Chapter 4
Ecology of *Teucrium* Species: Habitat Related Metal Content Dynamics

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Abstract The chapter reviews the available data about the effect of habitat related metal content on *Teucrium chamaedrys* and *T. montanum* (Lamiaceae). The study was focused on element concentrations in plant and soil samples, both on metalliferous and non-metalliferous soils. Metal concentrations varied depending on species and habitat type. The levels of elements in plant tissues from non-metalliferous localities were always lower, compared to those from metalliferous (serpentine) ones. None of the species could not hyperaccumulate metals although the metal concentration in some of them exceeded the range, which is naturally found in plants. Depending on the nickel accumulation, both analyzed species are classified as excluders. The level of tolerance was related to the amount of metals and their bioavailability in the soil. The metal concentrations for the toxic elements were above the permissible limits for the toxic elements, in both species. The populations of the studied species demonstrated some adaptations to the serpentine habitats related to their secondary metabolites and its morphology, which is known as serpentinomorphoses. As a result of the heavy metal profiles of the soils, significantly higher values and differences in the quantity of secondary metabolites were recorded in plant populations growing on serpentines compared to non-serpentine ones. *Teucrium chamaedrys* and *T. montanum* populations on metalliferous habitats, possess morphological differences in contrast of populations on non-metalliferous habitats.

Keywords *Teucrium* · Ecology · Distribution · Metal content · Soil · *Teucrium chamaedrys* · *Teucrium montanum*

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M. Stanković (ed.), *Teucrium Species: Biology and Applications*,
https://doi.org/10.1007/978-3-030-52159-2_4

Abbreviations

Al	Aluminium
B	Boron
BAF	Bioaccumulation factor
Ca	Calcium
Cd	Cadmium
CEC	Cation exchange capacity
Co	Cobalt
CO2	Carbon dioxide
Cr	Chromium
Cu	Copper
Fe	Iron
GR	Glutathione reductase
GSH	Reducted glutathione
H	Hydrogen
H_2O_2	Hydrogen peroxide
Hg	Mercury
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
ROS	Reactive oxygen species
Zn	Zinc

4.1 Introduction

The genus *Teucrium* L. is the one of the largest in Lamiaceae family. It is widely distributed in Europe, Asia, Africa and Australia and have two centers of richness located in the east and western regions of the Mediterranean area. The genus *Teucrium* comprises approximately 300 taxa of herbs and shrubs grouped in 9 sections according to Bentham (1833) with additions proposed by Boissier (1879) (Navarro and El Oualidi 2000). The Mediterranean area represents the major area of distribution of the genus, since it comprises about 90% of the total species in the world (Navarro and El Oualidi 2000; Navarro 2010).

Teucrium chamaedrys L. (wall germander) is the most important species of sect. *Chamaedrys* (Mill.) Schreb., distributed throughout the Mediterranean, mainly in the Balkan peninsula and Turkey. The species *Teucrium montanum* L. (mountain germander) is a representative of sect. *Polium* (Mill.) Schreb., subsect. *Rotundifolia* Berm. & Sanchez Crespo, distributed in South and East Europe, the west Mediterranean area and in the mountains of the south Arabian peninsula (Navarro and El Oualidi 2000).

Reichinger (1941) considered *Teucrium chamaedrys* as the most variable taxon in the *Teucrium* genus and described 15 subspecies, most of them locally distributed. These subspecies were not initially included in Flora Europaea (Tutin et al. 1972) but, it was later added by Greuter et al. (1986), and some of them in local floras (Ekim 1982; Peev 1989). This perennial plant occurs in open forests, shrublands and rocky slopes in semi-arid, arid and sub-humid climates (Navarro and El Oualidi 2000). In the Balkan countries, the plant inhabits rocky limestone areas, dry mountain meadows and pastures, edges of sparse oak and pine forests up to 1000 m in Serbia and up to 1500 m in Bulgaria, demonstrating a high degree of morphological diversity (Diklić 1974; Peev 1989; Markova 1992). It was also reported for the serpentine territories in Bulgaria, Serbia, Albania, Turkey (Ekim 1982; Shallari et al. 1998; Pavlova et al. 2003; Pavlova 2010). *Teucrium montanum* L. is a perennial, shrub-like plant with half ligneous branches, up to 25 cm high and inhabits thermophilic calcareous rocks, dry mountain meadows and edges of forests in Europe and Anatoly (Diklić 1974; Zlatić et al. 2017).

A large number of known medicinal species, belonging to the genus *Teucrium*, are used in folk medicine and pharmacy, in food industry as spices and for bitter beverages. The most popular species in the flora of Europe and Asia (*Teucrium chamaedrys*, *T. montanum* and *T. polium*) have a history of traditional use in the Balkan countries for herbal teas and basic medicinal healing treatments (Ivancheva and Stancheva 2001; Obratov-Petković et al. 2006, 2008; Evstatieva et al. 2007; Redzić 2010). For pharmaceutical purposes aboveground plant parts (stems, leaves, flowers) are collected from natural populations and from cultivated plants (Nikolov 2006). The mentioned plants are included in the list of medicinal plants permitted for export, and annually hundreds of tons are exported (Evstatieva et al. 2007). They are used in the treatment of digestive and respiratory disorders, abscesses, gout, conjunctivitis, in the stimulation of fat and cellulite decomposition. These species possess anti-inflammatory, antioxidative, antimicrobial, antidiabetic, and antihelmintic effects (Harborne et al. 1986; Stanković et al. 2011a, 2012; Grubešić et al. 2012; Milošević-Djordjević et al. 2013; Vlase et al. 2014; Zdraveva et al. 2018).

According to Obratov-Petković et al. (2006), the distribution and the degree of presence of medicinal plants are directly correlated to the edaphic factors. Moreover, the quantity of active substances in plant tissues depends of many ecological factors that affect the vegetative plant organs (Lombini et al. 1998). The properties of soil generally depended on the combined effects of climate, biological activity, topography and the mineralogical composition of the parent rock and play a central role in the distribution and ecology of plant species and their associated biota (Jenny 1980; Whitea and Claxtonb 2004). Usually, extreme edaphic conditions, like limestone, gypsum, dolomite, granite, guano deposits, salt marshes, and even mine tailings, provide ideal settings for examining the role of the edaphic factor in the distribution, ecology and natural selection of plants (Kruckeberg 1969; Rajakaruna 2004; Rajakaruna and Boyd 2008).

Fig. 4.1 *T. chamaedrys* L. on serpentine (left) and calcareous habitat (right). (Photo D. Pavlova and M. Stanković)

Fig. 4.2 *T. montanum* L. on serpentine (left) and calcareous habitat (right). (Photo M. Stanković and N. Zlatić)

The species *Teucrium chamaedrys* (Fig. 4.1) and *T. montanum* (Fig. 4.2) demonstrate a wide ecological tolerance as they were found in open grasslands, oak woodlands, pastures, rocky calcareous and siliceous terrains, and on serpentine outcrops. They occurred either on soils that are naturally metalliferous (e.g. serpentine soils) or in territories which secondarily have been enriched with metals (industrial plants emitting metals, mine tailings, etc.). As the serpentines and their soils are characterized by toxic quantities of heavy metals (particularly Ni and Cr), low Ca/Mg ratio, drought, and wide temperature fluctuations (Kruckeberg 1984, 1992; Brooks 1987; Brady et al. 2005), they probably have the most extreme habitat conditions for these species. Most populations of *Teucrium chamaedrys* and *T. montanum* grow on nonmetalliferous soils in their range and are quite sensitive to metal toxicity. There is a strong selection favoring the metal-tolerant genotypes, when plants colonize metalliferous soils, causing a rapid increase in the average tolerance of the local populations, which leads to the establishment of metal-tolerant "races" or ecotypes (Antonovics et al. 1971; Pollard et al. 2002). It could be concluded that their adaptive mechanisms includes exclusion of metals (either by restricting them to the roots or through the absence of any uptake mechanisms), compartmentalization of metals in various organs, or toxicity tolerance as it was summarized by Kay et al. (2011) for plants growing on such soils. The level of tolerance is related to the amount of metals in the soil and their bioavailability. The metal bioavailability in the system soil-plant could be an indicator of potential risk for environment and human health, especially for medicinal plants.

