine the effect of infection on Ni metal concentrations in the soil of the host. The samples were collected in the rhizosphere zone of the sampled infected and healthy *A. murale* plants.

Elemental analyses

Fresh roots, shoots, leaves and flowers were carefully washed with sodium metaphosphate (1%) for 24 h, then with deionized water and were finally oven-dried at 70 °C for 72 h. Dry weights for all plant parts were recorded. Dried plant samples were ground and elemental analyses were performed for N, P, K, Ca, Mg, Fe, Ni, Cr, Co, Mn, Zn, Cu, Na, and Al for all samples of *A. murale* and *O. nowackiana*.

The total N concentrations of the plant parts were analyzed by combustion at 900 °C with a CHNS analyzer (vario MICRO cube, Elementar Analysen systeme GmbH, Germany). For other elements, subsamples (0.5 g) of dry plant tissue were acid-digested at 95 °C in 2.5 ml of concentrated HNO_3 and 5 ml of H_2O_2 (30%). The final solutions were filtered (0.45 µm 164 Digi-FILTER) and made up to 25 ml with deionized water. Concentrations of the trace metals, P, K, Mg and Ca in the solution were all measured on the digested solutions with an inductively coupled plasma-atomic emission spectrometer (ICP-AES 166 Liberty II, Varian).

Soil samples were air dried, sieved to pass a 2-mm nylon mesh and ground. Total concentrations of N, P, and K were determined. The digestion procedure followed Kjeldahl (ISO 11261: 1995/2016). Colorimetric determinations were made for N and P, and flame photometry for K (Pr-EN 16169: 2011E; Pr-EN 14672: 2005; ISO 9964-3: 1993/2014). The trace metals, Mg and Ca concentrations in the soil after acid-digestion were

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also measured by ICP-AES. Chemically available Ni in each soil sample was assessed by an extraction with diethylenetriaminepentaacetic acid (DTPA). This has been shown to better assess the pool of soil Ni ions that are able to become soluble and possibly be absorbed by hyperaccumulator roots (Echevarria et al. 1998, 2006; Shallari et al. 2001). Extractions were thus performed with a DTPA-TEA solution (0.005 M DTPA, 0.01 M $CaCl_2$, 0.1 M triethanolamine, pH 7.3) according to Lindsay and Norvell (1978) and the Ni concentration in the extracts was measured by ICP-AES; Ni extracted by DTPA (NiDTPA) was calculated in mg kg^{-1}.

Data analyses

Univariate and multivariate statistical procedures were deployed to study the variation of the parameters. *t*-tests were used to test for significant differences in growth characteristics in both *A. murale*-infected and healthy, and elemental concentrations in their rhizosphere soil samples. The plant elemental data at first were subjected to *t*-tests to check the differences across years on the concentration of elements in the plant organs. After that an analysis of variance (ANOVA–single factor) was applied to determine whether elemental concentration varied between healthy, infected and parasite plants. ANOVA was applied for each element measured in different plant organs for each year. Post hoc analysis (Multiple Comparisons with Fisher's LSD) was performed to see if there were significant differences for each element between groups: *A. murale*-healthy/*A. murale*-infected and *A. murale*-infected/*O. nowackiana*. The capacity of the analyzed species for accumulation of a specific metal was defined by its accumulation factor, calculated as a ratio of elemental concentrations in aerial plant parts to that in the soil (Baker et al. 1994; Reeves 2006) and concentrations in parasite tissues to that of the host.

Results

Effects of *Orobanche* infection on the growth characteristics of *A. murale*

The effects of *Orobanche* infection were obvious and visible at the flowering stage. In all the organs of the host parasitized by *Orobanche*, we noticed a decline in the dry weight (Fig. 1). Maximum reduction of 84% in leaf biomass, 75% in shoots, and 86% in roots was noted. The growth of the whole plant in terms of its dry weight showed an overall 83% reduction. The weight of the infected host plant summed with the weight of parasite was not higher than that of the healthy non-host plant alone. We also noticed a reduction in the length of the main shoot, the length of the main root, and the number of primary branches of the host plants (Table 1). Maximum reduction of 39% in length of main shoot and 55% in the number of primary branches was noted. The least affected organ was the root system with a 28% reduction in length of the main root. Independent *t*-tests showed significant differences between host and healthy plants for growth parameters of shoots and roots and for the dry weight of each plant part (Table 2).

