

Mineral element composition in *Antirrhinum* subsection *Streptosepalum* (Plantaginaceae) in Western Europe (Iberian Peninsula)

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Abstract: García-Barriuso, M., Crespí, A.L., Nabais, C., Bernardos, S. & Amich, F. *Mineral element composition in Antirrhinum subsection Streptosepalum (Plantaginaceae) in Western Europe (Iberian Peninsula)*. *Lazaroa* 33: 19-26 (2012).

Antirrhinum rothmaleri (Pinto da Silva) Amich, Bernardos & García-Barriuso is a restricted and threatened endemic plant that inhabits the serpentines present in northern Portugal. *Antirrhinum braun-blanquetii* Rothm. and *A. meonanthum* Hoffmanns. & Link are also plants endemic to the western part of the Iberian Peninsula. Particulate trace concentrations of eight metals (Ba, Co, Cr, Cu, Ni, Pb, Sr and Zn) and ten major elements (Al, Ca, Fe, K, Mg, Mn, Na, P, Si and Ti) were determined in rock samples and dry leaf matter from these three species, belonging to the section *Streptosepalum* Rothm. of the genus *Antirrhinum* L. (Plantaginaceae). The habitat and ecology of *A. rothmaleri* are clearly reflected in its trace metal accumulation. These plants appear to be perfectly adapted to soils developed from different types of ophiolitic bedrock, whose chemical and physical properties have long been known to produce an environment hostile to most plant life. The clear morphological differences between *A. rothmaleri* and *A. braun-blanquetii* and *A. meonanthum*, probably reflect phenomena of genetic divergence and reproductive isolation rather than the nutritional imbalances and toxicity of the ultrabasic soil and rock where *A. rothmaleri* grows.

Keywords: *Antirrhinum*, Iberian Peninsula, major element, subsection *Streptosepalum*, trace element.

Resumen: García-Barriuso, M., Crespí, A.L., Nabais, C., Bernardos, S. & Amich, F. *Composición en elementos minerales en Antirrhinum subsección Streptosepalum (Plantaginaceae) en Europa Occidental (Península Ibérica)*. *Lazaroa* 33: 19-26 (2012).

Antirrhinum rothmaleri (Pinto da Silva) Amich, Bernardos & García-Barriuso es una planta endémica amenazada, y de distribución restringida a las serpentinas presentes en el norte de Portugal. *Antirrhinum braun-blanquetii* Rothm. y *A. meonanthum* Hoffmanns. & Link son asimismo taxa endémicos que viven en el occidente de la Península Ibérica. Las concentraciones de 8 metales traza (Ba, Co, Cr, Cu, Ni, Pb, Sr y Zn) y de 10 elementos mayores (Al, Ca, Fe, K, Mg, Mn, Na, P, Si y Ti) han sido determinados en muestras de rocas y en materia seca de las hojas de estas tres especies, pertenecientes a la sección *Streptosepalum* Rothm. del género *Antirrhinum* L. (Plantaginaceae). El habitat y la ecología de *A. rothmaleri* quedan claramente reflejados por la acumulación de metales traza. Dicho taxon parece estar perfectamente adaptado a los suelos y habitat que se desarrollan a partir de diferentes tipos de rocas ofiolíticas, cuyas propiedades físicas y químicas se conocen bien, porque producen un entorno hostil para la vida y el desarrollo de la mayor parte de los vegetales. Las claras diferencias morfológicas existentes entre *A. rothmaleri*, *A. braun-blanquetii* y *A. meonanthum*, son probablemente debidas a fenómenos de divergencia genética y consiguiente aislamiento reproductivo, producidos por los desequilibrios nutricionales y a la toxicidad de los suelos y rocas ofiolíticas sobre los que vive *A. rothmaleri*.

Palabras clave: *Antirrhinum*, Península Ibérica, elementos mayores, subsección *Streptosepalum*, elementos traza.