This chapter will describe the influence of soil metal concentration on the therapeutically important medicinal plants – *Teucrium chamaedrys* and *T. montanum* (Lamiaceae) throughout their range in the Mediterranean region. Additionally, this chapter will describe the components of edaphic factors and their role, through metal concentrations in different types of soils. Related to soil analysis, metal accumulation by *Teucrium chamaedry*s and *T. montanum* will be presented as well as plant-soil correlations with bioaccumulation factor of selected plant species. Furthermore, this chapter will include the morphological characteristics of presented species related to the substrate and the quantity of secondary metabolites as one of the mechanisms of species adaptation to the metalliferous soil.

4.2 Soils and Their Role in the Distribution of *Teucrium* Species

4.2.1 Components of the Edaphic Factor and Its Role

The edaphic factor as a combination of numerous physical and chemical properties of the substrate significantly determines plant species distribution, diversity and composition. Very significant soil factor is combination and concentration of mineral nutrients available in the soil. Bioavailability is the proportion of total metals or metal species that are available for incorporation into biota (bioaccumulation). Among the chemical elements two classes are considered essential for plants: macronutrients and micronutrients. Macronutrients like K and Mg are required in high quantities as they form the basis of crucial cellular components like proteins and nucleic acids. Micronutrients like Zn, Mn, Cu, Fe, Ni etc. often required as cofactors for enzyme activity are needed in small amounts and, as expected, in excess they become toxic. The highly toxic elements like Cd, Pb, Hg usually can not be distinguished by the nutrient uptake system and enter into the plant causing reduced uptake of the essential nutrients and significantly reduced plant growth and quality. Plant uptake of trace elements is dependent on (1) movement of elements from the soil to the plant root, (2) elements crossing the membrane of epidermal cells of the root, (3) transport of elements from the epidermal cells to the xylem, in which a solution of elements is transported from roots to shoots, and (4) possible mobilization, from leaves to storage tissues used as food (seeds, tubers, and fruits).

Many factors can affect the efficiency of chemical elements biouptake mainly from the soil through the plant roots. Among these are factors connected with specific plant biouptake mechanism and root system. Micro- and macronutrient homeostasis in plants is realized by various strategies for mobilization and uptake which differ for different plants species. Metal ions reach plant tissue through ion exchange, passive diffusion or through a membrane transporters localized in the root. Various defense mechanism has been developed by plants against toxic elements: extracellular immobilization near root region (using root exudates or mycorrhizal association), intracellular detoxification by the formation of complexes between metal ions and peptides – phytochelatins, or complexes with organic acids or aminoacids. On the other hand, metals tolerant for some plants are associated with increased synthesis of phytochelatins proline or antioxidant enzymes.

In addition to the factors connected with specific plant biouptake mechanism and root system, very important are factors connected with soil properties, edaphic factors and chemical elements bioavailability. Acid soils have contributed to a great extent to the solubilization of metals. It is clear that with increasing pH the solubility of Fe, Cr, Ni and Co in serpentine soils decreases. The order of increasing solubility is Fe, Cr, Ni, Co (Brooks 1987). According to Blake and Goulding (2002) Pb is not mobilized until $pH < 4.5$. Zinc is readily soluble relative to the other metals in soils and available in acid light mineral soils. It is considered that the Zn fraction associated with Fe and Mn oxides is likely to be the most available to plants. The greatest amounts of adsorbed Cu have always been found for Fe and Mn oxides, amorphous Fe and Al hydroxides, and clays (Kabata-Pendias 2011). The solubilization of Cd and its transformation into an available ionic form, which is preferentially absorbed by plant roots depend on soil pH and other soil factors (Sharma and Dubey 2006). In acid soils the mobility and availability of Cd is much higher than in non-calcareous, neutral and slightly alkaline soils. 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 (Obratov-Petković et al. 2006). The rapid increase in the Cd concentration at the 0.5 mg kg^{-1} level (in straw) is considered to be related to a break of the physiological barrier controlling a metabolic absorption of this metal (Kabata-Pendias 2011).

In most cases above separation of factors is quite operational as far as plant can to some extend regulate chemical elements biouptake as a respond to the changes in the soil properties and chemical elements bioavailability. The rhizosphere is a zone at the root-soil interface controlled by plant root and root released metabolites or exudates where additionally mutually interacting physical, chemical and biological processes take place. The health of both the plant and the soil associated is dependent on this rhizosphere region and its biochemical reactions.

Evidently, equilibria between different chemical elements in soil solid phase (mineral and organic/soil solution/plants rhizosphere) is extremely complex depending on various biotic and abiotic factors. Even more, reliable determination of bioavailable concentrations of nutrients and evaluation of their behavior is almost impossible. Although various operationally defined analytical procedures have been proposed and applied, the results obtained for chemical elements, their distribution between soil fractions and their chemical species are far from real situation in a complex soil-plants system. From such point of view the investigations on soils with unusual features (extreme pH, nutrient imbalances, limited depth, etc.) accepted as a strong selective force shaping plant evolution are very important lightening in a specific manner soil-plant connection and plant response to soil edaphic factors.

Together, these mechanisms allow plants to maximize their nutrient acquisition abilities while protecting against the accumulation of excess nutrients, which can be toxic to the plant. It is clear that the ability of plants to utilize such mechanisms exerts significant influence on plant community structure, soil ecology, ecosystem health, and biodiversity.

4.2.2 Metal Content in Serpentine Soil

Serpentine soil (formed after serpentine rocks (ultramafic rock consists of magnesium-iron silicate minerals, such as olivine and pyroxene) weathering under different climatic conditions) is a naturally occurring model system ideal for the study of the physiological responses of plants to edaphic factors. Serpentine soils are characterized with (i) low Ca content combined with elevated concentrations of Mg, which creates an unusually low Ca:Mg ratio; (ii) low concentrations of several of the macronutrients essential for plant growth, especially N, P, and K; (iii) high concentrations of toxic elements like Ni, Cr, Co and less toxic Mn. The parent material from which serpentine soil originates is highly variable leading to variations within them with respect to the absolute concentrations of Ca and Mg and the presence and concentration of toxic elements. Within serpentine ecosystems, additional abiotic stresses are also often present, including drought, low nutrient cycling rates, and shallow soil depth. This suite of abiotic factors and the sparse vegetation observed on serpentine soils has been described as the "serpentine syndrome" (Jenny 1980). It is a physiologically challenging environment for most plant species driving the development of unique physiological adaptations and high levels of endemism (Brooks 1987; Kruckeberg 1992).

Various hypotheses (reviewed by Brady et al. 2005; Kazakou et al. 2008), including tolerance to low Ca:Mg ratio, avoidance of Mg toxicity, an increased requirement for Mg, and tolerance/exclusion or hyperaccumulation of toxic elements have been discussed for plants that do grow on serpentine soil. Tolerances and adaptations range from those at the cellular level to those apparent to the naked eye. High concentrations of Ni are tolerated in some serpentine plants by exclusion, reduced transfer of Ni from root to shoot, or hyperaccumulation. Other plants adapt to a low Ca environment, by selectively up taking this nutrient rather than Mg.

Although physical-chemical features of serpentine soils where *Teucrium chamaedrys* and *T. montanum* are distributed vary considerably from site to site (Pavlova 2009; Pavlova and Karadjova 2013; Obratov-Petković et al. 2008; Branković et al. 2017; Zlatić et al. 2017) and within a site (Pavlova and Karadjova 2013), species populations are often found in open, steep landscapes with shallow and rocky soils with reduced moisture. Sparse plant cover on serpentines promotes elevated soil temperature and erosion (Kruckeberg 1992). Each of these factors poses an additional stress to plant life.