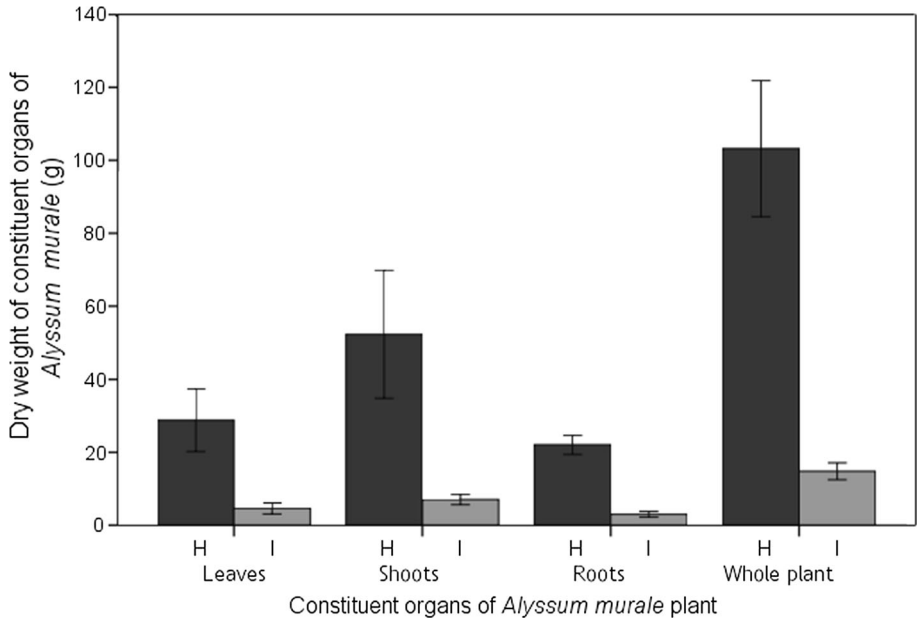

Fig. 1 Effects of *Orobanche* infection on the dry weight (g) of constituent organs of *Alyssum murale* plant at flowering stages of the parasite (Mean ± SD, $n = 12$). Abbreviations used: healthy *Alyssum murale* (H) and infected *Alyssum murale* (I)

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Table 1 Effects of *Orobanche* infection on studied parameters of *Alyssum murale*-healthy (H) and *Alyssum murale*-infected (I) plants (Mean ± SD, $n = 3$)

Parameters	Sampling area			
	Plot I	Plot II	Plot III	Plot IV
Length of main shoot (cm)				
Healthy (H)	40 ± 6	39 ± 4.5	37 ± 2.8	35 ± 3.6
Infected (I)	23 ± 2.7	22 ± 2	26 ± 3.1	21 ± 2.6
Length of main root (cm)				
Healthy (H)	40 ± 3.4	40 ± 2.9	34 ± 3.4	37 ± 4
Infected (I)	28 ± 3.4	30 ± 4	25 ± 20	25 ± 1.5
Number of main branches				
Healthy (H)	14 ± 2	10 ± 1	12 ± 2	11 ± 2
Infected (I)	7 ± 1	3 ± 1	5 ± 1	6 ± 1

Table 2 Significant differences for the growth parameters of constituent organs between the *Alyssum murale*-healthy and *Alyssum murale*-infected according to *t*-tests for equality of means

Independent samples test			
Growth parameters	*t*-test		
	t	*df*	*P* value
Length of the main shoot (cm)	9.82	22	< 0.001
Length of the root (cm)	7.20	22	< 0.001
Number of the main branches	7.85	22	< 0.001
Dry weight_Leaves	9.58	12	< 0.001
Dry weight_Shoots	8.92	11	< 0.001
Dry weight_Roots	23.9	13	< 0.001
Dry weight_Whole plant	16.3	11	< 0.001

Soil characteristics

The rhizosphere soils were characterized by elevated total concentrations of metals (such as Ni, Cr, Co) that are typical of ultramafic environments (Table 3). The total Ni concentrations were high, ranging from 3547 to 3864 mg kg^{-1} in the rhizospheric soil of *A. murale*-infected and healthy plants. All soils had high iron (Fe) concentrations (10%) as expected for soils of ultramafic origin (Bani et al. 2014; Echevarria 2018). The total Ca concentrations were also typical for an ultramafic soil, ranging from 0.36% to 0.42%, while total Mg concentrations were lower than those reported in other ultramafic soils of Albania, but typical for the Gramsh region (3.7–3.8%) as reported by Bani et al. (2013). The ultramafic soils are also rather infertile with regards to total potassium (K) and phosphorus (P). The levels of N, P, K are lower than other ultramafic sites, even in the well described site of Pojska, Pogradec (Bani et al. 2014). The levels of Co and Cr are high and also typical for serpentine sites of Albania (Bani et al. 2013, 2014). The zinc (Zn) concentrations fell within the ranges for normal soils lying in the range from 30 to 36 mg kg^{-1} (Kabata-Pendias and Pendias 1984). The total concentration of trace elements in soils provides limited information on their bioavailability, as this does not show

