COMPARATIVE CHEMICAL CHARACTERISATION OF SOILS AT *CYPRIPEDIUM CALCEOLUS* SITES IN LATVIA

Dace Kļaviņa^{1,#} and Anita Osvalde²

¹ National Botanic Garden of Latvia, Miera iela 1, Salapils, LV-2169, LATVIA;
dace.klavina@nbd.gov.lv

² Institute of Biology of the University of Latvia, Miera iela 3, Salapils, LV-2169, LATVIA;
augi@lu.lv

Corresponding author

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*The main aim of the study was to evaluate the soil chemical characteristics of *Cypripedium calceolus* sites in Latvia for understanding of the species ecology, and its conservation problems and possibilities. Soil for 27 *C. calceolus* sites in Latvia was analysed during 2007–2015. The concentration of plant available essential nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B), soil pH, electrical conductivity and concentration of organic matter were determined. Nutrient concentration of *C. calceolus* leaf samples from six sites were determined. The results demonstrated high heterogeneity in soil chemical composition with high variation for Ca, Mg, and Mn. The results suggest some relationship between soil organic matter content and size of the orchid population. Soil pH and Ca concentration did not affect the size and vitality of *C. calceolus* populations in Latvia. There was no significant correlation between nutrient concentration in soil and *C. calceolus* leaves suggesting strong impact of other soil-related factors in determining the availability of nutrients. In general, the levels of N, P, K, Ca, Mg, Fe, Mn, and Zn concentrations in *C. calceolus* leaves were adequate or slightly decreased for successful orchid growth. The study showed deficiency of S, Cu, and B in the vast majority of leaf samples. *C. calceolus* sites in Latvia occur mainly on organic soils or mineral soils with high content of organic matter. There was a weak positive correlation ($r = 0.21$) observed only between total number of ramets of *C. calceolus* in site and Ca concentration in the soil, suggesting that soil properties are not a main factor affecting species abundance in the site.*

Key words: *Cypripedium calceolus*, mineral nutrients, pH, organic matter, habitats.

INTRODUCTION

Cypripedium calceolus is a long-lived rhizomatous species with specific mycotrophic adaptation related to a functional type of rare plants with a narrow ecological niche (Mihalchuk, 2011). The initial development of young *C. calceolus* plants is very slow and the first flowering is delayed for at least 10 years. Adult plants survival is high. Species immigration and colonisation success is low and it subjected to strong interspecific competition (Devillers-Treschuren, 1999). *C. calceolus* inhabits temperate and boreal zones of Europe, Siberia and Central and East Asia (Cribb, 1997) and in most of its range it is a rare species both in number of sites and number of individuals (Devillers-Treschuren, 1999). It is one of the 14 vascular plant species specific for natural forest habitats in Latvia (Auniņš, 2013).

The species is protected in the majority of European countries (in Latvia since 1977), and included in the protected

plant lists of Bern and Washington conventions as well as in Annex II of the European Council Habitats Directive 92/43/EEC. It is included in Regulations No. 396 of the Cabinet of Minister of Latvia on protected species and in the second (vulnerable) threat category in the Red Data Book of Latvia (Andrušaitis, 2003). Strict species protection in many countries was enacted after its decline throughout almost its entire distribution range (Devillers-Treschuren, 1999).

Formerly it was known from 62 localities in Latvia (Cepurite, 2005). In chorological material of the Latvian flora (Ābele *et al.*, 1978), 42 sites for this species were listed, in Red Data Book — 53 (Andrušaitis, 2003), but *C. calceolus* is now present only in 27 sites, which were confirmed in the present study. The reduction of suitable habitats for this narrowly specialised species is a major threat (Devillers-Treschuren, 1999). The main factors limiting *C. calceolus* establishment and survival in particular microhabitats are

not known. Preliminary results of recent research on *C. calceolus* in Latvia were described by Kļaviņa *et al.* (2014), but no data referring all sites of this species in Latvia have been presented previously. One of the important abiotic factors for establishment and growth of a species is suitable soil conditions. Only few publications provide soil characteristics for *C. calceolus* sites (Arditti, 1992; Kull, 1999; 2003; Reshetjuk, 2011). Therefore, more detailed studies on the role of soil chemical composition, especially on significance of Ca and pH on distribution of this calcicole, are necessary. To gain better understanding of the ecology of the species and conservation issues and possibilities we started with analysis and characterisation of soil chemical composition of *C. calceolus* sites in Latvia. Soil studies are also important for comparing growth conditions in inhabited sites and in prospective sites (Ramsay, 1998) for re-introduction.

The aim of the study was to evaluate the soil chemical characteristics of *C. calceolus* sites in Latvia for better understanding of the ecology of the species and conservation issues and possibilities.

MATERIAL AND METHODS

Twenty seven sites in different types of habitats with *C. calceolus*, located throughout Latvia, were investigated during 2007–2015 (Fig. 1). Data on the species distribution were extracted from the data system OZOLS (Anonymous, 2012). A distribution map was prepared using a 10×10 km grid (Laiviņš and Krampis, 2004). The sites listed in Tables were arranged and numbered from west to east of Latvia, but in Figures the sites were marked with abbreviations of the site name.

The total number of ramets and generative ramets were recorded in each site (unpublished data). In the present paper

these data were used only partly for characterisation of the impact of soil organic matter and Ca content on the *C. calceolus* population in Latvia.