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INTRODUCTION

The serpentinization of ultramafic rock is a common process in which the original rock changes its mineralogical composition owing to hydration of the original phases (O'HANLEY, 1996). The chemical composition of serpentinites depends on the nature of the original rock and on the origin of the fluid phase (PEREIRA & al., 2003), and this origin will also be reflected in the newly formed serpentinite group minerals. The serpenticolous soils originated from different types of ultramafic rocks occur throughout the world and quite often in the form of ecological and/or pedological islands (LEFÈBVRE & VERNET, 1990). Due to different anomalies in their physical and chemical properties, these soils represent an environment that is very hostile for plant life (RAJAKARUNA & BOYD, 2009). The slow erosion of the rocks, the intense heating of such rocks under conditions of extreme insolation, the rapid loss of water and strong pedological stress all contribute to making serpentines a highly xeric and very hostile substrate for vegetation, such that these materials represent an important route for the evolution of certain plants; i.e., those known as "serpentinophytes" (RODRÍGUEZ-OUBIÑA & ORTIZ, 1995; IZCO & FERNÁNDEZ, 2001; LAZARUS & al., 2011).

Our work has focused on two important ultramafic massifs in the northwestern Iberian Peninsula: the Vinhais-Bragança and Morais Massifs. These ultrabasic areas of the north of Portugal occupy approximately 80 Km² (33 km² in the municipalities of Vinhais and Bragança, and 47 km² in the municipalities of Macedo de Cavaleiros and Mogadouro), and are situated between N 41° 25' and 41° 54', with an altitude between 450 and 1950 m.a.s.l. (PINTO DA SILVA, 1970).

The genus *Antirrhinum* consists of 24 perennial species that are native to the western Mediterranean (SUTTON, 1988; GUÊMES, 2009). Most occur as narrow endemics on the Iberian Peninsula (GUÊMES, 2009), and several are subject to different forms of threat (TORRES & al., 2003; BERNARDOS & al., 2006; GARCÍA-BARRIUSO & al., 2011).

Speciation within the genus probably occurred first on the Iberian Peninsula as a result of drought during the Cenozoic period and the climatic changes following the Last Glacial Maximum (LGM, ≈ 20.000 years BP), with a climate in the Mediterranean basin that was first cooler (between 15 and 5 kyr BP) and wetter during the Nabtian Pluvial (between 10 and 6 kyr BP, REEDER & al., 2002; DUCASSOU & al., 2009), thereafter becoming a warmer and drier climate at about 6000 years ago (REEDER & al., 2002; DUCASSOU & al., 2009). These climate changes created some fragmented distributional areas (ROTHMALER, 1956; VARGAS & al., 2004).

Antirrhinum section *Antirrhinum* subsection *Streptosepalum* (ROTHMALER, 1956) includes *A. braun-blanquetii* Rothm., *A. meoanthum* Hoffmanns. & Link, and *A. rothmaleri* (Pinto da Silva) Amich, Bernardos & García-Barruso (GARCÍA-BARRIUSO & al., 2012). The three species are endemic to the north and northwest of the Iberian Peninsula (GUÊMES, 2009; GARCÍA-BARRIUSO & al., 2012). *Antirrhinum rothmaleri* is a strict serpentinophyte of the Morais and Vinhais-Bragança massifs in the N of Portugal. *Antirrhinum braun-blanquetii* lives on limestone substrates, while *A. meoanthum* preferentially grows on siliceous materials (slates, schists and granites).

In this study, the accumulation of major and trace element composition (Ni, Ca, Mg, Fe, K) and their distribution in several plant species of *Antirrhinum* sect. *Streptosepalum* in the Iberian Peninsula was analysed. It is proposed that plant metal accumulation will be predictable from the soil bioavailable metal fraction.

The aims of the present work were: (1) to characterize the populations of *Antirrhinum rothmaleri* growing in areas of serpentinized rocks in NE Portugal and to compare them with Iberian populations of *Antirrhinum braun-blanquetii* and *A. meoanthum* via analyses of dry leaf matter and major and trace element contents, and (2) to characterize the pH of the soils and the composition of the rocks where populations of *Antirrhinum rothmaleri* are found, since this species behaves as a rupicolous or subrupicolous species.