Table 3 Total concentrations of heavy metals and macronutrients in the rhizosphere soil of *Alyssum murale*-healthy (H) and *Alyssum murale*-infected (I) plants (Mean ± SD, $n = 4$)

Soil	Year	Ca %	Mg	Fe	N	K	P mg kg^{-1}	Mn	Na	Cr	Co	Zn	Cu	Ni total	Ni DTPA
H	2016	0.40 ± 0.01	3.7 ± 0.2	9.75 ± 0.5	0.19 ± 0.01	0.13 ± 0.02	377 ± 64	1933 ± 18	97 ± 2.8	382 ± 14	125 ± 27	34 ± 3.3	13 ± 1	3864 ± 193	102 ± 3.4
	2017	0.42 ± 0.02	3.8 ± 0.2	10.3 ± 0.1	0.17 ± 0.04	0.14 ± 0.05	391 ± 84	1913 ± 10	93.5 ± 2	395 ± 23	116 ± 21	35.8 ± 4.4	12.7 ± 2	3750 ± 475	107.5 ± 4
I	2016	0.38 ± 0.01	3.6 ± 0.06	10.0 ± 0.15	0.17 ± 0.02	0.15 ± 0.01	363 ± 11	1903 ± 18	95 ± 3.5	374 ± 11	130 ± 30	31 ± 1.9	8 ± 2.3	3679 ± 270	100 ± 3.9
	2017	0.36 ± 0.05	3.7 ± 0.21	10.0 ± 0.28	0.15 ± 0.02	0.15 ± 0.04	385 ± 81	1905 ± 6	91 ± 2.6	363 ± 36	121 ± 44	30 ± 4	17 ± 1.8	3547 ± 337	97.5 ± 4.3

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Table 4 Significant differences for heavy metals concentration between soil of *Alyssum murale*-healthy and soil of *Alyssum murale*-infected according to *t*-test for equality of means

	2016 year			2017		
	t-test			*t*-test		
	t	*df*	*P* value	*t*	*df*	*P* value
Ca	− 1.83	6	0.11	− 2.46	6	0.05
Mg	− 1.82	6	0.12	− 0.91	6	0.39
Fe	0.89	6	0.41	0.84	6	0.43
N	− 2.22	6	0.07	− 1.23	6	0.26
K	0.98	6	0.36	0.29	6	0.78
P	− 0.45	6	0.66	− 0.08	6	0.93
Na	− 0.73	6	0.49	− 1.37	6	0.22
Mn	− 2.29	6	0.06	− 1.30	6	0.24
Cr	− 1.17	6	0.28	− 1.71	6	0.13
Co	0.20	6	0.84	0.22	6	0.83
Zn	− 1.58	6	0.16	− 1.74	6	0.13
Cu	3.12	6	0.02	2.90	6	0.03
Ni total	− 1.11	6	0.310	− 0.79	6	0.46
Ni DTPA	− 1.12	6	0.306	− 1.68	6	0.14

how strongly the element is bound to the reactive surfaces of soil constituents (Manceau et al. 1996). DTPA-extractable Ni was chosen as an estimate of soil Ni chemical availability. DTPA-extracted Ni in soils was relatively high ranging from 97.5 to 108 mg kg^{-1} (Table 3). This finding is in accordance with previous studies for the ultramafic soils of this region (Bani et al. 2013, 2014).

The *t*-tests for mean values for soil elements showed significant variations only for Ca for 2017 and Cu for 2016 between soil of healthy and infected *A. murale* samples (Table 4). The average level of Ca in the infected soil samples was lower than in the healthy ones, whereas for Cu it was the opposite.

Elemental composition of plant parts in *A. murale* and *O. nowackiana*

Nitrogen (N) concentration was lower in the infected *A. murale* than in the healthy ones, but *Orobanche* contained lower quantities of N than any part of the hosts (Table 5). Concentrations of P and K decreased in infected hosts, in all organs and as a result in whole plants (Table 5). The concentrations of K and P in the parasite were higher than in the host plants. The highest K and P concentration in plant organs was observed in flowers and leaves for *O. nowackiana* and in shoots and leaves of *A. murale*. Magnesium (Mg) concentration was lower in the infected *A. murale* than in healthy ones, but higher in the host than in *O. nowackiana* plants. Calcium concentration was lower in leaves and whole plant of the host plants. The highest Ca concentration in plant organs was observed in the leaves for both host and parasite plants. It was higher in *A. murale* -healthy plants than in infected ones (Table 5).