Sampling. *C. calceolus* soil and plant leaf samples were collected for laboratory analysis. The soil samples were taken from the root zone near *C. calceolus* plants to 20 cm depth. For each sample at least five sub-samples were collected and mixed into a single sample (0.5–1 L). During the study period, soil samples were taken at least two times at each location. A more detailed investigation was conducted in Ķemeri, Dunduri, Katleši, Lielie Kangari, Numerne, Silzemnieki, Slītere, and Sloka (the largest and/or nearest populations). Completely developed leaves from about 10–15 plants were taken for each leaf sample. Leaf samples from six sites were taken twice, in 2012 and 2013.

Laboratory analysis. Collected soil samples were air-dried and sieved through a 2-mm sieve. To determine available concentrations of 12 essential nutrients (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, and B) the soil samples were extracted with 1 M HCl solution (soil/extractant volume ratio 1:5), extraction time 1 h. Leaf samples of *C. calceolus* were dried at +60 °C temperature, ground, dry-ashed in concentrated HNO₃ vapour and re-dissolved in HCl solution (HCl — distilled water mixture 3:100) (Rinkis *et al.*, 1987).

The concentration of organic matter in soils was determined according to Tjurin (Rinkis *et al.*, 1987). Based on the concentration of organic matter, the soils were classified as mineral soils (0–10% organic matter), soils with high concentration of organic matter (11–60% organic matter), and organic soils (61–100% organic matter). In the Stikli site, organic matter in the soil was not detected.

The levels of Ca, Mg, Fe, Cu, Zn, and Mn were estimated by an atomic absorption spectrophotometer Perkin Elmer

Fig. 1. Location of sites of *Cypripedium calceolus* in Latvia.

AAAnalyst 700 (Page *et al.*, 1982; Anonymous, 2000), the levels of N, P, Mo, B by colorimetry, S by turbidimetry (spectrophotometer JENWAY 6300), and K with a flame photometer (Jenway PFP7) (Rinkis *et al.*, 1987).

Soil pH was measured in a 1 M KCl suspension and electrical conductivity (EC) using distilled water extract (Rinkis *et al.*, 1987).

The statistical analysis of the data was conducted using MS Excel 2013. Means and standard error were calculated. Heterogeneity of nutrient concentrations was characterised by the coefficient of variation (CV). One-way analysis of variance (ANOVA) was used to compare all soil parameters within the site, and among sites and soil groups (organic and mineral soils) (Fowler *et al.*, 1998). Pearson correlation coefficients were calculated to evaluate correlation between soil parameter and abundance of generative ramets of *C. calceolus*. To characterise *C. calceolus* abundance, three parameters were tested: total number of ramets, number of generative ramets and relative number of generative ramets (proportion of generative ramets among all ramets observed).

RESULTS

In general, a vast range of nutrient concentrations were found in different *C. calceolus* sites in Latvia (Tables 1, 2). Although soil samples were collected at different times (2007–2015), ANOVA analysis showed that the values of parameters within sites was homogenous. One-way analysis of variance (ANOVA) showed significant difference ($p < 0.05$) in concentration of all analysed chemical elements in soils between sites, excepting for potassium.

Among macronutrients, the highest coefficient of variation was found for Mn (77%) and the lowest for N (37%). Among micronutrients, the highest variability of concentration was found for Mo (197%) and Mn (77%), and lowest for Cu (38%). In general, a low range was characteristic for electrical conductivity (EC), to a large extent reflecting low variability of concentrations of mobile nutrients (mainly N and K) in soils at *C. calceolus* sites.

The pH_{KCl} values in soils (Fig. 2) from *C. calceolus* sites ranged in a comparatively wide amplitude: from moderately acidic (pH_{KCl} 5.19) to slightly alkaline (pH_{KCl} 7.39).

Table 1

CONCENTRATION (MEAN $\pm$ SE, MG/L, 1M HCL EXTRACTION) OF MACRONUTRIENTS IN AIR-DRY SOILS FROM DIFFERENT *CYPRIPEDIUM CALCEOLUS* SITES IN LATVIA