4.2.3 Metal Content in Calcareous Soil

Calcareous parent materials are the most widespread type of sedimentary rock in the world. They are made of calcium carbonate and originally divided into chemical, over gammon and rocks of organogenic origin. Usually, they contain primers of other metals such as Mn, Fe, Mg, clays, organic matter, sand, etc. Soils formed on the calcareous geological substrate contain calcium carbonate in free form (Ewald 2003). Calcareous soils are characterized with (i) high Ca content; (ii) high concentrations of several macronutrients essential for plant growth, especially K, P, and N; (iii) low concentrations of toxic elements like Co, Cr, and Ni, which is opposite to serpentine soils (Zlatić et al. 2017). This type of soil is characteristic for dry and semi-arid areas, as well as humid and moderately humid regions, especially in those areas where the parent material is rich in $CaCO_3$. At the chemical level, the presence of $CaCO_3$ determines the alkaline reaction in calcareous soils and affects the availability of certain metals, such as N, P, K, Mg, Zn, Cu, and Fe, to plants. The carbonates presented in the soil contribute to a alkaline pH value, ranging between 7.5 and 8.5. In addition to the chemical influence of $CaCO_3$ in calcareous soils, it also affects the physical characteristics of the soil (Lambers et al. 2008).

Plant species that inhabits limestone habitats are not exposed to the negative impact of heavy metals in the substrate. Plants that inhabit such habitats are adapted to other ecological factors by specific physiological mechanisms (Zlatić et al. 2017). These species are exposed to different stress conditions in opposed to the serpentine ones. The alkaline pH value of the calcareous soil, the deficiency of water in the substrate, the high temperature and erosion, are factors that plants needs to be adapt (Lambers et al. 2008).

4.3 Plant Life on Selected Edaphic Conditions – *Teucrium chamaedrys*

4.3.1 Metal Accumulation by Teucrium chamaedrys

The physiological response of plants to the stress provided by the elevated metal concentrations in the soil can be related to the different ways to cope with metals in the soil and to their genotypic response. Plants tolerate elevated metal concentrations on serpentine soils either by a constitutive trait present in all members of the species growing either on serpentine or non-serpentine substrate or by an adaptive mechanism present only in tolerant ecotypes (Antonovics et al. 1971; Kazakou et al.

2008). *Teucrium chamaedrys* absorbs a number of elements from soil both essential (Cu, Zn, Fe, Ni, Mn) and non-essential (Cr, Co, Pb) for its biological functions. The availability of metals in a soil-plant system depends on a number of factors which include pH of the soil, soil organic matter content, cationic exchange capacity as well as plant species, stage of development, and others (Farago 1994; Kassim and Rahim 2014). The species is widely distributed without preferences to any type of rock and should be considered as a "bodenvag" species, which are those widely distributed (Fig. 4.1) in serpentine and non-serpentine habitats (Kruckeberg 1992). The significant ecological differences between populations are related to the differences in edaphic conditions of the serpentine sites from the non-serpentine sites. According to Obratov-Petković et al. (2008) there are also differences in the heavy metal uptake between the individuals of the same species, which is first of all the consequence of climatic conditions and moisture regime.

The mechanism by which a serpentine-tolerant plant copes with elevated levels of Mg and relatively insufficient quantities of Ca in the soil is considered its most defining character (Brooks 1987; Brady et al. 2005; Kazakou et al. 2008). The ratios Ca:Mg are generally always above 1 in plants. However, serpentine plants are better able to maintain a greater than 1 ratio despite minimal levels of Ca found in the soils. They either have very efficient Ca uptake systems or ability to exclude Mg despite high concentrations in the soil. The low Ca:Mg ratio in serpentine soils has elicited a wide range of adaptive responses based on either ion exclusion at the root/soil interface, selective translocation of Ca from root to shoot, sequestration of Mg in the vacuole, or internal mechanisms of tolerance (Kay et al. 2011). Calcium concentrations found in tissues of *Teucrium chamaedrys* are between 2369 and 6345 mg kg^{-1} in serpentine plant populations in Bulgaria (Pavlova 2009; Pavlova and Karadjova 2012) and between 324 and 4872.2 mg kg^{-1} in plant populations from Serbia (Obratov-Petković et al. 2008; Branković et al. 2017; Zlatić et al. 2017). Similar to previous findings (Karataglis et al. 1982; Brooks 1987; Roberts and Proctor 1992; Kay et al. 2011; Pavlova and Karadjova 2013, Branković et al. 2017), while the amounts of Ca in the serpentine soils were small, the amounts of Ca taken up by the plant were higher. Serpentine species populations are much more tolerant to low Ca levels and elevated concentrations of Mg in the soil. Calcium concentrations in *Teucrium chamaedrys* populations are in all cases lower than in Ni hyperaccumulating plants distributed in the same areas such as *Alyssum* and *Thlaspi* species. The remarkable Ca uptake ability is considered an important feature in Ni-hyperaccumulator physiology (Broadhurst et al. 2004; Chaney et al. 2007).

Calcium is one of the elements contributing to the inhibition of the heavy-metal toxicity, and it is possible that plant takes up Ca to compensate the toxic action of different toxic metals (Brooks 1987; Lombini et al. 1998; Brady et al. 2005). Studying populations of *Buxus sempervirens* L. (Common Box) in Greece, Karataglis et al. (1982) suggested that the plant developed a mechanism to permit an excess soil Ca to be taken by plant.

Several species adapted to serpentine environments have higher external and internal Mg requirements than their non-serpentine relatives and variety of responses shown suggests that the physiological basis for tolerating low Ca:Mg may involve more than one mechanism in a given species (Brady et al. 2005; Kay et al. 2011). Normally, the high Mg of serpentine soils is reflected in high Mg concentrations in plant tissues, but this result might also indicate an unusual Mg and low Ca requirement (Proctor and Woodell 1975). The mean Mg concentrations for *Teucrium chamaedrys* in the range 0.13–0.35% and 0.11–0.19%, respectively for serpentine and non-serpentine sites, were reported by Pavlova and Karadjova (2012). The mean Mg concentrations for the species from Serbia were almost the same for serpentine and non-serpentine (calcareous) populations as reported by Zlatić et al. (2017).

Brooks and Yang (1984) found the concentration of Mg in plant tissue to be inversely proportional to the concentrations of other nutrients: B, Fe, Co, Mn, P, and Na. Their findings showed that these relationships were caused by high Mg levels in the soil; high Mg concentration is probably the major cause of infertility of serpentine soils and hence of the development of specialized serpentine floras adapted to this unfavorable edaphic condition (Kazakou et al. 2008). These data clearly suggest that the uptake of Mg comes at a cost to the plant as the uptake of other elemental nutrients is forfeited. More complex is this interaction in Ni-hyperaccumulator plants because Ni is also involved. Some publications demonstrated that in the presence of Ni internal Ca and Mg concentrations counteract Ni toxicity or in any case enhance Ni tolerance (Gabbrielli and Pandolfini 1984), or decrease Ca uptake and increase uptake of Mg (Kazakou et al. 2008). It is likely, therefore, that the Ca/Mg quotient of the soil solution has a strong influence on Ni absorption, translocation and accumulation.