Orobanche nowackiana contained higher quantities of Fe compared with the aboveground parts of the host but it was higher in *A. murale*-healthy plants. Bioaccumulation factors with values higher than 1 were found for K in *O. nowackiana*, and for N, K, and Ca in *A. murale*, in both infected and healthy plants.

Since *A. murale* is a Ni hyperaccumulator plant, it is not surprising that Ni concentration in the host is higher than in the parasite (Table 5). Nickel concentration was relatively higher in *O. nowackiana* leaves (299 mg kg^{-1}) than in above-ground plant parts of other types of plants in the area (Bani et al. 2013) and obviously it can tolerate and accumulate Ni. In the aboveground parts of the parasitic plant, concentrations of Na and Cr were higher than in the host. Concentrations of Zn, Co, Cu, Mn and Al were higher in the host than in the parasitic plant (Table 5). While the concentrations of Zn, Co and Al were slightly higher in the infected *A. murale*, those of Mn and Cu were higher in healthy individuals.

t-tests showed that in general there was no significant statistical difference between years of the concentration of elements in different plant organs. There were statistical differences between the years for N ($t = 3.92$, $df = 6$, $P = 0.008$), Cu ($t = 3.84$, $df = 6$ $P = 0.009$), only in leaves of healthy *A. murale* and P in the whole plant for infected *A. murale* ($t = 2.5$, $df = 6$, $P = 0.046$) and parasitic plants ($t = 2.6$, $df = 6$, $P = 0.040$).

One way ANOVA showed significant differences in concentrations of N, K, P, Mg, Ca, Fe, Zn, Na, Ni and Al in tissues between *A. murale*-healthy/*A. murale*-infected and *A. murale*–infected and parasitic plants for both years. Multiple comparisons with Fisher's LSD demonstrated that there were significant differences in macronutrient and micronutrient values between pairs *A. murale*-healthy/*A. murale*-infected, and *A. murale*-infected/*O. nowackiana* ($P < 0.05$) (Table 5).

There were no significant differences in the concentrations of Mn, Co or Cu between the *A. murale*-healthy and *A. murale*-infected plants (whole plants or above-

Table 5 Total concentrations of N, P, K, Ca, Mg (g kg^{-1}), Fe and heavy metals (mg kg^{-1}) in healthy and infected *Alyssum murale* plants and in *Orobanche nowackiana* (Mean ± SD, $n = 4$)