No.	Locality	Abbreviations	N	P	K	Ca	Mg	S	Mg/K
1	Klāpi	Klā	75.0 $\pm$ 25.0	50.5 $\pm$ 1.5	62.0 $\pm$ 7.0	11150 $\pm$ 4850	223 $\pm$ 78	19.0 $\pm$ 10.0	3.6
2	Slītere	Slit	87.7 $\pm$ 16.5	48 $\pm$ 2.6	97.7 $\pm$ 16.2	11867 $\pm$ 573	1517 $\pm$ 99	34.0 $\pm$ 8.4	15.5
3	Stikli	Stik	52.0 $\pm$ 10.0	47.5 $\pm$ 1.5	84.0 $\pm$ 12.0	5700 $\pm$ 50	770 $\pm$ 50	19.0 $\pm$ 2.0	10.0
4	Graviņas	Grav	44.5 $\pm$ 9.5	14.5 $\pm$ 6.5	58.0 $\pm$ 8.0	75750 $\pm$ 250	715 $\pm$ 65	25.5 $\pm$ 9.5	12.3
5	Svēte	Svēt	37.0 $\pm$ 5.5	109 $\pm$ 8.8	185.0 $\pm$ 15.0	4950 $\pm$ 190	800 $\pm$ 45	12.0 $\pm$ 1.5	6.5
6	Dubļukrogs	Dub	152.5 $\pm$ 22.5	93.0 $\pm$ 0.0	78.0 $\pm$ 2.0	7725 $\pm$ 25	508 $\pm$ 83	34.0 $\pm$ 1.0	6.5
7	Dunduri	Dun	49.8 $\pm$ 4.6	17.4 $\pm$ 7.8	48.6 $\pm$ 5.1	8218 $\pm$ 554	133 $\pm$ 25	93.2 $\pm$ 11.2	2.7
8	Ķemerī	Ķem	50.3 $\pm$ 6.9	26.0 $\pm$ 5.5	98.5 $\pm$ 20.3	40054 $\pm$ 3283	26705 $\pm$ 2207	33.6 $\pm$ 4.6	271.1
9	Sloka	Slo	55.4 $\pm$ 9.7	50.9 $\pm$ 2.7	84.6 $\pm$ 4.8	6432 $\pm$ 374	589 $\pm$ 229	56.6 $\pm$ 11.9	7.0
10	Tūja	Tūja	104.0 $\pm$ 15.5	113.0 $\pm$ 15.0	64.0 $\pm$ 5.8	8100 $\pm$ 525	570 $\pm$ 28	31.0 $\pm$ 5.2	8.9
11	Vitrupe	Vitr	96.0 $\pm$ 10.0	87.0 $\pm$ 6.5	59.0 $\pm$ 7.0	7200 $\pm$ 380	585 $\pm$ 60	29.0 $\pm$ 1.8	9.9
12	Lielie Kangari	LKan	64.7 $\pm$ 11.3	128.3 $\pm$ 21.7	49.0 $\pm$ 8.0	11750 $\pm$ 144	3990 $\pm$ 311	18.3 $\pm$ 4.1	81.4
13	Eglupe	Egl	48.0 $\pm$ 10.9	26.7 $\pm$ 7.1	109.0 $\pm$ 12.3	8125 $\pm$ 1500	575 $\pm$ 82	10.0 $\pm$ 0.9	5.3
14	Silzemnieki	Silz	76.6 $\pm$ 8.1	126.6 $\pm$ 6.7	69.2 $\pm$ 6.8	6471 $\pm$ 311	636 $\pm$ 31	20.2 $\pm$ 2.4	9.2
15	Ziemeļu purvi	Ziem	98.0 $\pm$ 8.5	61.0 $\pm$ 10.0	37.0 $\pm$ 4.0	7000 $\pm$ 380	690 $\pm$ 25	24.0 $\pm$ 2.5	18.7
16	Pērļupe	Pērļ	55.0 $\pm$ 5.8	42.0 $\pm$ 5.5	51.0 $\pm$ 7.5	79500 $\pm$ 650	635 $\pm$ 58	238.0 $\pm$ 25.0	12.5
17	Kazu grava	Kaz	80.0 $\pm$ 5.6	68.0 $\pm$ 7.5	195.0 $\pm$ 28.0	14850 $\pm$ 550	8950 $\pm$ 350	17.0 $\pm$ 1.5	45.9
18	Slapjo salu purvs	Slsp	88.0 $\pm$ 10.0	28.0 $\pm$ 3.5	90.0 $\pm$ 9.5	4320 $\pm$ 225	1080 $\pm$ 35	29.0 $\pm$ 2.5	12.0
19	Islīna	Isl	102.0 $\pm$ 9.4	50.0 $\pm$ 4.8	73.0 $\pm$ 5.5	5200 $\pm$ 450	790 $\pm$ 65	9.0 $\pm$ 0.5	10.8
20	Murmastiene	Mur	51.0 $\pm$ 7.2	97.0 $\pm$ 10.0	145.0 $\pm$ 11.8	4245 $\pm$ 328	565 $\pm$ 25	9.0 $\pm$ 1.0	3.9
21	Druviena	Druv	32.0 $\pm$ 3.5	94.0 $\pm$ 8.5	195.0 $\pm$ 19.5	16000 $\pm$ 625	4100 $\pm$ 115	13.0 $\pm$ 3.2	21.0
22	Zāgadi	Zāg	70.5 $\pm$ 1.5	74.5 $\pm$ 18.5	80.0 $\pm$ 1.0	7800 $\pm$ 350	840 $\pm$ 80	17.0 $\pm$ 4.0	10.5
23	Zeltiņi	Zelt	99.0 $\pm$ 11.0	70.0 $\pm$ 11.0	62.5 $\pm$ 3.5	7650 $\pm$ 550	568 $\pm$ 3	31.5 $\pm$ 3.5	9.1
24	Gulbene	Gulb	80.0 $\pm$ 0.0	67.5 $\pm$ 5.5	74.5 $\pm$ 2.5	8475 $\pm$ 225	753 $\pm$ 13	16.0 $\pm$ 2.0	10.1
25	Beja	Beja	62.0 $\pm$ 4.0	46.5 $\pm$ 27.0	76.5 $\pm$ 29.0	12981 $\pm$ 5037	850 $\pm$ 200	18.0 $\pm$ 12.0	11.1
26	Katleši	Katl	56.8 $\pm$ 6.8	50.3 $\pm$ 5.7	54.3 $\pm$ 5.7	5716 $\pm$ 725	635 $\pm$ 77	12.8 $\pm$ 1.4	11.7
27	Numerne	Num	49.8 $\pm$ 3.0	31.0 $\pm$ 6.7	67.4 $\pm$ 3.2	14393 $\pm$ 1182	1223 $\pm$ 118	18.6 $\pm$ 3.1	18.1
CV*, %			37.0	51.4	51.7	137.1	234.7	136.6	