The accumulation of Ni, Cr, and Co in the above-ground plant parts for all serpentine populations of *Teucrium chamaedrys* is higher than in non-serpentine ones which was proved by a number of studies (Shellari et al. 1998; Jurišić et al. 2001; Obratov-Petković et al. 2008; Pavlova 2009; Pavlova and Karadjova 2012, 2013; Branković et al. 2017; Zlatić et al. 2017). Metal concentrations also vary at population level in relation to soil properties, mainly soil metal availability. This species demonstrates, as many other, specific strategies of adaptation to elevated levels of toxic metals in soil probably result of mineral element imbalance characteristic of serpentine soils (Ater et al. 2000) or due to inter-population variation in parts of the uptake and translocation processes (Adamidis et al. 2014). Nickel concentrations measured in *Teucrium chamaedrys* serpentine populations in Bulgaria (Pavlova 2009; Pavlova and Karadjova 2012, 2013) are lower than the data of Shallari et al. (1998) for Albanian serpentine populations, and higher from data provided for this medicinal plant from Serbia (Obratov-Petković et al. 2008). It was shown that different Ni concentrations in serpentine populations correlate with the bioavailable fraction of trace elements in the soils (Pavlova and Karadjova 2013; Zlatić et al. 2017). The concentrations of Ni even higher for all serpentine populations of *Teucrium chamaedrys* were not exceptional. The amounts of Ni in *Teucrium chamaedrys* were low compared to the data for other plants growing on serpentines (Karataglis et al. 1982; Vergnano Gambi et al. 1982; Konstantinou and Babalonas 1996; Bani et al. 2013; Sawidis et al. 2014), but higher than levels normally considered to be toxic to most plants (Kabata-Pendias and Pendias 1984). The concentrations of Ni in plants from some of the investigated sites were higher than 60 mg kg^{-1} considered to be the threshold of physiological evidence of toxicity in plants of serpentine habitats (Kazakou et al. 2008, 2010). Concentrations of Ni above 100 mg kg^{-1} were documented for some serpentine populations of *Teucrium chamaedrys* in Bulgaria and Serbia (Pavlova and Karadjova 2012; Branković et al. 2017; Zlatić et al. 2017). Values from 145.7 mg kg^{-1} Ni found in *Teucrium chamaedrys* growing on serpentine in Goč Mt. (Serbia) give reason this species to be considered as accumulating plant of Ni (Branković et al. 2017). Despite of high Ni values documented they are rarely found in species populations and can be considered exception for the species. Only a few species appear to show extreme variations in metal uptake, even when confined to metalliferous soils. This behavior may be a reflection of widely varying metal availability caused by variations in pH or other soil properties (Reeves 2017). The first record for Ni accumulation above 1000 mg kg^{-1}, threshold for Ni hyperaccumulation, reported for the species of *Teucrium* were those of Hossain (2007) after Kassim and Rahim (2014) who studied metal accumulation in *Teucrium polium* populations in Iran. The values 8140 mg kg^{-1} in leaves and 2300 mg kg^{-1} in stem are reported for serpentine populations of the species. Later on, Ni concentrations between 9678 and 14.110 mg kg^{-1} in above ground parts of *Teucrium polium* grown in serpentine soils in Turkey were reported by Yaman (2014). The same species is included in the list of accumulating species that belong to the Italian flora (Bazan and Galizia 2018), altogether with Pb accumulator *Teucrium flavum* subsp. *glaucum* and Hg accumulator *Teucrium scorodonia*.

The populations of *Teucrium chamaedrys* growing on non-serpentine soil types also differ in their ability to accumulate Ni and in most cases reported values are below 1 mg kg^{-1} (Obratov-Petković et al. 2008; Pavlova 2009; Pavlova and Karadjova 2012). An exception is the data provided for a calcareous site from Kopaonik Mt. (Zlatić et al. 2017) where a value of 9.65 mg kg^{-1} was measured. In all cases Ni concentrations were below the levels considered threshold for Ni toxicity.

The Ellenberg's indicator values used to describe the ecological relationship amongst accumulating plants, climate and soil conditions demonstrate that most of the phytoextractors naturally grow in neutral to basic soils (Bazan and Galizia 2018). The experiments with two *Alyssum* species (including *A. murale*) showed that increasing soil pH through liming increased Ni uptake when plants are grown on Ni-rich non-serpentine soils. However, liming an serpentine soil decreased Ni uptake (Kukier et al. 2004).

The phytotoxic Ni concentrations range widely among native species populations and cultivars of *Teucrium chamaedrys* growing on different substrates (Jurišić et al. 2001; Bazan and Galizia 2018). The higher concentrations were found in cultivated plants, while in *Teucrium montanum* the highest quantities of Ni and other metal ions (Cr and Cu) were found in wild species populations. This indicates that abilities of handling metal cations are not specific for serpentine populations, but can also be found in non-serpentine populations (Vicić et al. 2013a). Hence, Ni toxicity did not appear to be a universal feature of serpentine soils, but was dependent on other variables, including Mg concentration in particular; the influence of Ni sometimes even varied within a site (Kazakou et al. 2008).

In *Teucrium chamaedrys* from serpentine sites Ni is taken up in higher quantities than Cr, and the Ni/Cr ratio in plant tissues is always much higher than in the soil. Most data from Bulgarian and Serbian serpentines demonstrated low Cr concentrations in populations of *Teucrium chamaedrys* (Obratov-Petković et al. 2008; Pavlova 2009; Pavlova and Karadjova 2012). These results corroborate the data of Brooks (1987) that serpentine plants contain only trace quantities of Cr. The phytoextraction of Cr from serpentine plant populations in Goč Mt. (Serbia) was above the threshold of 50 mg kg^{-1} and according to Branković et al. (2017) it may be considered as accumulation. However, even on serpentine soils, Cr concentrations > 50 mg kg^{-1} are so uncommon that this may be used as an indicator of soil contamination (Reeves 2006). Low mobility of this metal in the soil also serves to possible dust contamination. Several native plants (from different families) from areas of serpentine or chromite deposits were found to accumulate Cr as much as 0.3% or 3.4% (Kabata-Pendias 2011).

Although, most soils contain significant amounts of Cr, its availability to plants is highly limited and controlled mainly by the soluble Cr contents of the soils. Cr bioaccessibility is a function of soil type and retention time and not easily translocated within plants, thus it is concentrated mainly in roots (Kabata-Pendias 2011). Data provided for some *Teucrium* species (Pavlova and Alexandrov 2003; Vicić et al. 2013a) confirmed this conclusion. There is a great difference in the accumulation of Cr by shoots and roots of various plants and in vegetable crops the ratio shoot/root varies widely from 0.005 to 0.027 (Kabata-Pendias 2011).

Besides the elevated concentrations for Ni and Cr measured for *Teucrium chamaedrys* from serpentine soils such high concentrations were reported also for Fe (Obratov-Petković et al. 2008; Pavlova and Karadjova 2012, 2013; Branković et al. 2017; Zlatić et al. 2017). The quantity of Fe in the tissues of the species growing on serpentines varied in wide range from extremely high unusual concentrations (1781.9 mg kg^{-1}) to almost typical contents for the species from Serbia (Obratov-Petković et al. 2008; Branković et al. 2017, Zlatić et al. 2017) and in range 538–2740 mg kg^{-1} for the species from Bulgaria (Pavlova and Karadjova 2012). The same high concentrations were also reported for different species growing on metalliferous soils (Cornara et al. 2007; Dudić et al. 2007; Golubović and Blagojević 2013; Franco et al. 2013, Sawidis et al. 2014). Values > 1000 mg kg^{-1} often indicate contamination by serpentine soil or dust, not easily removed by simple washing procedures (Reeves et al. 1999). However, higher Fe concentration seems to be characteristic for the serpentine flora of the Balkans and more attention should be given on this phenomenon (Babalonas et al. 1984). Such behavior of some serpentine plants might be explained with their ability to acidify the rhizosphere and to increase the solubility of ferric ions and their reduction to even more soluble ferrous ion. However, where Fe is easily soluble, plants may take up a very large amount of Fe and this is clearly shown by vegetation grown in soils derived from serpentine and excessive Fe uptake can produce toxic effects in plants. The variation among plants in their ability to absorb Fe is not always consistent and is affected by abiotic factors – soil, climate, and biotic conditions like the stages of plant growth and the ability to accumulate Fe (Kabata-Pendias and Pendias 1984; Kabata-Pendias 2011). In most cases *Teucrium chamaedrys* growing on serpentines demonstrates abnormal levels of Fe (> 200 mg kg^{-1}) in tissues according to the Element Concentration Cadastre in Ecosystems presented at the 25th General Assembly of the International Union of Biological Sciences (Lieth and Markert 1988). The accumulation of the metal in plant organs is different depending on the species, for example Sawidis et al. (2014) mentioned that Fe in the root was more in tree, shrub and cultivated plant samples but in non-cultivated and aquatic plants the leaves showed the highest Fe values. Also, Fe concentrations in roots of *Teucrium montanum* and *T. polium* were higher than in the leaves, stems and flowers (Pavlova and Alexandrov 2003; Kassim and Rahim 2014).

The concentrations of Co in *Teucrium chamaedrys* were lower than Cr in samples suggesting that the plant adapted to serpentine soil did not accumulate Co (Wallace et al. 1982). The concentrations for Co seldom reached 10 mg kg^{-1} in plants on normal soils and even on serpentine soil this level is not often exceeded. Co concentrations in *Teucrium chamaedrys* were in range 0.9–9.4 mg kg^{-1} for serpentine populations and 0.07–0.4 mg kg^{-1} in non-serpentine populations in Bulgaria (Pavlova and Karadjova 2012). Data from serpentines in Serbia (Branković et al. 2017) also fall in this range.