Element	Year	*O. nowackiana*					*A. murale*-infected				*A. murale*-healthy			
		Flowers	Leaves	Shoot	Tubercles and roots	Whole plant	Leaves	Shoot	Root	Whole plant	Leaves	Shoot	Root	Whole plant
N	2016	19.1 ± 0.5	12.8 ± 0.1**	9.2 ± 0.4**	10.4 ± 1.4	11.9 ± 1.2	27.7 ± 0.8*,**	14.7 ± 1.1**	9.8 ± 1.5*	20.9 ± 0.9*	30.5 ± 1.3*	20 ± 1.7	17.8 ± 0.7*	25 ± 0.9*
	2017	21.6 ± 0.4	12.6 ± 1.9**	8.60 ± 0.8**	8.34 ± 1.8	10.9 ± 1.2	24.2 ± 2.8*,**	19.7 ± 1.2**	9.54 ± 1.0*	21.6 ± 0.6*	35.5 ± 2.2*	21.9 ± 1.9	19.6 ± 1.5*	27 ± 2.5*
K	2016	26.2 ± 2.0	16 ± 0.60**	17.8 ± 0.5**	7.9 ± 0.5	19.8 ± 1.7**	8.7 ± 0.5**	7.2 ± 0.6**	5.7 ± 0.5	7.4 ± 0.2**	9 ± 0.17	9.3 ± 0.4	6.4 ± 0.4	9.2 ± 0.4
	2017	27.9 ± 3.9	18.7 ± 4.9**	17.2 ± 2.7**	8.2 ± 2.0	18.1 ± 3.9**	7.6 ± 0.8**	8.1 ± 1.0**	5.4 ± 1.2	7.6 ± 0.8**	9.34 ± 0.5	9.7 ± 0.4	6.2 ± 0.7	9.4 ± 0.4
P	2016	3.30 ± 0.2	2.16 ± 0.2**	1.6 ± 0.07	0.75 ± 0.13**	2.62 ± 3**	1.4 ± 0.07*,**	1.3 ± 0.05	0.59 ± 0.05**	1.3 ± 0.08**	1.94 ± 0.05*	1.52 ± 0.08	0.6 ± 0.03	1.7 ± 0.04
	2017	4.30 ± 0.87	2.12 ± 0.16**	1.5 ± 0.08	0.8 ± 0.08**	2.2 ± 0.12**	1.26 ± 0.1*,**	1.2 ± 0.09	0.5 ± 0.008**	1.1 ± 0.13**	1.82 ± 0.38*	1.3 ± 0.4	0.66 ± 0.1	1.6 ± 0.34
Mg	2016	1.19 ± 0.07	1.29 ± 0.06**	1.1 ± 0.06**	1.2 ± 0.140	1.2 ± 0.03**	5.08 ± 126*,**	1.9 ± 0.19**	1.74 ± 0.11	2.62 ± 0.13*,**	6.7 ± 0.195*	1.14 ± 0.09	2.27 ± 0.16	3.6 ± 0.22*
	2017	1.30 ± 0.10	1.21 ± 0.12**	1.24 ± 0.03**	1.07 ± 0.07	1.29 ± 0.05**	5.1 ± 351*,**	1.69 ± 0.38**	1.84 ± 0.69	3.39 ± 0.55*,**	6.04 ± 0.75*	1.29 ± 0.05	1.95 ± 0.67	4.1 ± 0.3*
Ca	2016	1.84 ± 0.11	4.31 ± 0.15**	1.32 ± 0.15**	1.45 ± 0.09**	1.65 ± 0.06**	27.3 ± 0.96*,**	6.7 ± 0.44**	3.0 ± 0.47*,**	18 ± 0.740*,**	33.5 ± 1.12*	6.3 ± 0.32	2.46 ± 0.3*	26.4 ± 1.7*
	2017	2.93 ± 0.2	4.14 ± 0.11**	2.35 ± 0.22**	2.43 ± 0.23**	1.65 ± 0.02**	27.5 ± 3.5*,**	7.1 ± 0.92**	3.48 ± 0.5*,**	19 ± 0.235*,**	33.2 ± 0.48*	6.25 ± 0.52	2.7 ± 0.53*	24.2 ± 4.6*
Fe	2016	504 ± 126	827 ± 51**	386 ± 21	1253 ± 42	553 ± 21	549 ± 67*,**	345 ± 38*	1423 ± 79**	414 ± 20*	972 ± 46*	238 ± 41*	1603 ± 32*	615 ± 72*
	2017	515 ± 12	815 ± 70**	362 ± 27	1190 ± 21**	524 ± 43	463 ± 201*,**	325 ± 72*	1438 ± 153*,**	424 ± 118*	948 ± 32*	212 ± 18*	1644 ± 53*	605 ± 14*
Mn	2016	17.2 ± 1.9	26 ± 1.3	14 ± 1.4**	28 ± 1.5	21 ± 0.8**	57.6 ± 4.4	28 ± 3.9*,**	33.8 ± 2	31.6 ± 1.8**	80 ± 7.1	16.9 ± 2.1*	44.3 ± 5.9	43.4 ± 5.9