MATERIAL AND METHODS

SAMPLING FOR ROCK AND SOIL ANALYSES

Rock samples were taken from the sites of all the populations of *A. rothmaleri* encountered (in Alimonde, Chacim-Balsemão and Gralhós). Major and trace elements were determined using acid treatment (HNO₃ plus HF) under pressure in an Ethos Sel microwave digester from Milestone. The diluted solution was buffered with boric acid. Determination was performed with an Ultima 2 Jobin Yvon optical emission plasma spectrometer at the Chemical Analysis Laboratory of the University of Salamanca.

Loss-on-Ignition (LOI) is a test used to measure the amount of moisture that a sample loses when it is ignited at certain temperatures (PEREIRA & al., 2008). For this work, the LOI was calculated by measuring a few grams of powdered rock in a crucible, heating it to 950 °C in a

muffle furnace, and allowing the water and other volatile elements to escape. After cooling in a controlled atmosphere, mass was re-determined and the difference was considered to be the LOI value.

Soil pH was determined in a soil/water (1:2.5) suspension with a pH-meter.

TAXONOMIC SAMPLING FOR ANALYSES OF MAJOR AND TRACE ELEMENTS IN DRY LEAF MATTER

Depending on the size the populations, between twenty one and thirty four plant samples (young green leaves) of each species were collected from natural environments of ultramafic areas in northern Portugal (*A. rothmaleri*), the province of León (*A. braun-blanquetii*) and the province of Salamanca (*A. meonanthum*) for analyses of major and trace elements (see Table 1, Fig. 1). Samples were air-dried and ground. About 0.1g of homogenized material was wet-

Table 1

List of populations and number of plants (N) of *Antirrhinum* representatives used for analyses of major and trace elements in leaves (EL, *), and voucher number in SALA. Abbreviations: PO, Portugal; SPA, Spain. Collectors: FA, F. Amich; SB, S. Bernardos; MGB, M. García-Barriuso.

Taxon /Number and code of populations /Origin and collection data	N	EL	Voucher
<i>Antirrhinum braun-blanquetii</i>			
ABB1. SPA, León, Barrios de Luna, 1125 m, N 42° 54' 21.1", W 5° 53' 18.3", 29.06.2007, FA & SB	23	*	SALA 135487
ABB2. SPA, León, Busdongo de Arbás, 1205 m, 30TTN8062 (N 42° 59' 3.9", W 5° 41' 34.4"), 15.07.2008, FA & SB	21	*	—
Subtotal	44		
<i>Antirrhinum meonanthum</i>			
AME1. SPA, Salamanca, San Esteban de la Sierra, 750 m, 30TTK5388 (N 40° 30' 13.03", W 5° 54' 25.78"), 01.07.2008, FA & SB	33	*	SALA 135491
Subtotal	33		
<i>Antirrhinum rothmaleri</i>			
ARO1. PO, Trás-os-Montes, Macedo de Cavaleiros, Gralhós, near Nuestra Señora de La Salette, 625 m, 29TPF8799 (N 41° 31' 28.0", W 6° 44' 52.0"), 16.07.2007, FA, SB & MGB	34	*	SALA 135485
ARO2. PO, Trás-os-Montes, Macedo de Cavaleiros, Chacim, Monastery de Balsemão, 525 m, 29TPF7993 (N 41° 28' 30.1", W 6° 51' 21."), 26.07.2008, FA & SB	21	*	SALA 135484
ARO3. PO, Trás-os-Montes, Bragança, Alimonde, 730 m, 29TPG7429 (N 41° 47' 37.8", W 6° 53' 3.58"), 16.07.2007, FA, SB & MGB	31	*	SALA 135483
ARO4. PO, Trás-os-Montes, Bragança, Alimonde, road to Vila Boa, 670 m, 29TPG7429 (N 41° 47' 54.7", W 6° 54' 12.9"), 03.07.2008, FA & SB	--		SALA 135486
Subtotal	86		
TOTAL	163		



