Ecobiological study of medicinal plants in some regions of Serbia

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ABSTRACT

Ecobiological study of medicinal plants includes the analysis of particular soil features and the ecological indexes of plant species as site indicators. Two experimental serpentine areas in northwest and central Serbia were selected to identify the potential limiting factors for medicinal plant harvesting. Soil analysis is done according to ICP methodology – ICP Forest Manual, Part III, Soil Sampling and Analysis (1998). Floristic and phytocoenological investigations were carried out by Wasthoff-van der Maarel (1973). The assessment of soil quality is based on the calculation of indicator values of available nitrogen (N), heavy metals, and the sensitivity to acidification. According to our results, N (12.1–17.5), acidification (7–12), and indicator values for some heavy metals (0.3–46.5) show a low biological availability. Medicinal plant species at the investigated areas have low values of ecological indexes: N (2.41–2.82), moisture (2.45–2.70) and soil acidity (3.35–3.70). Hydrothermic conditions indicate the predominant presence of sub-xerophytes, semi-sciophytes and mesothermic species. These types of plant species are recommended for exploitation.

Keywords: serpentine parent rock; degraded meadow ecosystem; soil quality; plant indicator values

The use of medicinal plants is limited by the quality of active substances they contain. This quality depends on many ecological factors that affect the photophilous, but also the geophilous plant organs (Lombini et al. 1999). The distribution and the degree of presence of medicinal plants are directly correlated with the ecosystem conditions, especially the soil quality.

The relations between the habitat characteristics and the presence of medicinal plants were studied at two areas in Serbia: Mt. Kosmaj (northwest Serbia) and Mt. Goč (central Serbia). The parent rock of the localities is mainly serpentine. Serpentine is characterised by the presence of a smaller number of plant species in comparison with limestone (Harrison et al. 2000, Batianoff and Singh 2001, Green et al. 2003). The calcifugous plant species can grow on serpentine bedrock due to pH (5.5–8), the higher concentration of Mg, Cr, Ni, Co, and the lower concentration of essential macronutrients, such as Ca, K and P. Physical conditions of serpentine soils are unfavourable for many plants.

The changes in the soil that affect the ecosystem functions are gradual and hardly noticeable during a short time period. Long-term soil acidification by atmospheric deposition causes an irreversible reduction in cation exchange capacity and the mobilization to potentially toxic concentrations of heavy metals (Blake and Goulding 2002). The studies of ecological soil quality, as an important element of sustainable soil management, have been intensified. For example, (Vanmechelen et al. 1997) analyse the soil ecological quality related to the availability of plant nutrients (nitrogen, phosphorus, base cations), sensitivity to acidification and availability of heavy metals (Zn, Pb and Cd).

Our study predominately deals with the responses of medicinal plant populations developed at the particular localities to chemical processes of soil degradation. This kind of investigation has been performed in order to identify the potentials for further exploitation of medicinal plants.

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MATERIAL AND METHODS

Two experimental plots were chosen on Mt. Kosmaj: Beli Kamen and Sedlar (northwest Serbia) and two plots on Mt. Goč, locality Ravnine (central Serbia). Both areas are mostly on the serpentine parent rock.

The climate of these regions is temperate continental. The average altitude of the studied localities is 600 m on Mt. Kosmaj and 980 m on Mt. Goč.

Two soil profiles were opened at Beli Kamen, three at Sedlar on Kosmaj, and two profiles on Goč. The soil samples were taken from the fixed depths: 0–10 cm, 10–20 cm and 20–40 cm in five repetitions. The soil analysis is focused on the group of basic characteristics, including morphological characteristics, the analysis of standard chemical parameters and general pedological features. Soil characteristics were analysed according to JDPZ standards (1966) harmonised with the criteria of ICP methodology (ICP Forest Manual, Part III, Soil Sampling and Analysis (1998).