The concentrations of essential elements Mn, Cu and Zn measured in *Teucrium chamaedrys* from Bulgaria and Serbia (Obratov-Petković et al. 2008; Pavlova 2009; Pavlova and Karadjova 2012, 2013; Branković et al. 2017; Zlatić et al. 2017) were within the ranges considered normal for plants both from serpentine and non-serpentine sites (Kabata-Pendias and Pendias 1984; Reeves 2006). Usually, serpentine soils are not rich in Cu, Zn, and Pb, and typical concentrations of these elements in *Teucrium chamaedrys* tissues are below the limits for toxicity. However, results for Zn and Pb concentrations were higher in species populations from Bulgaria in comparison with the data from Serbia (Obratov-Petković et al. 2008; Branković et al. 2017) and from Albania (Shallari et al. 1998) result of possible contamination from recent polymetal mining activities and aerosol deposition due to heavy road traffic near some sites. Also, the quantity of Zn in plants changes with the growing season and often shows an increase throughout the season (Antonovics et al. 1971).

Cadmium is also not part of the "serpentine syndrome" and in most serpentine soils demonstrates relatively lower levels close to typical ranges for unpolluted regions. The concentrations of this element in tissues of *Teucrium chamaedrys* reported from some serpentine sites in Bulgaria were between 0.2 and 1.43 mg kg^{-1} (Pavlova and Karadjova 2012), while in Serbia Cd was below the toxic levels (Branković et al. 2017). The degree to which plants are able to take up Cd depends on its concentration in the soil and its bioavailability modulated by the presence of organic matter, pH, redox potential, temperature and concentration of other elements (Di Toppi and Gabbrielli 1999). Cd and Pb as well, are considered to be effectively absorbed by the root and leaf systems showing a great difference in the ability of various species to accumulate them (Kabata-Pendias and Pendias 1984). In general, Cd concentration in plant tissues is in the order:

root > stem > leaves > grains, although possible absorption directly from the atmosphere can change it (Zhang et al. 2006). Cadmium is variable within species populations of *Teucrium chamaedrys* and the variability of Cd accumulation within populations can be of genetic origin or due to local environmental factors (Reeves 2006). The absorption and accumulation of Cd in plants is also dependent on the growth stage and metabolic activity (Zhang et al. 2006). Based on the difference in absorption, transport and accumulation of heavy metals, *Teucrium chamaedrys* can be classified as an excluder.

Over the diverse range of sites, sampling times of the species and climatic conditions involved in the field collections, it is not appropriate to try to draw conclusions about the detailed interactions of the major and trace elements.

4.3.2 Plant-Soil Correlations

Correlations between elements in the aerial plant parts and elements in the rhizosphere serpentine soils were calculated, taking into account all studied soils and plants (*Teucrium chamaedrys*, *Teucrium polium* and *T. montanum*) as average values for Bulgaria (Pavlova and Karadjova 2012, 2013). Positive correlation coefficients were found for Ni, Cr, Fe, Co, and Cu concentrations in plants with Ca in the soil. Significant negative correlations were found between Mg in plants and Mg in soil confirming plant ability to exclude Mg from soil. However it should be also emphasized that the correlations between measured elemental concentrations in plant populations and their rhizosphere soils are species specific (Pavlova and Karadjova 2013). Highly significant correlations at level $p < 0.01$ were observed between Fe, Ni, Cr, and Co in *Teucrium chamaedrys* samples and Fe in the soils for Bulgarian serpentine sites. However, it should be mention that all these correlation patterns are for total element content in soils, which most probably will be changed if their bioavailable fractions have been considered.

4.3.3 Bioaccumulation Factors

The uptake of heavy metals depends on their concentration 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 (Kashem and Singh 2002). The bioaccumulation factor (BAF) calculated as a ratio between the concentrations of elements in plants and in the respective soils provides information or potential bioavailability modes of absorption and accumulation of specific elements in plant tissues. For most cases in *Teucrium chamaedrys* BAF has a value below 1 (Pavlova and Karadjova 2013; Zlatić et al. 2017). BAF values higher than 1 were reported for K both from serpentine and calcareous populations of *Teucrium montanum* and *T. chamaedrys* by Zlatić et al. (2017), whereas BAF values higher than 1 were calculated for Ca from serpentine populations of the same species. The bioaccumulation trends in the species are the following: Zn > Cu > Cd > Pb > Ni > Co > Mn > Cr > Fe. It is worth mentioning that the bioaccumulation trend follows the bioavailability of trace elements. Higher BAFs could be expected for elements like Zn, Cu, Ni, and Pb. Extractable fraction for these elements is reported to be between 2% and 8%. BAFs for elements like Fe and Cr with extractable fraction below 0.05% are much lower (Pavlova and Karadjova 2013). Almost the same is the bioaccumulation trend for the species shown by Zlatić et al. (2017) and this fact confirms the specific adaptation strategy of the species to the metals. Zinc and Cu are the metals with the highest biouptake abilities, while Cr and Fe have the lowest. Zn is regarded highly mobile from some authors, while others consider Zn to have intermediate mobility. Soluble forms of Zn are readily available to plants and uptake of Zn was reported to be linear with concentration in soils and in nutrient solution (Kabata-Pendias 2011). The mechanism of Cu uptake is different from the uptake of Zn (Antonovics et al. 1971). In the above ground plant parts of *Teucrium chamaedrys,* both from serpentine and non-serpentine soils, Cu uptake stays low and almost constant at low levels of soil Cu. The plant reaction to Cu in the soil suggests that the mechanism of exclusion is available. Plant Cu concentrations are controlled within a remarkably narrow range and extractable Cu was about 2% (Pavlova and Karadjova 2013). The bioavailability of Pb in *Teucrium chamaedrys* above-ground plant parts determined through extractable fraction in 0.43 M acetic acid was about 3%, higher compared to extractable Cu (Pavlova and Karadjova 2013). It is considered that plants are able to take up Pb from soils to a limited extent even under contaminated soil conditions (Kabata-Pendias 2011).

Ni is considered to be a major contributor to the serpentine factor because of the very low availability of Cr and the relatively low abundance of Co compared to Ni (Brooks 1987). The uptake of Ni in *Teucrium chamaedrys* populations was demonstrated to be variable and extractable Ni was about 5% (Pavlova and Karadjova 2013). Nickel is usually easily extracted from soils by plants and its contents of plants are functions of Ni forms in soils. Both plant and pedological factors affect these processes and the most pronounced factor is soil pH (Kabata-Pendias 2011). Although only 1% of the total Ni in soils is available to plants, this far exceeds the percentage of available Cr (Brooks 1987). The low solubility of Cr is reflected in the very slight uptake of this element by plants. In the case of *Teucrium chamaedrys* Cr demonstrates very low extractable fraction, below 0.05%, similarly to Fe. According to Brooks (1987) Fe in serpentine soils is very insoluble and typically only about 0.01% of the total Fe is extracted. It is possible that *Teucrium chamaedrys* strictly selects elements and the absorbed amount and this mechanism enables survival. Thus, in addition to the internal factors of control of mineral content in plant such as genetic specificity, the properties of the root system and leaves, ontogenetic development of the species, external factors should also be considered as very important (Rašić et al. 2006). It is known that the availability of some heavy metals decreases with rising pH of the soil, organic matter and clay content (Mengel and Kirkby 1987; Obratov-Petković et al. 2006; Kabata-Pendias 2011). Among the external factors soil acidity has a strong influence on ion mobility and their uptake.

Weak acidic to weak alkaline conditions of the rhizosphere soil favor strong binding of toxic element in the soil, on one hand, and optimal bioavailability of nutrients as they are essential elements (Rašić et al. 2006).