	2017	18 ± 4.5	25.5 ± 4.8	14.6 ± 1.3**	30 ± 1.6	22.5 ± 2.4**	51 ± 27	33 ± 13*,**	35.8 ± 9	38.5 ± 13**	62 ± 18	17.5 ± 7.1*	41.5 ± 16	41.8 ± 4.5
Zn	2016	27 ± 1.7	22 ± 1.9**	14 ± 1.4**	17.5 ± 1**	23 ± 0.7**	133 ± 2.8*,**	88 ± 1.5**	41 ± 2.99**	100 ± 3.6*,**	105 ± 4.3*	76.3 ± 3	31.4 ± 2.9	83 ± 4.6*
	2017	26 ± 2.3	23 ± 1.2**	13.2 ± 2.4**	18 ± 2.8**	23.5 ± 2.6**	132 ± 13*,**	83.1 ± 5.8**	42 ± 128**	109 ± 10*,**	103 ± 19 *	72.8 ± 16	35.8 ± 7.5	89 ± 11*
Na	2016	177 ± 2	326 ± 15**	448 ± 10	616 ± 30**	315 ± 11**	79.9 ± 3.7**	92.8 ± 3.6	73.5 ± 2*	85.3 ± 1.7*,**	87 ± 4.8	125 ± 6.1	88.4 ± 3	117 ± 5.9*
	2017	175 ± 13	313 ± 16**	460 ± 39	658 ± 47**	303 ± 16**	89.9 ± 3.3**	101.5 ± 7.7	85 ± 7*	97.8 ± 6*,**	89 ± 5.6	131 ± 14	91 ± 4.9	122 ± 16*
Co	2016	1.89 ± 0.2	1.16 ± 0.2**	2 ± 0.25**	3.1 ± 0.4	1.75 ± 0.3**	17 ± 0.9*,**	7.7 ± 0.8*,**	5.7 ± 1.8	10.2 ± 0.9**	13.3 ± 2.1*	4.1 ± 9.3*	3.6 ± 0.4	9.3 ± 0.7
	2017	1.7 ± 0.2	1.2 ± 0.15**	2.3 ± 0.12**	3.6 ± 0.2	2.15 ± 0.2**	18.9 ± 0.7*,**	8.2 ± 2*,**	4.3 ± 0.66	11 ± 0.86**	15.4 ± 2.*	3.9 ± 0.6*	4.18 ± 1.7	10.3 ± 1.8
Cr	2016	7.4 ± 0.7	8.9 ± 0.2**	4.2 ± 0.8	8.6 ± 0.8**	6.2 ± 0.5	3.17 ± 0.3*,**	4.00 ± 0.5	7.8 ± 0.8*,**	3.4 ± 0.5*	7.3 ± 0.23	4.8 ± 0.04	10 ± 0.5*	6 ± 0.6
	2017	6.5 ± 0.4	9.6 ± 3.1**	3.6 ± 1.4	9.8 ± 3.7**	5.2 ± 1.2	3.2 ± 1.2*,**	4.15 ± 2.4	9.2 ± 2.2*,**	4.3 ± 0.4*	8.6 ± 3.2	5.85 ± 0.9	11.9 ± 5.4*	7.3 ± 1.3*
Cu	2016	2.8 ± 0.5	2.8 ± 0.5	1.75 ± 0.2	4 ± 0.18	2.6 ± 0.6	3 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.8 ± 0.4	5.5 ± 0.6	2.8 ± 0.3	2.7 ± 0.5	4.6 ± 1.4
	2017	3.19 ± 1.4	2.93 ± 0.5	1.59 ± 1	3.88 ± 16	2.85 ± 0.6	3.43 ± 0.6	2.4 ± 0.4	2.0 ± 0.1	3.21 ± 0.2	4.2 ± 0.13	3.29 ± 0.5	2.09 ± 12	3.6 ± 0.5
Ni	2016	276 ± 55	255 ± 52**	160 ± 12**	531 ± 66**	226 ± 26**	10,600 ± 594*,**	4033 ± 197**	3522. ± 98*,**	6042 ± 580*,**	11,974 ± 572*	4169 ± 127	2339 ± 120*	7004 ± 212*
	2017	199 ± 72	299 ± 14**	184 ± 62**	601 ± 208**	250 ± 150**	11,872 ± 297*,**	3815 ± 478**	3390 ± 143*,**	5851 ± 319*,**	12,459 ± 1135*	3982 ± 508	2230 ± 162*	6873 ± 376*
Al	2016	80.6 ± 5.7	133 ± 13**	57 ± 3.8**	211 ± 12**	87 ± 5.7**	307 ± 10*,**	84 ± 8.3*,**	250.5 ± 10**	260 ± 13*,**	189 ± 14*	27 ± 2.9*	246 ± 15	145 ± 6*
	2017	79 ± 6.7	141 ± 13**	58 ± 3.7**	228 ± 20**	91 ± 8**	361 ± 34*,**	73 ± 14*,**	262 ± 16**	246 ± 24*,**	200 ± 47*	28.4 ± 8*	249 ± 21	158 ± 23*