Figure 1. – Distribution of the sampled populations of *Antirrhinum* section *Antirrhinum* subsection *Streptosepalum* in the Iberian Peninsula.

ashed with 2 ml of concentrated HNO_3 in a Teflon pressure vessel at 150°C for 10 h. The digest was finally made up to 10 ml with ultra-pure water. The solutions of rock samples were analyzed for their element composition (Al, Ca, Fe, K, Mg, Mn, Na, P, Si and Ti) using flame atomic absorption spectrophotometry (PERKIN-ELMER 2380; ASLIN, 1976; FLETCHER, 1981; KABATA-PENDIAS, 2001) at the University of Coimbra. The same rocks were analyzed for trace elements (Ba, Co, Cr, Cu, Ni, Pb, Sr and Zn) by ICP-MS at the Chemical Analysis Laboratory of the University of Salamanca. The solutions of plant samples were also analyzed for Ca, Fe, K, Mg and Ni by ICP-MS at the Chemical Analysis Laboratory of the University of Salamanca.

We performed an ANOVA test to analyse the differences between the three species of *Antirrhinum*, taking the major and trace element concentrations in leaf dry matter as explanatory variables (Ca, Fe, K, Mg, Ni), by means of SPSS (ANON., 2009).

A Garmin e-map GPS was used to locate the populations geographically using 1×1 km UTM and geographical coordinates. A representative voucher specimen from each population was collected and deposited at SALA.

RESULTS

ROCK AND SOIL ANALYSES

The serpentized rocks of NE Portugal are massive, dense, and have a homogeneous structure. They often contain chromite crystals (VASCONCELOS FERREIRA, 1965). Tables 2 and 3 show the chemical composition in terms of the major and trace element concentrations of the ultramafic rocks where *A. rothmaleri* was found, together with the results reported by MENEZES DE SEQUEIRA (1969) for comparative purposes.

The degree of serpentization is marked by the loss-on-ignition (LOI) parameter, in view of the high H_2O content in the serpentine structure ($\approx 13\% \text{H}_2\text{O}$) (D'ANTONIO & KRISTENSEN, 2004) (Table 2). The samples consisted of one serpentine (Alimonde), and two serpentized peridotites (Chacim-Balsemão and Gralhós).

The general characteristics of the serpentinites, including those of Portugal, are: (1) a high level of heavy metals, especially nickel; (2) high levels of Fe_2O_3 and, in particular, of MgO; and (3) low levels of calcium, potassium, and phosphorous (Tables 2 and 3).

The pH value in water showed little variation, varying between 7.33 and 7.40 (Table 4).

Table 2
Major element composition (%) of three rock samples in the studied area. (b.d.l. = below detection limit)

Samples	LOI	SiO ₂	TiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	MnO	CaO	Na ₂ O	K ₂ O	P ₂ O ₅
Menezes de Sequeira (1969)	--	27-50	Traces-0.6	0.05-7.6	6-13	14-39	0.06-0.20	0.05-25	0.25-1.17	0.02-0.17	--
Alimonde (ARO3)	12.9	39.22 ± 2.88	0.01 ± 0.01	0.85 ± 0.11	7.61 ± 0.53	38.84 ± 2.98	0.09 ± 0.02	0.23 ± 0.04	0.05 ± 0.02	0.01 ± 0.00	0.01 ± 0.01
Gralhós (ARO1)	11.98	35.96 ± 2.76	0.12 ± 0.21	1.90 ± 0.23	10.83 ± 0.63	38.06 ± 2.96	0.10 ± 0.03	0.28 ± 0.04	0.01 ± 0.00	b.d.l.	0.03 ± 0.01
Chacim-Balsemao (ARO2)	11.77	39.02 ± 2.77	0.05 ± 0.02	1.41 ± 0.18	8.96 ± 0.59	38.16 ± 2.95	0.11 ± 0.02	0.84 ± 0.09	0.04 ± 0.01	b.d.l.	0.01 ± 0.00