* Coefficient of variation

Table 2

MICRONUTRIENT CONCENTRATION (MEAN $\pm$ SE, MG/L, 1M HCL EXTRACTION) IN AIR-DRY SOILS AND ELECTRICAL CONDUCTIVITY (WATER EXTRACT) FROM DIFFERENT *CYPRIPEDIUM CALCEOLUS* SITES IN LATVIA

	Locality	Fe	Mn	Zn	Cu	Mo	B	EC mS/cm	Fe/Mn
1	Klāņi	340 $\pm$ 130	62 $\pm$ 8	6.8 $\pm$ 0.3	1.9 $\pm$ 0.5	0.04 $\pm$ 0.01	1.1 $\pm$ 0.2	0.56 $\pm$ 0.16	5.5
2	Slitere	1223 $\pm$ 278	130 $\pm$ 13	11.8 $\pm$ 2.0	1.0 $\pm$ 0.1	0.03 $\pm$ 0.00	1.8 $\pm$ 0.2	0.90 $\pm$ 0.12	9.4
3	Stikli	485 $\pm$ 135	1458 $\pm$ 85	9.0 $\pm$ 1.5	0.9 $\pm$ 0.1	0.03 $\pm$ 0.00	2. $\pm$ 0.1	0.56 $\pm$ 0.05	3.7
4	Graviņas	29 $\pm$ 17	108 $\pm$ 13	2.4 $\pm$ 0.7	0.8 $\pm$ 0.3	0.11 $\pm$ 0.09	0.9 $\pm$ 0.6	0.92 $\pm$ 0.03	0.3
5	Svēte	630 $\pm$ 55	340 $\pm$ 35	10.5 $\pm$ 0.9	1.8 $\pm$ 0.2	0.02 $\pm$ 0.00	0.9 $\pm$ 0.2	0.54 $\pm$ 0.04	1.9
6	Dubļukrogs	383 $\pm$ 23	283 $\pm$ 13	6.0 $\pm$ 0.0	1.1 $\pm$ 0.05	0.02 $\pm$ 0.00	1.5 $\pm$ 0.1	0.62 $\pm$ 0.10	1.4
7	Dunduri	112 $\pm$ 12	18 $\pm$ 7	3.9 $\pm$ 0.9	0.5 $\pm$ 0.1	0.03 $\pm$ 0.00	3.8 $\pm$ 0.7	1.38 $\pm$ 0.17	6.1
8	Ķēmeri	609 $\pm$ 113	229 $\pm$ 13	8.3 $\pm$ 1.3	1.4 $\pm$ 0.4	0.02 $\pm$ 0.00	0.4 $\pm$ 0.1	0.78 $\pm$ 0.11	2.7
9	Sloka	374 $\pm$ 43	22 $\pm$ 2	3.1 $\pm$ 0.3	1.7 $\pm$ 0.1	0.03 $\pm$ 0.01	1.5 $\pm$ 0.2	0.90 $\pm$ 0.08	17.3
10	Tūja	895 $\pm$ 95	355 $\pm$ 30	6.5 $\pm$ 0.4	1.2 $\pm$ 0.2	0.02 $\pm$ 0.00	2.1 $\pm$ 0.2	0.61 $\pm$ 0.03	2.5
11	Vitrupe	1680 $\pm$ 120	680 $\pm$ 58	8.5 $\pm$ 0.6	1.8 $\pm$ 0.1	0.07 $\pm$ 0.01	1.4 $\pm$ 0.2	0.53 $\pm$ 0.03	2.5
12	Lielie Kangari	837 $\pm$ 149	227 $\pm$ 27	15.2 $\pm$ 5.4	1.1 $\pm$ 0.2	0.03 $\pm$ 0.00	0.1 $\pm$ 0.0	0.52 $\pm$ 0.05	3.6
13	Egļupe	1343 $\pm$ 436	331 $\pm$ 112	3.2 $\pm$ 0.3	1.1 $\pm$ 0.2	0.03 $\pm$ 0.00	1.0 $\pm$ 0.2	0.58 $\pm$ 0.04	4.1
14	Silzemnieki	316 $\pm$ 42	192 $\pm$ 16	6.3 $\pm$ 0.8	1.4 $\pm$ 0.1	0.03 $\pm$ 0.00	1.3 $\pm$ 0.3	0.63 $\pm$ 0.07	1.7
15	Ziemeļu purvi	760 $\pm$ 100	230 $\pm$ 20	1.5 $\pm$ 0.1	1.5 $\pm$ 0.2	0.04 $\pm$ 0.00	1.0 $\pm$ 0.2	0.62 $\pm$ 0.04	3.3
16	Pērļupe	17 $\pm$ 5	60 $\pm$ 6	3.6 $\pm$ 0.2	0.9 $\pm$ 0.1	0.02 $\pm$ 0.00	1.8 $\pm$ 0.3	0.76 $\pm$ 0.05	0.3
17	Kazu grava	400 $\pm$ 45	430 $\pm$ 35	3.5 $\pm$ 0.4	1.6 $\pm$ 0.2	0.03 $\pm$ 0.00	0.7 $\pm$ 0.2	0.53 $\pm$ 0.02	0.9
18	Slapjo salu purvs	260 $\pm$ 30	170 $\pm$ 15	1.9 $\pm$ 0.3	0.8 $\pm$ 0.1	0.07 $\pm$ 0.01	1.1 $\pm$ 0.1	0.78 $\pm$ 0.06	1.5
19	Isliena	1605 $\pm$ 150	185 $\pm$ 18	3.5 $\pm$ 0.3	1.0 $\pm$ 0.2	0.02 $\pm$ 0.00	1.5 $\pm$ 0.3	0.66 $\pm$ 0.05	8.7
20	Murmastiene	1340 $\pm$ 150	105 $\pm$ 9	4.9 $\pm$ 0.5	3.0 $\pm$ 0.2	0.04 $\pm$ 0.00	0.8 $\pm$ 0.1	0.41 $\pm$ 0.02	12.8
21	Druviena	695 $\pm$ 80	65 $\pm$ 5.5	3.2 $\pm$ 0.4	1.5 $\pm$ 0.1	0.03 $\pm$ 0.00	0.1 $\pm$ 0.0	0.52 $\pm$ 0.05	10.7
22	Zāgadi	553 $\pm$ 243	283 $\pm$ 63	5.0 $\pm$ 0.0	1.6 $\pm$ 0.4	0.04 $\pm$ 0.00	1.9 $\pm$ 0.5	0.66 $\pm$ 0.02	2.0
23	Zeltiņi	1305 $\pm$ 220	332 $\pm$ 138	3.5 $\pm$ 0.5	1.5 $\pm$ 0.1	0.06 $\pm$ 0.00	1.3 $\pm$ 0.1	0.62 $\pm$ 0.04	3.9
24	Gulbene	1298 $\pm$ 138	653 $\pm$ 8	2.3 $\pm$ 0.8	1.6 $\pm$ 0.2	0.06 $\pm$ 0.03	2.0 $\pm$ 0.1	0.73 $\pm$ 0.07	2.0
25	Beja	525 $\pm$ 100	145 $\pm$ 85	12.3 $\pm$ 3.5	1.6 $\pm$ 0.4	0.07 $\pm$ 0.05	2.0 $\pm$ 0.7	0.67 $\pm$ 0.22	3.7
26	Katleši	1028 $\pm$ 130	62 $\pm$ 7	2.9 $\pm$ 0.9	2.0 $\pm$ 1.9	0.04 $\pm$ 0.001	0.8 $\pm$ 0.2	0.37 $\pm$ 0.01	16.5
27	Numerne	329 $\pm$ 89	112 $\pm$ 18	6.2 $\pm$ 0.8	0.8 $\pm$ 0.1	0.03 $\pm$ 0.00	1.3 $\pm$ 0.2	0.73 $\pm$ 0.05	2.9
	CV*, %	66.5	75.7	61.4	37.9	52.6	56.6	29.8	