Parameters and reference methods of soil analyses:

- pH ($CaCl_2$) – UNEP-UN/ECE Method 9103SA, Reference method LABEX 8703-01-1-1; SO/TC190/SC3/GT8;
- $CaCO_3$ – UNEP-UN/ECE Method 9102SA, Reference method AFNOR X 31-105 by calcimeter;
- Total N – UNEP-UN/ECE Method 9105SA, (wet oxidation Kjeldahl-method – K_2SO_4:$CuSO_4$ 1:10);
- Org. C – UNEP-UN/ECE Method 9104SA, Reference method ($K_2Cr_2O_7$, H_2SO_4); for organic layer by Ansttets method with modification by Ponomareva and Plotnikova;
- Content of Zn, Cu, Pb – The total heavy metal concentration is determined by the method of atomic absorption spectrophotometry, apparatus Varian AA10;
- Conservation and preparation of samples for pseudo total content of Zn, Pb and Cd is according to UNEP – UN/ECE Method 9107SA (the soil is treated with the mixture of the concentrated HCl, HNO_3 and H_2O_2, ratio 3:1:2). The following emission lines were used (nm): Cd = 231.2, Pb = 219.1, Zn = 215.9;
- CEC and base saturation – UNEP-UN/ECE Method 9109SA;
- Exchangeable acid cations (ACE) Fe^{2+} and Mn^{2+} extraction in $BaCl_2$, measured by AAS-Varian AA-10, emission lines were used (nm): Fe = 250.5, Mn = 282.1, Al^{3+} and H^+ extraction in 1 MKCl, measured by titration;
- Exchangeable base cations (BCE) Ca, Mg, K and Na extraction in $BaCl_2$, measured: Ca and Mg with $La(NO_3)_2$ – AAS-Varian AA-10, emission lines were used (nm): Ca = 429.9, Mg = 287.4;
- K and Na – flame spectrophotometry aperture Bruno-Lange GMBh M6a.

Calculation of indicator values of the soil ecological quality is given in Table 1.

The parameters in the above equations are: Nc – cumulative value of the class of nitrogen concentration in mineral layers of soil; RC/N – cumulative value of C/N ratio in organic and mineral layers; RCZ – value for the climate zone; pHc – cumulative value of the class of pH in organic and mineral topsoil layer; (CaCO)c – the class of $CaCO_3$ concentration in mineral topsoil layer; M – concentration of heavy metals (Zn, Pb, Cd) in organic layer; RpH – relative mobility of Zn, Pb and Cd as the function of soil pH; CECc – CEC class in topsoil layer.

The general floristic study includes the whole flora of Kosmaj and Goč, whereas the specific ecological investigations were conducted on the experimental areas during three years (2002–2005). About 50 samples of each medicinal plant were collected and prepared for the analyses during the flowering phenophases.

The determination of plant species was performed by standard floristic methods. The identification of medicinal plants was performed according to Sarić (1989). The phytocoenological investigations were performed by the composite scale of abundance and the degree of coverage after Westhoff van der Maarel (1973), ranging from 1 to 9.

Table 1. Calculation of indicator values of the soil ecological quality

Indicator values	Basic equations
Availability of nitrogen, IN	IN = Nc + RC/N + RCZ
Status of acidification, IAS	IAS = pHc + BSc + ($CaCO_3$)c
Availability of heavy metals, IHM	IHM = M.RpH/CECc

Table 2. Chemical characteristics of the study soils

Locality	Layer (cm)	Zn	Pb	Cd	pH	CEC cmol/kg	BS (%)	C	N	C/N	$CaCO_3$ (%)
		(mg/kg)						(g/kg)			
Beli Kamen	organic layer	66.43	35.40	0.50	4.00			182.9	15.1	12.1	
	0–10	78.25	44.25	0.55	4.10	21.62	63.69	14.79	5.70	2.59	
	10–20	79.01	45.50	0.54	4.10	23.48	61.33	11.02	3.40	3.20	
	20–40	87,00	39.00	0.46	4.20	26.62	70.14	7.40	0.90	8.20	
Sedlar	organic layer	88.45	87.16	1.16	6.00			84.70	5.50	15.40	
	0–10	106.33	86.86	1.46	6.50	43.65	83.05	63.97	6.20	10.32	19.70
	10–20	74.99	51.16	1.24	6.90	31.46	86.18	23.08	2.00	11.54	> 25
Goč	organic layer	48.95	38.97	0.34	4.70			166.5	9.00	18.50	
	0–10	62.50	39.25	0.18	6.30	26.59	99.83	27.70	2.80	9.89	
	10–20	60.56	38.58	0.18	6.87	27.82	99.95	18.20	2.50	7.28	