Table 3
Composition in terms of trace-element concentration (ppm) of three rock samples in the study area. (b.d.l. = below detection limit)

Samples	Ba	Co	Cr	Cu	Ni	Pb	Sr	Zn
Alimonde (ARO3)	11 ± 1.1	66 ± 7.01	778 ± 18.9	b.d.l.	2215 ± 140.4	b.d.l.	70 ± 7.05	21 ± 2.1
Gralhós (ARO1)	b.d.l.	63 ± 6.87	2264 ± 141.1	b.d.l.	1835 ± 135.6	b.d.l.	63 ± 7.01	22 ± 2.1
Chacim-Balsemao (ARO2)	b.d.l.	61 ± 6.99	2536 ± 143.2	b.d.l.	1961 ± 136.8	b.d.l.	64 ± 6.88	32 ± 2.7

Table 4
Soil pH values for four populations of *Antirrhinum rothmaleri*

Population	pH
ARO1	7.33
ARO2	7.38
ARO3	7.40
ARO4	7.39

ANALYSES OF MAJOR AND TRACE ELEMENTS IN DRY LEAF MATTER

The concentrations of Ni and Mg were much higher in *A. rothmaleri* than in *A. braun-blauquetii* and *A. meonanthum* (Table 5), while the Ca concentration was much lower in *A. rothmaleri*. The Mg²⁺/Ca²⁺ ratio was < 0.5 in *A. braun-blauquetii* and *A. meonanthum* but > 1 in *A. rothmaleri*. Two of the three *A. rothmaleri* populations studied had clearly lower Fe and K concentrations than those seen for *A. braun-blauquetii* and *A. meonanthum*, while the remaining population (ARO 2) showed concentrations similar to those of the other taxa. Although all three *A. rothmaleri* populations grew on soils very rich in Fe (see

Table 5), the ARO1 and ARO3 populations showed low concentrations of this element (8.6 and 7.5 % respectively), as they did for K (0.985 and 0.441 % respectively).

In light of the results of the analysis of variance, it may be concluded that the two explanatory variables were significant: Ni (F= 11.78, p= 0.038) y Ca (F= 19.12, and p= 0.020), but not for the other variables analysed: Mg (F= 1.041, p= 0.454), Fe (F= 3.530, p= 0.163), K (F= 0.783, p= 0.533).

The analyses revealed that even though the chemical composition of the serpentinized ultramafic rocks was similar at all three *A. rothmaleri* locations (Tables 2 and 3).

Despite some small differences, the habitat and ecology of *A. rothmaleri* are clearly reflected in its trace metal accumulation (Table 5). These plants appear to be perfectly adapted to soils developed from different types of ophiolitic bedrock, whose chemical and physical properties have long been known to produce an environment hostile to most plant life (SELVI, 2007). Unlike other serpentinophytes (i.e. *Alyssum lusitanicum* subsp. *lusitanicum*), *A. rothmaleri* does not thrive in soils disturbed by ploughing, mining, etc. (FUENTE & al., 2007).

Table 5
Major and trace element composition (%) in leaf dry matter of *Antirrhinum* subsection *Streptosepalum*