4.3.4 Species Adaptations to Metalliferous Soils: Quantity of Secondary Metabolites

Secondary metabolites play a major role in the environmental adaptation of plants. The concentrations of various secondary plant products are strongly dependent on the growing conditions and their accumulation occurs in plants subjected to stresses including various elicitors or signal molecules (Nasim and Dhir 2010; Akula and Ravishankar 2011). Heavy metals, as well as other abiotic stress factors (temperature, salinity, water, radiation, etc.), can influence biosynthetic pathways of secondary metabolites (Mahajan and Tuteja 2005) and suppress or stimulate their production. The impact of heavy metals on plant secondary metabolite production is in relation to their concentration and exposure time (Berni et al. 2018). The plant secondary metabolites play numerous roles in plant protection under different biotic and abiotic influences (Ibrahim et al. 2017). Drought resistance is attributed to flavonoids or other phenolic compounds which may also help plants to tolerate soils that are rich in toxic metals (Treutter 2006). It is confirmed that due to the presence of heavy metals certain plants increase the synthesis of phenolics (Michalak 2006; Zlatić et al. 2017; Zdraveva et al. 2018).

As a result of the specific heavy metal profiles of the soils, differences in the quantity of secondary metabolites in *Teucrium chamaedris* were found. In all serpentine plant populations significantly higher values of secondary metabolites were reported compared to non-serpentine ones (Stanković et al. 2010; Zlatić et al. 2017; Zdraveva et al. 2018). The serpentine populations of *Teucrium chamaedrys* are enriched in phenolic compounds similarly to other medicinal plants growing on serpentine substrates (Stanković et al. 2011a, b, 2015; Veličković et al. 2014) and they could be considered as appropriate sources of natural antioxidants. The total amount of phenolic compounds and flavonoids both in extracts and plant material (above-ground parts) of *Teucrium chamaedrys* populations from serpentines were higher compared to non-serpentine (calcareous) populations according to Zlatić et al. (2017). The data presented for Bulgaria (Zdraveva et al. 2018) also confirmed higher values of these compounds in serpentine populations. The content of phenols and flavonoids in plant extracts of *Teucrium chamaedrys* depends on the type of extracts and polarity of solvents used for extractions (Stanković et al. 2010).

The concentrations of phenols and flavonoids vary in the plant body of the species. Stanković et al. (2010) showed the highest concentrations of phenols and flavonoids in leaf extracts in water, acetone and petroleum ether, smaller in flower extracts, and lowest in stem extracts. The large number of glandular hairs, rich in phenolic components, is considered as a reason for higher concentrations of phenols found in some stem extracts of *Teucrium chamaedrys* var. *glanduliferum* from Serbia (Stanković et al. 2010). The concentrations of phenols and flavonoids in different plant parts compared to those of the whole plant are also different.

The phenolic compounds contribute to the antioxidant potential of plants by neutralizing free radicals and preventing decomposition of hydroperoxides into free radicals (Jain et al. 2014). The high concentration of phenolics, as well as the properties of these molecules and the positive correlation found between the concentration of phenolic compounds and antioxidant activity of different plant extracts of *Teucrium chamaedrys*, indicated that these compounds contributed to the strong antioxidant activity (Stanković et al. 2010). The highest antiradical effect has been also detected in purified methanol *Teucrium chamaedrys* leaf and root extracts from Italy (Pacifico et al. 2009) but the methanol extracts collected from serpentine *Teucrium chamaedrys* populations possessed stronger antioxidant capacity. According to Stanković et al. (2010) both methanol and acetone extracts of *Teucrium chamaedrys* have high concentration of total phenols and flavonoids, which is in correlation with the intense antioxidant activity of these extracts and have a crucial role in the adaptation of this plant to the adverse effects of heavy metals.

The plant extracts of *Teucrium chamaedrys* populations demonstrated variation in the concentrations of tannins as well (Zdraveva et al. 2018). The tannin content was higher in serpentine samples and it was comparable to data for Croatia reported by Grubešić et al. (2012). The amounts of total polyphenols and tannins was found to vary also among the *Teucrium* species (Grubešić et al. 2012; Maleš et al. 2015) and their quantity was higher in native than in cultivated samples in coherence with the environmental factors (temperature, humidity, light intensity, water, minerals and CO_2). The content of total polyphenols and tannins in the samples of *Teucrium chamaedrys* and other studied species reported by Maleš et al. (2015) is significantly lower in comparison to data presented by Grubešić et al. (2012) because of the different method used for quantitative analysis. However, polyphenols represent most abundant and widely distributed antioxidants that have beneficial health promoting effects and confirm that *Teucrium* species are good source of polyphenols (Maleš et al. 2015).

Exposure to heavy metals leads to accumulation of harmful reactive oxygen species (ROS). Reactive oxygen species (ROS), generated during heavy metal stress, may cause lipid peroxidation that stimulates formation of highly active signaling compounds capable of triggering production of bioactive compounds (secondary metabolites) that enhance the medicinal value of the plant (Nasim and Dhir 2010; Berni et al. 2018).

Phenolic compounds and other secondary metabolites have numerous pharmacological properties and thus any environmental condition that affects either the quantity or composition of phytochemical compounds may potentially influence the efficacy of the medicinal plant product. There is limited information on specific physiological responses of medicinal plants to heavy metals in soils and the resulting changes in the efficacy of the plant (Ibrahim et al. 2017).

4.3.5 Species Adaptations to Metalliferous Soils: Morphology

Plants growing on metalliferous soils often possess morphologies distinct from their relatives growing on normal soils. Soil nutrients, moisture stress, photoperiodism, temperature effects, photosynthesis and respiration, all have elicited racial differentiation in a variety of seed plants (Kruckeberg 1969). Intraspecific variation leading to local adaptation (i.e. ecotypic differentiation) has been frequently cited (Štepankova 1996, 1997; Rajaharuna and Bohm 1999; O'Dell and Rajakaruna 2011) for species found on and off serpentine soils. The specific serpentinomorphoses are described and summarized by many authors (Pichi-Sermolli 1948; Krause 1958; Ritter-Studnička 1968). The morphological characteristics of serpentine plants are three main groups: xeromorphic foliage, reduction in stature, and increase in root system (Kruckeberg 1984). Several of these traits have been shown to be genetically determined (Westerbergh 1994).

Although *Teucrium chamaedrys* is widely distributed in serpentine and non-serpentine habitats it has high abundance and potential preference for alkaline soils (Obratov-Petković et al. 2006). The growth habit of *Teucrium chamaedrys* differs between serpentine and non-serpentine sites as it was documented for many species (Brooks 1987; Vergnano Gambi 1992; Westerbergh and Saura 1992; Mayer and Soltis 1994; Štepankova 1996, 1997; Westerbergh and Rune 1996; Bratteler et al. 2006; Wright et al. 2006; Boyd et al. 2009; Salmerorn-Sarnchez et al. 2018). The plants are typically adapted to dry soils and are of smaller stature than non-serpentine relatives. *Teucrium chamaedrys* populations are dense and well developed in mesophilous habitats on calcareous and siliceous terrains compared to serpentine populations. The serpentine populations are characterized by their reduced size – lower stems, smaller leaves, and shorter internodes compared to the non-serpentine populations (Pavlova 2009). The variation is higher for the vegetative features and not clearly expressed for the generative ones. The positive correlations within the floral and vegetative trait groups do not indicate functional relationships among traits. Confirming Conner and Sterling (1996) it was concluded that correlations between both groups of traits were due at least in part to overall size relationship and reflect common developmental pathways.

Studying ecobiology of *Teucrium chamaedrys* in some regions of Serbia Obratov-Petković et al. (2006) noted that populations of *Teucrium chamaedrys* growing on serpentines were less compact than those growing on limestone; the plants growing on serpentine were smaller than the same species on limestone; the leaves of plants on serpentine were smaller, covered with more hairs and many of them were grey-green in comparison with the plants on limestone. The leaf indumentum has adaptive value for species because the hairs allow to gain a higher rate of carbon under arid condition than the leaf acquire without hairs to avoid potentially lethal condition of soil and loss of water which allows the plant to extend its growth for a longer period into the drought (Azmat et al. 2009). Important characteristic for the individuals growing on serpentines is denser indumentum and longer simple or glandular hairs covering the stems, leaves, and bracts which is related to the dry habitat conditions provided by the serpentine. For most serpentine populations of *Teucrium chamaedrys* are reported 3–5 celled glandular hairs densely covered plant parts – leaves, stem and bracts (Pavlova 2009). Such kind of hairs is not mentioned by Bini-Maleci and Servettaz (1991), Navarro and El Oualidi (2000) or Grubešić et al. (2007) for this species and can be considered specific for the serpentine populations. Because hairs can play an important role in various aspects of plant physiology and ecology in *Teucrium*, further investigations are needed to demonstrate the ecotypic differentiation in *Teucrium chamaedrys* found on serpentine and non-serpentine soils (Pavlova 2009).