Ecological indices of humidity (F), soil acidity (R), nitrogen quantity (N), light (L), and temperature (T) are according to the formula:

$$\sum_{i=1}^{n} Ni, k \times EOi$$

where: *Ni*, *k* represents the number of species in a community, and *EOi* is the ecological optimum for the given species (Kojić et al. 1997). The average values of ecological indices are used for discussion.

RESULTS

Soil research

At the locality Beli Kamen, brown soil lessive on sandstone (luvisol) is described, and at the locality Sedlar, rendzina on flysch. Eutric cambisol is described at the locality Ravnine on Goč. Two soil profiles were opened at Beli Kamen, three at Sedlar on Kosmaj, and two profiles on Goč. The average values of chemical characteristics of the studied soils are shown in Table 2.

Indicator values of some parameters were calculated by the equations (Table 1) based on the analysis of soil chemical characteristics. The mean values of indicators for the availability of nitrogen, phosphorus, exchangeable base cations and sensitivity to acidification are shown in Table 3.

Biological availability of Zn, Pb and Cd in forest ecosystems and in natural ecosystems in general is highly dependent on the soil characteristics, such as soil reaction – pH (and the related content of $CaCl_2$) and cation exchange capacity (CEC), as the most important ones. The indicator of biological availability (IHM), as one of the key factors of soil quality, is defined based on the above-described characteristics and based on the concentration of elements in topsoil layer. The calculated indicator values of heavy metal (Zn, Pb and Cd) availability are shown in Table 4.

Floristic research

The investigation of flora and vegetation is performed with special reference to medicinal and aromatic plants. The localities on Kosmaj represent the degradation phases of meadow community *Festucetum vallesiacae*, and they are characterised by very complex successions and regressions. Also, the abundance, sociability and the presence degree of medicinal plants are the most expressed at these two localities. From the floristic aspect,

Table 3. The mean values of indicators of availability and acidification status

Indicator values	Soil type		
	Kosmaj		Goč
	luvisol (Beli Kamen)	rendzina (Sedlar)	eutric cambisol (Ravnine)
IN	15.0	14.0	14
ISA	9	16	13

Table 4. The mean values of indicators of Zn, Pb and Cd availability

Soil type	Locality	Indicator values		
		Zn	Pb	Cd
Luvisol	Kosmaj (Beli Kamen)	46.5	8.85	0.75
Rendzina	Kosmaj (Sedlar)	22.1	17.4	0.3
Eutric cambisol	Goč	18.6	7.8	0.3

the locality on Goč is very specific. The floristic structure of the endemic community *Helleboro serbicae-Danthonietum calycinae* contains five endemic species. The dominant species are typical serpentinophytes, such as *Danthonia calycina*, *Helleborus serbicus*, *Alyssum margrafii*, *Potentilla visianii*, etc.

Locality Kosmaj. The largest area of Kosmaj is covered by forest communities that are significantly thinned, degraded and devastated. The most widespread are beech forests (*Fagetum montanum*), which descend occasionally down to 180 m a.s.l. Oak forests are mainly represented by the association *Quercetum confertae-cerris* which is situated on gently sloping sites on brown forest soil. Meadows occupy a smaller area. In forest and meadow associations of Mt. Kosmaj, the total number of identified species is 264 species from 51 families. Kosmaj is characterised by a relative floristic poverty, resulting mainly from the impact of bedrock (mostly serpentine) and site degradation. Two meadow communities were analysed on Kosmaj at two localities. At the locality Beli Kamen, the degradation phase of meadow community *Festucetum vallesiacae* contains the dominant species *Holcus mollis*. At the locality Sedlar, the degradation phase of meadow community *Festucetum vallesiacae* is described with *Rubus cae-*