* Coefficient of variation

Fig. 2. pH_{KCl} and Ca:Mg ratio in soils from *Cypripedium calceolus* sites in Latvia.

Fig. 3. Organic matter content in soils from *Cypripedium calceolus* sites in Latvia.

* the largest populations of *Cypripedium calceolus*: Ķemeri – 1400 ramets, Katleši – 1135 ramets, Dunduri – 511 ramets, Numerne – 304 ramets, Slitere – 200 ramets.

Among macronutrients, there were low mean concentrations of N at about half of the study sites. On the other hand, soils of sites Dubļukrogs, Tūja, and Zeltiņi were rich in nitrogen (N 100–153 mg/L), compared to the other study sites. High organic matter content was also characteristic for those sites (Fig. 3). A mean concentration of P higher than 120 mg/L was found only at two study sites (Silzemnieki and Lielie Kangari), while at about half of the sites the level of P did not exceed 50 mg/L. A high K concentration occurred at Svēte, Kazu grava, Murmastiene, and Druviena) and S only in few *C. calceolus* sites (Dunduri and Sloka) located mainly on mineral soils. An extremely high S concentration was observed at Pērļupe — 238 mg/L. Along with variable pH_{KCl} values, the concentrations of Ca and Mg varied by 19 and 200 times, respectively, when comparing minimum and maximum values.

Although high heterogeneity was found for all micronutrients, the highest differences were found for Fe and Mn, which were mainly due to specific soil characteristics in *C. calceolus* habitats (mineral or organic soil, degree of organic matter decomposition).

Electrical conductivity, which characterises the concentration of water soluble ions in soil extract, showed moderate availability of these nutrients in most sites (Table 2), except in soil from Dunduri (EC 1.38 mS/cm). The raised electrical conductivity at this site was due to high S content caused by gypsum deposits in this site (high Ca and S and low Mg content, Table 1).

Analysis of nutrient ratios, which is an important factor in plant mineral nutrition, showed that in soil samples from *C. calceolus* sites the concentration of Mg was about 3–271 times higher than the K concentrations (Table 1). The high Mg concentrations occurred in soils with soil reaction close to neutral. The Ca:Mg ratio in soils of the investigated sites varied from 1.5 (Ķemeri — dolomite) up to 125 (Pērļupe — limestone) (Fig. 2) indicating the presence of diverse parent soil in different sites.

Some extremely low Fe:Mn ratio (Table 2) were found for slope forests at Graviņas, Pērļupe, Kazu grava, where the concentration of Mn was higher than Fe.