4.4 Plant Life on Selected Edaphic Conditions – *Teucrium montanum*

4.4.1 Metal Accumulation by Teucrium montanum

Plant species are exposed to different environmental factors. Among them, the influence of geological substrate and soil was considered as the most important. The effects of the edaphic factor originate from the abiotic and biotic components of the soil as a dynamic system. The process of pedogenesis is a complex of physical and chemical process determined by the type of geological substrate, climatic conditions, living organisms, etc. Edaphic factors in plants affect the appearance of various morpho-anatomical and ecophysiological properties in the function of adaptation. Differentiated groups of species was adapted to a wider range of edaphic conditions variation, as well as groups of species specialized for a substrate making special edaphic ecotypes. Due to the development of complex adaptation mechanisms to a certain type of substrate, the distribution of some ecological groups was completely determined by the type of substrate, while for some groups, there is a certain degree of tolerance in terms of variation of edaphic conditions.

Plant species, that are widespread on habitats with a certain type of substrate, developed the ability to tolerate habitats that are characterized by completely different edaphic conditions. Soil formed on the calcareous geological substrate contained calcium carbonate in free form, which determined the alkaline reaction and limited availability of certain elements to plants, such as N, P, K, Mg, Zn, Cu, and Fe. The soil formed on the serpentine geological substrate represents a special substrate, with extreme physical and chemical characteristics. The disturbed mineral regime, the disturbed Ca:Mg ratio, the lack of essential nutrients Ca, P, N, K, increased concentrations of heavy metals, such as Mn, Ni, Cr and the variation in pH from acidic to strong alkaline, are one of the most significant characteristics of serpentine soils (Zlatić et al. 2017).

Teucrium montanum L. (Lamiaceae) is a facultative serpentinophyte (Fig. 4.2) and it is an ideal model organism for comparative analysis of ecological differentiation relative to the substrate such as serpentine and calcareous substrate (Fig. 4.3).

Fig. 4.3 Serpentine (left) and calcareous (right) habitat of *T. montanum* L. (Photo M. Stanković)

Teucrium montanum absorbs a large number of elements from soil, essential (Cu, Fe, Mn, Ni, Zn) and non-essential (Cr, Co, Pb) for its biological functions. Many authors state that the most stressful component of the plant was disturbed Ca:Mg ratio, that affects the establishment of viable populations of *Teucrium montanum* species on serpentine habitats. The Ca:Mg ratio is generally in values above 1 in plants, while the plants present on the serpentine could have values higher than 1, regardless of the minimum concentration of Ca in the soil. It has been confirmed that Ca deficiency had an influence on root elongation and shoots growth (Marschner 1995). Since Ca and Mg are available to the plants through the roots, plants need a special mechanisms to maintain the adequate Ca:Mg ratio in the species that grow on serpentine. These mechanisms allow selective Ca and Mg exclusion, despite high concentrations in the soil, as well as the translocation of Ca and Mg from the root into the above-ground plant parts (Vicić et al. 2013b).

In the study of *Teucrium montanum* populations on the serpentine localities in Serbia and Bulgaria, similar amounts of accumulated Ca and Mg was found in the above-ground plant parts. Plant populations sampled from serpentine soil accumulated higher quantities of Ca than the population sampled from calcareous soil (Pavlova and Karadjova 2012). Juranović-Cindrić et al. (2013) described that the population of *Teucrium montanum* accumulated between 30 and 40 times more Mg than Ca, which indicated that the species process a high ability to absorb Mg in habitats where the concentration of Mg is lower than the concentration of Ca.

Calcium concentrations found in tissues of *Teucrium montanum* were between 1356.5 and 2237.7 mg kg^{-1} in serpentine plant populations from Serbia (Zlatić et al. 2017), and between 2980 and 4798 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results in presented research indicated that the amount of Ca, in soil samples and in samples of plants on the calcareous substrate, is higher in relation to the quantity in samples from the serpentine substrate. The increased amount of Ca in the soil affects the amount of Ca in the plants (Zlatić et al. 2017). Calcium is involved in 0.1–2.0% of dry matter of plants. The normal concentrations of this element in the above-ground parts of the plants were 5 g kg^{-1} (Shallari et al. 1998). The content of Ca in plants growing on serpentine soil is lower than 0.8% (Kataeva et al. 2004). Concentration of calcium was higher in the aboveground parts of plants compared to the substrate (serpentine or calcareous), although the serpentine species are better adapted to low Ca concentrations. The plants, which have normal growth, the Ca:Mg ratio is higher than 1. The serpentine species are adapted to low Ca concentrations relative to Mg, since Mg prevents the absorption of Ca (Brooks 1987). Calcium is an essential macronutrient which participates in the construction of the cell wall, provides normal transport through membrane proteins and acts as a cofactor for many enzymes (Marschner 1995). Differences in concentration of Ca in soil and plant material from serpentine habitats could be due to the presence of organic matter and the effects of various ecological factors presented on a specific type of ecosystem (Zlatić et al. 2017).

The amount of Mg in plant samples of *Teucrium montanum* was in the range from 1476.6 to 3651.4 mg kg^{-1} (Zlatić et al. 2017), and in the range from 1932 to 4626 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results of the study indicated that the amount of Mg in the plant samples on the serpentine substrate (Goč and Kamenica) was higher than in the samples from the calcareous substrate (Kopaonik and Durmitor) (Zlatić et al. 2017). Increased concentrations of Mg in serpentine plants population indicated their ability to adapt and survive under stressful conditions. The differences in quantity between Mg and Ca affects the appearance of undeveloped vegetation on serpentine soils. Plants could grow successfully, if the ratio of the total amount of Ca:Mg in the soil is at least in ratio 1:1 (Brooks 1987). Magnesium is macronutrient that plants used for their growth and development in large quantities (Karley and White 2009). Also, this element is located at the center of the chlorophyll molecule and had a significant function for many enzymes and chelating nucleotide formations (Shaul 2002). Lack of Mg in plants could affects the increase in the activity of antioxidant mechanisms in order to neutralize harmful effects (Cakmak and Kirkby 2008). The obtained results of the comparative analysis showed that the samples of soil and plant material from the serpentine geological substrate had a higher concentration of Mg from the calcareous substrate. Certain amounts of Mg are required by plant nutrition, and studies showed that plants could metabolically control the absorption of Mg from the soil. The differences in the concentration of Mg between serpentine localities could be attributed to the different influence of pedological processes and ecological factors that were dominate on the investigated habitats (Zlatić et al. 2017).

The amount of K in plant samples of *Teucrium montanum* was in the range from 6221.4 to 12338.6 mg kg^{-1} (Zlatić et al. 2017). The results of this study indicated that the amount of K in soil and plant samples from calcareous substrate, was higher in relation to the quantity in samples from the serpentine substrate. The increased amount of K in the soil influenced the amount of K in the plants (Zlatić et al. 2017). Potassium is one of the main nutrient, which affect the growth and development of plants. It is the most important element in plant nutrition of all alkali elements found in the soil (K, Ca, Mg, and Na) (Haby et al. 1990). Due to the disturbed water regime, plants require higher concentrations of K for adaptation to stress caused by photo-oxidative damage. Potassium had an important role in the protection of plants, which are presented in arid habitats (Sen Gupta et al. 1989).