Table 5. Medicinal plants – abundance and ecological indices for moisture (F), soil acidity (R), nitrogen (N), light (L), temperature (T) at the locality Beli Kamen (luvisol)

Plant species	Abundance	F	R	N	L	T
Calamintha vulgaris Druce	7	3	4	2	4	3
Centaurium umbellatum Gillib.	5	3	3	3	4	3
Achillea millefolium L.	3	2	3	3	4	3
Mentha arvensis L.	3	4	3	3	4	4
Thymus pulegioides L.	3	2	3	1	4	3
Hypericum perforatum L.	3	2	3	3	3	3
Prunella vulgaris L.	3	3	3	3	4	3
Galium verum Scop.	3	2	4	2	4	3
Cichorium intybus L.	3	2	4	3	5	4
Artemisia vulgaris L.	3	3	3	4	4	3
Matricaria chamomilla L.	2	3	3	3	4	4
Solidago virgaurea L.	2	3	3	3	2	3
Eupatorium cannabinum L.	2	4	4	3	3	3
Agrimonia eupatoria L.	2	2	4	3	4	3
Verbascum phlomoides L.	2	2	4	3	4	5
Lysimachia vulgaris L.	2	4	3	3	3	3
Anchusa officinalis L.	2	2	3	3	4	4
Total/Average	17	2.70	3.35	2.82	3.76	3.35

Table 6. Medicinal plants – abundance and ecological indices for moisture (F), soil acidity (R), nitrogen (N), light (L), temperature (T) at the locality Sedlar (rendzina)

Plant species	Abundance	F	R	N	L	T
Rubus caesius L.	8	4	3	2	3	3
Galium verum L.	7	2	4	2	4	3
Origanum vulgare L.	7	2	3	2	3	3
Campanula glomerata L.	5	2	4	3	4	3
Calamintha officinalis Moench.	5	3	4	2	4	3
Teucrium chamaedrys L.	5	1	4	1	4	3
Achillea millefolium L.	3	2	3	3	4	3
Medicago falcata L.	3	2	4	2	4	4
Hypericum perforatum L.	3	2	3	3	3	3
Stachys recta L.	3	1	4	2	4	3
Clematis vitalba L.	3	3	4	3	3	3
Agrimonia eupatoria L.	3	2	4	3	4	3
Potentilla recta L.	3	1	3	2	4	5
Campanula rapunculus L.	2	2	4	3	3	3
Althaea officinalis L.	2	3	3	3	4	5
Digitalis lanata Ehrh.	2	2	5	2	4	4
Verbascum phlomoides L.	2	2	4	3	4	5
Total/Average	17	2.65	3.70	2.41	3.29	3.47

sius as the dominant species. Tables 5 and 6 show the floristic composition of medicinal plants, the abundance and the ecological indices for moisture (F), soil acidity (R), nitrogen (N), light (L), and temperature (T). At the locality Beli Kamen, 42% of the total number of plant species are medicinal plants. Among them, *Calamintha vulgaris* is the most numerous species, the degree of presence 7. At the locality Sedlar, in addition to the species *Rubus caesius*, the following species occur with a high degree of presence: *Galium verum*, *Campanula glomerata*, *Calamintha officinalis*, *Origanum vulgare*, *Teucrium chamaedrys*, etc.

Locality Goč. A specific endemic association *Helleboro serbicae-Danthinietum calycinae* is developed at the locality Ravnine on Mt. Goč serpentinite. This association consists of 76 species altogether of which 22 species are medicinal plants. The association is located in the reclamation unit and it belongs to the degradation phase of the forest community *Abieti-Fagetum serbicum*.

Table 7 shows the floristic composition of medicinal plants, the abundance and the ecological indices for moisture (F), soil acidity (R), nitrogen (N), light (L), and temperature (T).

DISCUSSION

The comparative analysis is based on the results of floristic investigations, ecological indices for medicinal plants and soil characteristics. Most of the attempts to identify and to analyse the so-called plant-available fractions of various nutrients with different extractants or incubation techniques were not very successful (Rehfuess and Prietzel 1998). The next step is to use the concentrations or the contents of total nutrients in the soils as fertility indicators.