The soils of the studied *C. calceolus* sites in Latvia mainly (14 sites) had high concentration of organic matter (11–56%). Seven sites were found on organic soils (organic matter content 66–85%) and five sites on typical mineral soils with organic content less than 10% (Fig. 3). Potassium concentration, electrical conductivity and organic content significantly differed among these three soil groups ($p < 0.05$). The results showed some relationship between soil organic matter content and size of orchid population. The largest *C. calceolus* populations in Latvia Ķemeri (1400 ramets) and Katleši (1135 ramets) occurred on soils with high concentration of organic matter (organic matter content 17%), and three other large populations: Dunduri (511 ramets), Slitere (200 ramets), Numerne (304 ramets) on organic soils (organic matter content 84–85%) (Figure 3).

A negative correlation ($r = -0.44$; $p < 0.05$) was observed between the relative number of generative ramets of *C. calceolus* and Ca concentration in the soil (Fig. 4) and a weak positive correlation ($r = 0.21$) — between total number of ramets of *C. calceolus* in a site and Ca concentration in the soil.

To get more detailed information about nutrient status of *C. calceolus*, concentration of 12 biogenous elements were determined also in orchid leaf samples from selected sites in 2010–2013 (Table 3). In general, heterogeneity of leaf nutrient concentrations was lower than for the concentrations in soil. Among macronutrients, the minimum and maximum concentration of K in leaves differed by 2.6 times; among micronutrients the largest difference between maximum and minimum concentrations was found for Cu (4.0 times) and Mn (2.4 times).

Some incoherence was found between nutrient concentrations in soils and leaves at *C. calceolus* sites (Tables 1–3). For example, K concentration in plants at the Katleši site where K content in soils was low (54 mg/L) was the same as that in plants from Ķemeri, where the K content in soil was almost twofold higher (99 mg/L). The similar situation was found for Mn concentration in orchid leaves from the Sloka site, which was similar to that at Silzemnieli, L. Kan-

Fig. 4. Correlation between Ca concentration in soils and abundance of generative ramets of *C. calceolus* (the largest populations of *Cypripedium calceolus*: Kemeris – 1400 ramets, Katleši – 1135 ramets, Dunduri – 511 ramets, Numerne – 304 ramets, Slitere – 200 ramets).

Table 3

CONCENTRATION OF NUTRIENTS IN AIR-DRY *CYPRIPEDIUM CALCEOLUS* LEAVES FROM DIFFERENT SITES

Elements	Kemeris	Sloka	Silzem-nieki	L. Kangari	Eglupe	Katleši	Max/ Min
Macronutrients, %							
N	2.20	2.27	2.28	1.75	1.80	1.93	1.30
P	0.26	0.24	0.24	0.20	0.14	0.34	2.43
K	2.12	2.07	1.86	0.82	1.94	2.09	2.58
Ca	0.81	1.18	1.22	1.71	1.59	0.83	2.11
Mg	0.33	0.24	0.29	0.41	0.28	0.22	1.86
S	0.10	0.22	0.13	0.12	0.09	0.10	2.44
Micronutrients, mg/kg							
Fe	109	88	78	160	125	160	2.05
Mn	31	48	45	46	46	74	2.41
Zn	43	35	42	30	24	45	1.89
Cu	6.4	6.1	3.9	5.0	3.8	15	4.00
Mo	0.23	0.23	0.22	0.25	0.25	0.30	1.36
B	15	21	30	18	16	17	2.00

gari, and Eglupe, while soil Mn concentration 22 mg/L, mentioned in Sloka, was tenfold lower than in the other sites.

DISCUSSION

C. calceolus has been reported to grow in moderately moist, nutrient-poor to moderately nutrient-rich, particularly nitrogen-poor, alkaline-neutral to moderately acid soils with pH 5.4–7.9 (Kull, 1999; 2003). However, Arditti (1992) reported that *C. calceolus* is a north temperate terrestrial species that naturally grows in soils that are low in macronutrient content and with high soil reaction (pH 7.1–7.4). Therefore, soil conditions are likely not strongly limiting for this species.

In Latvia *C. calceolus* localities are very fragmented. They are found in various forest types — Myrtilloso-polytrichosa,

Myrtilloso-sphagnosa, Dripterioso-caricosa, Caricoso-phragmitosa, and Oxalidos turf. mel. Myrtilloso-turf. mel. with significantly heterogeneous soils on parent soil containing carbonate (Liepa, 2005). The sites Slapjo salu purvs, Druviena, Lielie Kangari, Kazu grava, and Kemeris have soils developed on dolomite bedrock with Ca:Mg from 1.5 to 4.0, while Pērļupe, Graviņas, Dunduri, Klāņi are on limestone bedrock with Ca:Mg of 50–125 (Fig. 2). This is also highly consistent with the geological data for Latvia (Juškevičs *et al.*, 1999). The results of soil analysis showed that Ca and Mg concentrations are sufficient for successful plant growth (with the exception of Mg in the Dunduri site), regardless of soil pH. The concentrations of Ca and Mg in soils correspond to adequate or high levels for crops (Rinķis and Ramane 1989) and flower cultures (Nollendorfs (1983). The Ca and partly Mg concentrations in *C. calceolus* leaves were found to be sufficient and conformed to reference values for epiphytic orchids (Jones *et al.*, 1991) and for bulbous flowers (Nollendorfs, 1983).

For plant nutrition the most optimal Ca:Mg ratio in soils using 1 M HCl extraction is 5–8:1 (Rinkis and Nollendorf, 1982). There were nine sites (Slitere, Murmastiene, Stikli, Silzemnieki, Ziemeļu purvi, Īsliena, Zāgadi, Katleši, Svēte) with favourable Ca:Mg ratio between 6 and 10 for nutrient uptake from the soil.