Populations	Ni	Ca	Mg	Fe	K	Mg ²⁺ /Ca ²⁺
<i>A. braun-blanquetii</i> (ABB1)	0.656 ± 0.09	2.18 ± 0.30	0.95 ± 0.11	0.44	42.8 ± 4.1	1.667 ± 0.28
<i>A. braun-blanquetii</i> (ABB2)	0.750 ± 0.08	1.85 ± 0.29	0.31 ± 0.06	0.17	21.7 ± 2.1	1.542 ± 0.25
<i>A. meonanthum</i> (AME1)	0.358 ± 0.05	1.93 ± 0.30	0.76 ± 0.09	0.39	11.5 ± 1.2	1.814 ± 0.28
<i>A. rothmaleri</i> (ARO1)	2.860 ± 0.39	0.84 ± 0.11	1.01 ± 0.12	1.20	8.6 ± 0.9	0.985 ± 0.26
<i>A. rothmaleri</i> (ARO2)	1.770 ± 0.27	1.08 ± 0.27	0.92 ± 0.11	0.85	42.1 ± 3.9	1.855 ± 0.29
<i>A. rothmaleri</i> (ARO3)	2.500 ± 0.31	0.64 ± 0.06	1.93 ± 0.29	3.09	7.5 ± 0.9	0.441 ± 0.06

DISCUSSION

The adaptation of plant species to different soil types has been recognized as a consequence of the strong natural selection imposed by ecological discontinuities (WALLACE, 1858). The ecology of the three plant species analyzed here differs considerably: *A. braun-blanquetii* grows in the northern part of the Iberian Peninsula, on alkaline substrates in communities of the alliance *Saxifragion trifurcato-canaliculatae* (RIVAS-MARTÍNEZ & al., 2002); *A. meonanthum* is distributed over the NW quadrant of the Iberian Peninsula (slates, gneisses, granites) in phytocenoses corresponding to the alliance *Parietario-Galion muralis* (RIVAS-MARTÍNEZ & al., 2002); and *A. rothmaleri* lives on serpentinized ultramafic rocks in the northern of Portugal, in communities of the alliance *Armerion eriophyllae* (GARCÍA-BARRIUSO & al., 2012, see Table V).

Apart from their different ecologies, the clear morphological differences between *A. rothmaleri* and *A. braun-blanquetii* and *A. meonanthum* (GARCÍA-BARRIUSO & al., 2011) probably reflect phenomena of genetic divergence and reproductive isolation rather than the nutritional imbalances and toxicity of the ultrabasic soil where *A. rothmaleri* grows. A number of plant species endemic to metalliferous soils, such as *A. rothmaleri*, have been found to accumulate metals at extraordinarily high levels (> 1%), in contrast to normal concentrations in plants. This can be seen clearly in the values shown in Table 5.

Although examples of serpentinophytes have not been reported either for the family Plantagi-

naceae, in general, or in *Antirrhinum* in particular, it could be speculated, as has been proposed for other taxa (such as *Silene dioica*, WESTERBERGH & SAURA, 1992), that *A. braun-blanquetii* could have colonized serpentinicolous terrains several times independently, and could probably have been the ancestor of *A. rothmaleri*, with which it is closely linked (GARCÍA-BARRIUSO & al., 2011).

It has been reported that the evolutionary transition of the serpentinicolous endemism may sometimes be exclusively unidirectional (ANACKER & al., 2011). If such speciation requires a relevant array of physiological and ecological adaptations and strategies to become adapted to serpenticolous soils this could lead to exclusion from non-serpentine zones and that this adaptation would be irreversible. The results obtained here suggest that in *A. rothmaleri* such adaptations and strategies did indeed occur and that it is now a restricted serpentinicolous endemism.

As in the case of other serpentinophytes, such as *Notholaena marantae*, and *Onosma* sp. pl. (PINTO DA SILVA, 1970; PICHI SERMOLLI, 1948; CECCHI & al., 2011), *Antirrhinum rothmaleri* could represent a pre-glacial relict, with its origin towards the beginning of the Pleistocene, about 2.7 million years ago. As mentioned above, serpentines for an extremely xeric substrate, and those located in the Vinhais-Bragança-Morais massifs could have served as a refuge for different species inhabiting the northern part of the Iberian Peninsula during cold periods of the Pleistocene. Similar examples have been indicated for some taxa in other areas of Europe (STE-

VANOVIĆ & al., 2003; SELVI, 2007). Under these conditions, the populations could have been isolated and a strong selective pressure exerted by serpenticolous soils could have led to adaptive speciation.

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