Indicator values for nitrogen (Vanmechelen et al. 1997) for all soil types in Europe indicate the low capacity (12.1.–17.5.). Indicator values for nitrogen represent total potential of organic material that may be potentially used by plant in certain climatic conditions. However, the nitrogen quantity that might be used by plants depends also on the conditions of nitrogen loss from the soil.

In the case of rendzina at the locality Sedlar, as well as eutric cambisol at the locality Ravnine, where the reaction of soil samples is neutral, the conditions for nitrogen loss are more favourable.

Table 7. Medicinal plants – abundance and ecological indices for moisture (F), soil acidity (R), nitrogen (N), light (L), temperature (T) at the locality Ravnine (Goč)

Plant species	Abundance	F	R	N	L	T
Stachys officinalis (L.) Trevis.	9	2	3	3	5	3
Achillea millefolium L.	9	2	3	3	4	3
Primula veris subsp. *columnae* Huds.	8	3	4	3	5	3
Anthyllis vulneraria L.	8	1	4	2	4	3
Sanguisorba minor Scop.	8	2	4	2	4	3
Potentilla erecta (L.) Rausch.	8	3	3	2	3	2
Thymus moesiacus Bernh.	8	1	3	2	3	5
Anemone nemorosa L.	7	3	3	3	2	3
Centaurea jacea L.	7	3	3	3	4	3
Filipendula hexapetalla Gilib.	7	4	3	3	3	3
Ranunculus bulbosus L.	7	2	4	2	4	3
Galium verum L.	7	2	4	2	4	3
Agropyrum repens (L.) Beauv.	7	3	3	4	3	3
Calamintha vulgaris Moench.	7	3	4	2	4	3
Centaurium umbellatum Gilib.	5	3	3	3	4	3
Euphrasia stricta Wolff.	5	2	3	2	3	3
Taraxacum officinalis Weber	5	2	3	3	4	3
Erica carnea L.	5	2	4	2	4	4
Primula vulgaris Huds.	5	3	4	3	5	3
Colchicum autumnale L.	5	2	4	3	5	3
Angelica silvestris L.	3	3	3	3	4	4
Juniperus communis L.	3	3	4	3	4	2
Total/Average	22	2.45	3.45	2.5	3.86	3.1

At the locality Beli Kamen, situated on luvisol, the degradation phase of meadow community *Festucetum vallesiacae* is characterised by a significant presence of the species *Holcus mollis*, as the dominant species. According to the indicator value of nitrogen availability $I_N = 15.0$ (Kadović and Knežević 2003), the soil may be classified as class 1 (low availability), while the content of total nitrogen in the soil belongs to the medium class (3). Element cycling by plants results in a net movement of nutrients to the soil surface through the transport within the plant and the release via litterfall and direct leaching from the leaves by throughfall. As long as the nutrient uptake by plants takes place at greater depths than nutrient return to the soil, a net uplift should be expected as a result of cycling. Nutrient uplift could also interact with other influences of plants on the dynamics of lithospheric elements (Jogobay and Jackson 2004).

The flora of the above meadow community has the average value of ecological index of nitrogen 2.82. The values of most species in this community are 2 and 3, e.g.: *Centaurium umbellatum*, *Achillea millefolium*, *Mentha arvensis*, *Hypericum perforatum*, etc. *Artemisia vulgaris* has the highest value of ecological index of nitrogen, while *Thymus pulegioides* has the lowest index.

At the locality Sedlar (Table 6) the degradation phase of meadow community *Festucetum vallesiacae* is described with the dominant species *Rubus caesius*. At this locality, the soil (rendzina) has neutral reaction. Similar pH conditions are found on Goč, where the soil (eutric cambisol) is characterised by weak acid to neutral reaction. Because of that, the conditions for nitrogen loss are more favourable (I_N = 14.0) at these sites. Neutral reaction enables volatilisation of NH_4^+, and at the same time, nitrification processes are not stopped.