Soil pH can play an important role in the distribution of terrestrial orchids by affecting germination, growth of seedlings and plants (Arditti, 1992). Soils rich in calcium and with limestone parent soil (Graviņas, Pērļupe) are more able to neutralise acid deposition than sand or gravel. Carbonates are more alkaline and produce a higher pH when dissolved in water and can buffer acidity of the rain water or metabolic processes acidifying soil. For *C. calceolus* in Latvia, soil pH is not a major factor affecting population size and vitality. The two largest populations, in Kemeris and Katleši, were found on soils with different soil reaction: pH_{KCl} 5.65 and 7.09, accordingly. The three next largest populations, at Dunduri, Slitere, and Numerne, occur on soils with pH_{KCl} 6.0–6.4. Soil chemical analyses from

C. calceolus localities in Estonia also showed a similar range for soil pH (5.4–7.5) (Kull 1999; 2003), while in central Europe and Ukraine the pH in *C. calceolus* habitats is higher than 7.0 (Kull, 1999; Reshetjuk, 2011).

Plant growth requires sufficient macronutrient level in soil. The proportion of organic matter in soils can also affect mineral nutrient uptake by plants. Although soils where *C. calceolus* grows in Latvia were not rich in nitrogen, the concentrations were sufficient when compared to those reported for European terrestrial orchid habitats (Arditti, 1992, Kull, 1999). Significantly higher concentration of N was found for *C. calceolus* in Ukraine (Reshetjuk, 2011). As *C. calceolus* sites in Latvia occur mainly on organic soils or mineral soils with high content of organic matter, additional supply with nitrogen during summer might arise from mineralisation of organic matter and nitrification. The N concentrations in orchid leaves varied around 2.00% and corresponded with a low to sufficient level reported for orchids (Jones *et al.*, 1991; Arditti, 1992). It is well established that nitrogen concentration in leaves has positive correlation with photosynthesis capacity (Zhang *et al.*, 2006) and some *Cypripedium* species have a greater photosynthesis adaptation ability than narrowly specific species (Zhang *et al.*, 2006a).

Since *C. calceolus* has relatively low requirements for nutrients (Kull, 1999), in some cases high P content in soil (Svēte, Tūja, Lielie Kangari, Silzemnieki) possibly depressed seed germination. There were no or only a few seedlings observed in these sites. A similar phenomenon was observed for terrestrial orchids in the USA (Sheviak, 1983). On the other hand, *C. calceolus* is adapted to very heterogenic environment conditions and as a mycotrophic plant can receive P and other nutrients from symbiotic relationships in poor soils (Dearnaley, 2007). The great majority of mycorrhizal fungi associated with the *Cypripedium* genus are members of narrow clades within the fungal family *Tulasnellaceae* (Shefferson *et al.*, 2005). It is possible that rare occurrence of the fungus or its specific environmental demands may affect occurrence of orchid species (McCormick and Jacquemyn, 2014). Phosphorous concentration in orchid leaves was sufficient in all of the sites studied, with values from 0.20 to 0.24, except at Eglupe, where the soil P concentration was also low. It is interesting that a low P concentration in soil was also found in the Kemeris site, where leaf P concentration was significantly higher, indicating impact of other factors than nutrient level in soils on nutrient uptake in plants.

Similar as for nitrogen, the concentrations of K in soils of *C. calceolus* habitats in Latvia were comparable with those in Estonia and Germany, but were significantly lower than in Ukraine (Kull, 1999; Reshetjuk, 2011). K concentration in *C. calceolus* leaves in Latvia varied between 1.86% and 2.12% which indicates the minimal sufficiency requirements (2.10–3.5%) for epiphytic orchids (Jones 1991). A serious K deficiency was found in L. Kangari with a K level in leaves of only 0.82%. This site occurs on mineral soil with low organic matter and K content, while Mg concen-

tration was very high. The most favourable Mg and K ratio in soil (1 M HCl extract) for optimal plant mineral nutrition is 2 : 1 (Rinkis and Nollendorf, 1982). Thus, high levels of Mg might retard K uptake from soil.

There is almost no information on required S concentration in soils for terrestrial orchids. Our study showed high heterogeneity in soil S levels; S concentration in *C. calceolus* leaves was below 0.15% in most samples, which is considered low (Nollendorfs, 1983; Jones Jr. *et al.*, 1991).

In general, a wide concentration range of mobile forms of micronutrients Fe, Mn, Zn, Cu, Mo, and B in 1 M HCl soil extract were found in *C. calceolus* sites in Latvia, due to different types of soils, forests, as well as other biotic factors.

A neutral and alkaline soil reaction is considered to be one of the main factors causing deficiency of micronutrients in plants (Marshner, 1995). In some sites (Graviņas, Pērļupe) there is potential risk of Fe deficiency due to high soil reaction and very low Fe concentration in soil. A non-optimal Fe:Mn ratio was also found for these sites. While optimal Fe : Mn ratio in soils using 1 M HCl extraction is more than 10:1 (Rinkis and Nollendorf, 1982), in *C. calceolus* habitats on slope forests a ratio Fe : Mn < 1 was observed. Considering the reported leaf sufficiency levels (Nollendorfs, 1983; Jones Jr *et al.*, 1991; Arditti, 1992), the Fe, Mn, and Zn concentrations in *C. calceolus* leaves analysed were sufficient for successful orchid growth.