Values with letters (**, *) indicate significant difference between plant organs of study plants and inverse for values without letter (ANOVA one way, Multiple Comparisons with Fisher's LSD at the $P < 0.05$ level)
*Significant differences between *Alyssum murale* infected and healthy; **significant differences between *Alyssum murale* infected and parasite

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Table 6 Nickel yield from healthy and infected *Alyssum murale* from the natural plots in serpentine soil of the eastern shore of Dushku Lake, Albania

Plot no	Average plant mass		Ni concentration in plants		Biomass Yield		Ni Yield	
	g plant^{-1}		g kg^{-1}		kg ha^{-1}		kg ha^{-1}	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
1	120.0	15.18	6.83	6.08	4800.0	607.0	32.8	3.69
2	112.0	15.76	7.38	5.39	4480.0	630.0	33.1	3.40
3	101.0	12.90	6.47	6.06	4040.0	516.0	26.1	3.13
4	79.4	15.34	6.80	5.86	3176.0	614.0	21.6	3.59

ground parts). Also there were no significant differences in concentrations of Cu between infected *A. murale* and *O. nowackiana*. Multiple Comparisons with Fisher´s LSD showed no significant differences in Ni accumulation abilities in *A. murale* and *O. nowackiana* in samples collected in different years.

Biomass and Ni yields for healthy and infected *A. murale*

The biomass of *A. murale*, as the average of the whole biomass harvested in each of the four plots, ranged from 3176 to 4800 kg ha^{-1} for healthy *A. murale*, which was much higher than for infected ones (515–630 kg ha^{-1}) with a highly significant difference ($t = -9.9$, $df = 6$, $P < 0.001$). The Ni yields in the biomass of healthy *A. murale* (21–33 kg ha^{-1}) were higher than in infected *A. murale* (3.1–3.2 kg ha^{-1}) with a highly significant difference ($t = -8.9$, $df = 6$, $P < 0.001$) (Table 6).

Discussion

Effects of *Orobanche* infection on host growth and biomass yield

It is evident from the data that the *Orobanche* infection brings about a marked decrease in the growth of *A. murale* plants. Judging from the loss of about 83% of its dry weight, the biomass yield of infected *A. murale* was about 7 times less than the healthy ones. Similar data were reported by Singh et al. (1971) for tomato plants infected by *Orobanche cernua* Loeffl. but the reduction of the weight (28%) mentioned by the authors was much less than in this study. Significant reduction in the growth of sunflower (Andreev 1930), tomato (Mauromicale et al. 2008), faba bean, field pea, and grass pea (Fernández-Aparicio et al. 2016) has also been recorded due to *Orobanche* infection. The dry weight and the length of constituent organs of infected *A. murale* plants changed significantly compared to healthy ones indicating that the parasite may be mobilising resources from the host plant. Moreover, Fernández-Aparicio et al. (2016) mentioned that reductions in host biomass occurred not only in vegetative organs but also in reproductive ones such as for faba bean, field pea, and grass pea where the reproductive organs were more affected. Thus, *Orobanche* appears to be a serious parasite as evidenced from the losses of dry weight of *Alyssum* plants: total host biomass reduction is explained by the parasitic sink activity as mentioned by Singh et al. (1971) and Fernández-Aparicio et al. (2016).

Effects of infection on the mineral composition of the host

The elemental composition differed between healthy and infected plants. Healthy whole *A. murale* plants had higher concentrations of N, K, P, Fe, Ca and Mg than infected ones. The effects of infection were more marked in leaves than in other parts. Transfer of these essential elements to the parasite appears to lead to retarded growth of the host as well as the lowering of the concentrations of these elements in the infected host, relative to the healthy host. Our data differ from those of Boyd et al. (1999) who recorded higher concentrations of Ca in the infected host Ni hyperaccumulator *Streptanthus polygaloides*.

In the present work *A. murale* (like many *Alyssum* species on ultramafic soils) is found to have a high concentration of both Ca and Mg. Attention has been drawn previously (e.g. Reeves et al. 1997; Lombini et al. 1998) to the fact that Ni hyperaccumulators in *Alyssum* have remarkably high leaf Ca concentrations, especially considering the usual deficiency of this element in ultramafic soils. These species therefore have a remarkable ability to take up Ca as well as Ni. The levels of both Ca and Mg in *A. murale* appear to be far beyond physiologically required levels, as evidenced by the storage of Ca as Ca-oxalate crystals in the trichomes (McNear et al. 2005). However, remarkably high Ca concentrations must also be present elsewhere in the leaves, as the trichomes constitute only a small proportion of the leaf mass.

It is difficult to use the absolute elemental concentrations of the host and parasite to draw inferences about the operation of the transfer process, because of

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differences between the two species in the structure of the various analysed plant parts. We can, however, consider relative differences among the elements measured here.