Regarding the ecological index of nitrogen (2.41), the value of the most frequent species of the community is 2, e.g. *Rubus caesius*, *Galium verum* and *Origanum vulgare*. Another group of species has value 3: *Campanula glomerata*, *Achillea millefolium*, *Hypericum perforatum*, *Clematis vitalba*, etc., while *Teucrium chamaedrys* has value 1. There are no species with the index above 3. This might be related to fairly small needs for nitrogen.

Soil acidification is an important link between air pollution and the damage to the terrestrial and aquatic ecosystems. This process has been related to forest dieback via its effect in the tree root zone, but also due to the ability of soil to buffer acid deposition as a key factor in regulating the long-term surface water and groundwater acidification. Soil characteristics, such as pH or carbonates, may change according to the specific characteristics of the contamination source. Sorption patterns depend on the concentration of heavy metals (Sastre et al. 2006).

Acidification processes in rendzina are weak, which is shown by the indicator value I_{SA} = 35 (Kadović and Knežević 2003). This value indicates a very low sensitivity to this process, which is typical for the class with very high pH values (> 6.0 pH). The average ecological index of soil acidity (R) of the study plants (3.70) shows the same effect. Ecological analysis of species of this community shows that there are no species with ecological index of acidity of 1 or 2, 11 species have index 3, and only the species *Digitalis lanata* has index 5. Medicinal species with high abundance and potential preference for alkaline soils are: *Calamintha vulgaris*, *Galium verum*, *Cichorium intybus*, *Eupatorium cannabinum*, *Agrimonia eupatoria*, *Campanula glomerata*, *Calamintha officinalis*, *Teucrium chamaedrys*, *Primula veris*, *Anthylis vulneraria*, *Sanguisorba minor*, *Ranunculus bulbosus*, etc.

Hydrothermic conditions of the soil influence significantly the processes of aminization, ammonification and nitrification. There is a correlation between hydrothermic conditions of the soil and the ecological indices of plants for moisture (F), light (L) and temperature (T). The average value of ecological index of moisture (F) shows the predominant presence of subxerophytes (2.45 to 2.70) in both communities. Ecological index of light (L) shows that plant species are between semisciophytes and heliophytes (3.29–3.86), and ecological index of temperature (T) shows the dominance of mesothermic species (3.35–3.86).

The contents of heavy metals in the soil depend on numerous factors, such as: specific ability of some plants to over-accumulate various toxic heavy metals, chemical and physical characteristics of soil and their interactions etc. (Sústriková and Hecl 2004). Although the total concentrations of heavy metals indicate the loads of particular elements, generally there is not sufficient information on the risks of toxicity for plants; on the contrary, the low content and/or availability of microelements may lead to potential deficiency in plants.

The heavy metal concentrations in these soils are compared to the critical levels in most European countries, which are in compliance with the concept of multifunctional land use, and according to De Vries and Bakker (1998) they range as follows: Pb 25–100, Cd 0.3–2, in mg/kg.

The uptake of heavy metals depends on the concentration of heavy metals in the soil solution and the rate of the transfer from the solid phase into soil solution for replenishment of the heavy metals taken up by the plant roots. According to Kashem and Singh (2002), total uptake of Cd and Zn in plants increases with time in contrast to solution concentration.

Acidification to pH – 4 mobilizes 60–90% of the total soil Cd, but it is adsorbed on ion exchange surfaces and complexed with soil organic matter. According to Blake and Goulding (2002) Pb is not mobilised until pH < 4.5.

Complexing reactions of metals released by the buffer reaction with dissolved organic matter, which impedes an equivalent H^+ released from the ion-exchange resin, may be the reason for the higher pH at the beginning. Schwarz et al. (1999) concluded in their experiments that in a delay of release into solution: Pb > Cu > Zn > Cd, reflecting the competitiveness for exchanger sites.