Significant deficiency of Cu and B was found in *C. calceolus* leaves for at least half of the samples analysed. In general, the results of soil analyses also revealed low levels of Cu (less than 2.0 mg/L) in soils with *C. calceolus*. In addition, Cu is readily and tightly complexed by organic matter (Rinkis and Ramane, 1989; Marshner, 1995), suggesting reduced Cu availability due to the high level of organic matter found in majority of soils from *C. calceolus* sites.

Very high B sufficiency levels were reported for epiphytic orchid leaves: from 40 mg/kg to 200 mg/kg (Jones *et al.*, 1991; Arditti, 1992). Boron levels found in our study varied between 15 and 30 mg/kg and only in the Silzemnieki site corresponded to optimal levels for flower cultures (Nollendorfs, 1983; Mills and Jones, 1996). In contrast, almost optimal soil B concentrations in 1M HCl extraction (1.0–2.0 mg/L) for bulbous flowers and main crop plants (Nollendorfs, 1983; Rinkis *et al.*, 1989) were found for *C. calceolus* soils in Latvia. The availability of B to plants is affected by a variety of factors, including soil pH and Ca : B ratio. A high Ca concentration in soil, independent of soil pH, can reduce B uptake (Marshner, 1995; Fageria, 2009).

There are very limited recommendations for optimal Mo levels in soils and leaves for temperate terrestrial orchids. Although high soil reaction can promote Mo accumulation in plants, the results of our study showed that Mo concentration in *C. calceolus* leaves did not reach 0.5 mg/kg, the

lower recommended leaf tissue concentration for various plants (Nollendorfs, 1983; Riņķis and Ramane, 1989).

A negative correlation ($r = -0.44$; $p > 0.05$) was observed between relative number of generative ramets of *C. calceolus* and Ca concentration in the soil (Fig. 4) and weak positive correlation ($r = 0.21$) between total number of ramets of *C. calceolus* in a site and Ca concentration in the soil. Thus, in habitats with more calcareous soils (higher Ca concentrations in the soil), *C. calceolus* tended to produce more ramets but relative abundance of generative ramets of *C. calceolus* tended to be lower. It should be stressed that there was no linear correlation between percentage of generative ramets and Ca concentration at the soil, the Ca concentration range of 4245–16 000 mg/L is typical for most of the *C. calceolus* habitats in Latvia, indicating Ca sufficiency and/or species adaptation to wide-ranging Ca supply. However, the highest proportions of generative ramets (50–70 %) were found in *C. calceolus* sites with the Ca concentration in soil in the range 4300–8500 mg/L.

Overall the obtained results from 27 sites showed high heterogeneity in soil chemical composition for *Cypripedium calceolus* in Latvia. Although the concentrations of nutrients were comparable with levels obtained in Estonia and Germany (Kull, 1999), the range of variation for Ca, Mg, Mn was higher. The results showed a weak relationship between soil organic matter concentration and size of orchid population. It was found that soil pH and Ca concentration are not the major factors affecting the size and vitality of *C. calceolus* populations in Latvia.

There was no significant correlation between nutrient concentration in soils and *C. calceolus* leaves, suggesting strong impact of other soil-related factors determining the availability of nutrients. In general, the levels of N, P, K, Ca, Mg, Fe, Mn, and Zn concentrations in *C. calceolus* leaves were sufficient or slightly decreased for successful orchid growth. The study showed deficiency of S, Cu and B in the vast majority of leaf samples.

The determined diverse soil conditions in *C. calceolus* habitats helps to understand species growth and sustainability conditions in relation with various plant communities in different forest types.

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AUGŠŅU ĶĪMISKAIS RAKSTUROJUMS *CYPRIPEDIUM CALCEOLUS* AUGTENĒM LATVIJĀ

Ilgākā laika periodā (2007–2015) pētītas Eiropas direktīvu aizsargātas sugas dzeltenās dzegužkurpītes *Cypripedium calceolus* visas 27 Latvijas atradnes. Lai noskaidrotu *C. calceolus* izplatības limitējošos faktoros, sākām ar augteņu augšņu analīzi. Konstatēta augsta augšņu ķīmiskā sastāva heterogenitāte ar īpaši plašu Ca, Mg, Mn variāciju. *C. calceolus* Latvijas atradnes atrodas galvenokārt uz organiskām augsnēm vai uz augsnēm ar augstu organiskās vielas saturu. Iegūtie rezultāti uzrāda saistību starp organiskās vielas saturu un šīs orhidejas populācijas lielumu. Turpretī konstatēts, ka augsnes pH nav izšķirošais faktors, kas nosaka rametu skaitu augtenē. Tā kā netika konstatēta cieša saistība starp minerālelementu saturu auga lapās un augsnē, var secināt, ka barības vielu uzņemšanā liela nozīme ir ne tikai elementu koncentrācijai, bet arī citiem ar augsni saistītiem faktoriem. Kopumā N, P, K, Ca, Mg, Fe, Mn un Zn saturs *C. calceolus* lapās vērtējams kā augšanai adekvāts vai nedaudz pazemināts. Vairumā lapu paraugu tika identificēts S, Cu un B deficīts. Konstatēta vāja pozitīva korelācija ($r = 0.21$) starp *C. calceolus* kopējo rametu skaitu augtenē un Ca koncentrāciju augsnē, kas arī liecina, ka augsnes īpašības nav galvenais faktors, kas nosaka sugas augšanas sekmes katrā konkrētā augtenē.