The particularly high Ca concentration in the host is not reflected in the parasite. Lower Mg concentrations in the parasite are not surprising in view of its lack of chlorophyll. Most noteworthy, however, is the highly significant elevation of K and P concentrations in all parts of the parasite, especially the leaves. This confirms the observation of Singh et al. (1971) who also found that concentrations of K and P in *Orobanche* species were greater in the parasite than the host, suggesting that specific levels of these elements may be required by the parasite, and that some degree of metabolic regulation is involved. Concentrations of N, Ni and of trace elements such as Co, Zn, Mn and Al are also significantly lower in the parasite, suggesting a rather low requirement of the parasite for these elements.

Singh et al. (1971) considered that a part of the mineral requirement of *Orobanche* is met by the host while other minerals are also obtained by direct absorption from the soil through its own root system. *Orobanche* species have a rudimentary cap without a root hair zone that makes them unable to take up water and mineral compounds by themselves from the soil (Delavault 2015). Thus in *Orobanche* all nutrients needed for their development must be derived from the host phloem. Because of their low transpiration rate, Hibberd et al. (1999) consider that it is almost impossible for *Orobanche* to obtain nutrients by diverting the flow of xylem from the host, as is the case for hemiparasites. In that case *O. nowackiana* can be considered a phloem-feeding plant and is expected to take up minerals only from its host. As *Orobanche* always maintains higher P and K concentrations and lower N, Ca and Mg in the plant organs than the host, this suggests selective withdrawal of minerals from the host, with effects not only on the concentration of some elements in the host but also in the plant biomass. The greater quantity of K in the parasite might be related to the fact that K is transferred from host to parasite together with organic metabolites, as Coic et al. (1965) assumed. It was also shown that Ni in *A. murale* is strongly redistributed within the plant by phloem flow (Estrade et al. 2015).

Ni uptake by parasite and *A. murale*

Nickel hyperaccumulation did not prevent *A. murale* from being attacked by *O. nowackiana*. *Orobanche nowackiana* appears to be Ni tolerant, since these plants were able to take up a high concentration of Ni from the phloem of *A. murale*. Reeves (1992) suggested that unadapted plants growing on serpentine soil contain < 100 mg kg^{-1} Ni in their tissues. The mean Ni concentration reported in our study for *O. nowackiana* parasitizing *A. murale* is well over that value (i.e. 299 mg kg^{-1} in leaves and 601 mg kg^{-1} in tubercles and roots). The concentration of Ni found here in *O. nowackiana* tissues is high, but lower than that reported by Reeves (1992), who stated that the synonymous *O. rechingeri* that parasitized *Alyssum lesbiacum* could accumulate more than 600 mg kg^{-1} Ni, and lower than that found by Boyd et al. (1999) in *Cuscuta californica* parasitizing *Streptanthus polygaloides* (800 mg kg^{-1} Ni). Moreover, in Greece, this species often parasitises Ni-hyperaccumulator species that also have extremely high Ni concentrations in their tissues (Bani et al. 2009, 2010). The fact that *O. nowackiana* attacks metal hyperaccumulator plants suggests sufficient mechanisms of tolerance of the parasite. It is evident from the data that the *Orobanche* infection decreases the Ni concentration in leaves and whole plants of infected *A. murale*. Consequently the Ni phytoextraction yield is reduced eightfold compared to its level in a healthy plant. This is for two reasons: the parasite accumulates nickel, reducing the Ni concentration in the host, but also greatly reduces the weight of the leaves, the organs where *A. murale* accumulates most nickel (Bani et al. 2010, 2015a, b).

Conclusions

It is evident from the data that *O. nowackiana* infection of *A. murale* brings a marked decrease in host growth, reducing the dry weight, the length of the main shoot, the length of the root, and the number of branches. The infection also decreases mineral concentrations in the host, especially in the leaves. The P and K concentrations diminish in the infected host, a fact related to the higher translocation to the parasite. *Orobanche* is consistently found with higher P and K concentrations in its tissues than the host, indicating a selective transfer of these two elements and suggesting a metabolic role in the parasite, while the N, Ca, Mg, Ni, Zn, Co, Mn and Al concentrations are lower than in the host. The best candidate for agromining in Albanian ultramafic soils, *A. murale*, is not free from attack by *O. nowackiana*. This attack affects the levels of Ni in *A. murale* and strongly decreases its biomass production and Ni phytoextraction yield. For successful agromining we have to ensure that crops of *A. murale* are protected against parasitic plants. However, this parasitic infection seems to be limited to higher elevations in Albania whereas phytomining is mostly applicable on lowland soils.

Acknowledgements We would like to acknowledge the technical team of "Laboratoire Sols et Environnement", Nancy, France. The authors also wish to thank Prof. Dr. Alfred Mullaj for help with identifying the sampling area of *Orobanche nowackiana*.

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