According to Vanmechelen et al. (1997) (Table 4), mean values for Zn in luvisol indicate the class of low availability, and in rendzina and eutric cambisol, they indicate the risk of Zn deficiency (< 34.7). The values for Pb indicate the class of low availability, similarly the values for Cd (< 1.8). By substitution of cations with metals, topsoil layers of soil immobilise the heavy metals and thus protect plants from direct toxic effects. Content and type of clay and organic matter determine the exchange capacity. It is shown that concentrations of Zn, Pb and Cd in the soil are highly correlated with CEC (Vanmechelen et al. 1997), and that process of cation exchange is a basic mechanism of immobilisation of these elements. High values of CEC in topsoil layer indicate the ability of soil to keep these elements, regardless of their relatively high total concentrations in the layer (0–10 cm), e.g. Zn (78.25 mg/kg in luvisol and 62.5 mg/kg in eutric cambisol) and Pb (44.25 mg/kg in luvisol and 86.9 mg/kg in rendzina and 39 mg/kg in eutric cambisol).

Plant species are mostly identical in all the experimental areas. It can be explained by edaphic and phylogenetic reasons. The edaphic specialization and plant adaptation to serpentine soils is phylogenetically and geographically widespread (Brady et al. 2005). Plant species adapted to serpentine soils often posses somewhat distinct morphology from closely related species not adapted to serpentine sites (Cooke 1994).

On Mt. Kosmaj and Mt. Goč, serpentinite is not the only geological layer. There are small areas composed of limestone. Morphologically, we compared the medicinal plants sampled on serpentinite with the same species sampled on limestone and noticed the differences between them. The turfs of plants growing on serpentinite are less compact than those of plants growing on limestone (*Teucrium chamaedrys*, *Anthyllis vulneraria*, *Prunella vulgaris*, *Thymus pulegioides*, etc.); the plants growing on serpentinite are smaller than the same species on limestone; the leaves of plants on serpentinite are smaller, covered with more hairs and many of them are grey-green in comparison with the plants on limestone; some of the sampled plants on serpentinite have better developed roots (*Calamintha vulgaris, Achillea millefolium, Hypericum perforatum, Primula vulgaris*, etc.) than on limestone.

Biologically, serpentine sites frequently host a depauperate flora compared to the surrounding regions. Sparse plant cover also encourages erosion and promotes elevated soil temperature (Kruckeberg 2002). Each of these factors poses an additional stress to plant life. Together, the chemical, physical and biotic components of the edaphic factor produce the "serpentine syndrome" (Jenny 1980). This combination creates a patchwork of microhabitats in the serpentine grassland that results in a variation in species structure within a small area (Mc Carten 1992).

At the same time, high levels of genetic differentiation were detected between the populations within one region, as well as between the populations of different regions (Patterson and Givnish 2004). Plant populations growing on serpentine have a strong divergent selection, the subsequent genetic differentiation of the populations renders them reproductively isolated, and in extreme cases, results in ecological speciation (Schluter 2001).

Boyd and Martens (1998), after Brady et al. (2005) offer three theories to explain the "preadaptive" nature of nonserpentine populations to serpentine conditions: a) high rates of gene flow from serpentine to nonserpentine populations bring serpentine tolerance alleles into latter populations, b) a constitutive serpentine adaptive trait presents little or no cost to a plant, c) a serpentine adaptive trait is adaptive for more than one function.

Prezygotic isolating mechanisms between plant species include shifts in flowering time, a switch to primarily self-fertilization from out-crossing, and alternations in flower morphology that affect pollinator attraction and/or visitation (Macnair 1998). Peak flowering time in serpentine and nonserpentine populations differs significantly. These are the reasons why similar plant species grow on all the investigated areas.

The comparative analysis of the three investigated localities, based on the research of soil and ecological indices, shows the existence of correlative relations. The following medicinal species could be recommended for the purposes of harvesting from the wild: *Calamintha vulgaris*, *Centaurium umbellatum*, *Achillea millefolium*, *Mentha arvensis*, *Thymus pulegioides*, *Hypericum perforatum*, *Galium verum*, *Cichorium intybus*, *Origanum vulgare*, *Teucrium chamaedrys*, *Artemisia vulgaris*, *Stachys recta*, *Potentilla erecta*, *Thymus moesiacus* and *Euphrasia stricta*.

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