The accumulation of Ni, Cr, and Co in the above-ground plant parts for all serpentine populations of *Teucrium montanum* were higher than in non-serpentine ones (Pavlova and Karadjova 2012; Branković et al. 2017; Zlatić et al. 2017). Element concentrations vary between the populations in relation to soil properties. Nickel quantity in plant samples for *Teucrium montanum* was in the range from 9.8 to 108.6 mg kg^{-1} (Zlatić et al. 2017). The results of this study indicated that the amount of Ni, in soil samples and in samples of plants on the serpentine substrate, was higher than the quantity in samples from the calcareous substrate, as well as that the increased amount of Ni in the soil influenced the amount of Ni in the plants. In the plant species from serpentine, the absorption of certain nutrients had been changed because of stress induced by the presence of Ni. Certain concentrations of this element in plant tissue inhibited the process of photosynthesis and transpiration. In natural conditions, the toxicity of Ni is in conjunction with serpentine soils which possess great amounts of this metal (Kabata-Pendias 2011). Nickel is very mobile in the soil and showed the ability to cross from one soil layer to another. Fe and Mg oxides could accumulate large amounts of Ni. After this accumulation, Ni is available to plants. Influence on the bioavailability of Ni in the soil depended of the presence of organic matter, clay fractions and pH values (Kastori 1993). It has been showed that the soils formed on the serpentine substrate had a higher concentration of Ni than those on limestone (Zlatić et al. 2017). The composition of the geological substrate plays an important role in the presence of Ni in the soil. The difference between localities with the same geological substrate could be attributed to the various environmental factors, that affect the certain habitat, as well as the chemical processes in the upper layers of the soil.

The amount of Cr in plant samples of *Teucrium montanum* was in the range from 4.15 to 45.3 mg kg^{-1} (Zlatić et al. 2017) and between 0.3 and 28 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results of presented research indicated that the amount of Cr, in soil samples and in samples of plants on the serpentine substrate, was higher than the amount in samples from the calcareous substrate on the territory of Bulgaria and Serbia. The increased quantity of Cr in the soil affects the quantity of Cr in plants (Zlatić et al. 2017). Chromium content in plants is affected by the amount of soluble Cr in the soil. In the mentioned studies, there is no data based on the important role of Cr in plant metabolism. However, there are data which described the positive influence of Cr on plant growth. The most accessible for plants is Cr^{6+}, which in certain soils is unstable and whose distribution depends on the characteristics of the soil, its texture, and pH value. In some plant species from the serpentine region, the Cr concentration varies from 0.3% to 3.4% (Kabata-Pendias 2011).

The amount of Fe in the plant samples of *Teucrium montanum* was in the range from 351.9 to 2673.1 mg kg^{-1} (Zlatić et al. 2017) and between 148.5 and 1490 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). In the presented studies the amount of Fe, in soil samples and in samples of plants on the serpentine substrate, was higher than the quantity in samples from the calcareous substrate. Iron is a reactive element and it is similar like Co and Ni. Studies have confirmed that the reactivity of Fe depends on the pH value and the oxidative degree of the Fe compound (Kabata-Pendias 2011). The distribution of the Fe compound in the soil can be useful in determination the type and characteristics of the soil (Zonn 1982). The concentration of Fe in the soil solution at usual pH value varies between 30 and 550μg l^{-1}. In very acidic soils, it could be over concentration of 2000μg l^{-1}. Therefore, in calcareous soil types, with pH from 7 to 7.8, concentration of Fe ranged from 100 to 200μg l^{-1}. In sandy substrates, whose pH varies from 2.5 to 4.5, concentration of Fe ranged between 1000 and 2223μg l^{-1}. The solubility level of Fe is minimal in alkaline soils. Acidic soils are rich in soluble inorganic Fe in relation to soil from neutral and limestone substrates. Reduced quantities of Fe are characteristic for dry areas (Kabata-Pendias 2011). Although the Fe compounds in the soil are mobile, there is a tendency for the formation of mobile organic compounds and chelates. These compounds caused the distribution of Fe in the soil layers, as well as the transition of ion Fe from one layer to another that plants absorb through the root system. The quantity of Fe, that plants could absorb, depends on the changing conditions of the soil and climate, and the stage of plant development. However, when Fe is soluble, plants may absorb a higher amount (Kabata-Pendias 2011). This was clearly demonstrated in plant samples from serpentine habitats where the Fe concentration in the tested grasses were between 2127 and 3580 mg kg^{-1} (Johnston and Proctor 1977). Fe oxides bind to Mn compounds and this is the reason of why Fe is more represented in the serpentine substrate (Kabata-Pendias 2011). Certain amounts of Fe are required by plants in the nutrition. Plants regulate the concentration of the same in their tissues through metabolism. The concentration of Fe affects the composition of the parent material, pedogenetic processes, the presence of organic matter and other factors. The difference in the concentration of Fe between similar sites could be explained through different environmental factors, which are dominated in the specific habitat.

The concentrations of essential elements, Mn, Cu and Zn measured in *Teucrium montanum* from Serbia and Bulgaria (Pavlova and Karadjova 2012; Zlatić et al. 2017) were within the ranges considered normal for plants from serpentine and non-serpentine sites (Kabata-Pendias 2011). Usually, serpentine soils are not rich in Cu, Zn, and Pb. The amount of Mn in the plant samples of *Teucrium montanum* was in the range from 22.16 to 78.3 mg kg^{-1} (Zlatić et al. 2017) and between 16 and 99 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results of the study indicated that the amount of Mn, in soil samples and in samples of plants on the serpentine substrate, was higher than the quantity in samples from the calcareous substrate. By reducing the amount of Mn in the soil, the amount of Mn increases in the above-ground parts of the plants. High levels of Mn are observed in soils that are present on serpentine soil, in soil rich in Fe and organic matter, as well as in arid and semi-arid regions of the earth (Kabata-Pendias 2011). Certain quantities of Mn are required in plant nutrition, and studies showed that plants could metabolically control the absorption of Mn from the soil (Skinner et al. 2005). Numerous studies showed the relationship between the amount of Mn and Fe represented in the soil. Manganese was significantly more present in areas where Fe oxides are present (Kabata-Pendias 2011).

The amount of Cu in plant samples of *Teucrium montanum* was in the range from 1.68 to 8.35 mg kg^{-1} (Zlatić et al. 2017) and between 5.7 and 15.4 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The quantity of Cu, in the soil samples and in the samples of plants on the serpentine substrate (Goč and Kamenica), was higher than the quantity in the samples from the calcareous substrate (Kopaonik and Durmitor). With reducing the amount of Cu in the soil, the amount of Cu in plants grows. Plants sampled on serpentine soil had a higher concentration of Cu than those on limestone. However, minor deviations were observed, which is in accordance with the above data on the bioavailability of Cu (Zlatić et al. 2017). The concentration of Cu was influenced by the presence of organic matter, pedogenetic processes, and the composition of the parent material.

According to the results presented by the study from Zlatić et al. (2017), the quantity of Zn in the above-ground plant parts of the species *Teucrium montanum* parts varied from 14.79 to 39.75 mg kg^{-1} in populations from Serbia, and between 29 and 70 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results indicated that the quantity of Zn, in soil samples and in samples of plants from the calcareous substrate, was higher than the quantity in samples from the serpentine substrate, and that the increased amount of Zn in the soil influenced the amount of Zn in the plants. The composition of the parent material, the structure of the soil, the presence of organic matter, the ecological factors, the pH value are one of the factors that influenced the amount of Zn in the soil. The concentration of Zn in plant tissues varies between 15 and 150 mg kg^{-1}, while maximum values were up to 300 mg kg^{-1} (Brunetti et al. 2009). In the acidic environment, Zn was very soluble (Kastori 1993). In previous studies, it has been found that the lack of Zn disrupted the absorption of Fe (Graham et al. 1987). Tolerant plant species had ability to reduce the effect of increased concentrations of Zn. Mechanisms of defense are reflected in the adaptation of metabolic reactions in the plant organism. Plants accumulate Zn in some parts of the cell and tissue (Kabata-Pendias 2011).

The amount of Pb in plant samples of *Teucrium montanum* was between the detection limits to 6.02 mg kg^{-1} (Zlatić et al. 2017), and between 0.6 and 21.7 mg kg^{-1} in plant populations from Bulgaria (Pavlova and Karadjova 2012). The results of the authors suggested that the amount of Pb in the soil samples from the calcareous substrate was higher than the quantity in the samples from the serpentine substrate, while the opposite results were showed in the plant samples. Lead is the least mobile among heavy metals in the soil, although its sorption was lower than Zn and Cu (Vega et al. 2007). The Pb concentrations in soils formed on the calcareous substrate are always higher than those formed on the serpentine (Kabata-Pendias 2011). Lead behaves like Ca, therefore, it had less in plants on calcareous soil. The obtained results were in accordance with numerous studies related to the absorption of Pb by plants present on the serpentine substrate (Reeves et al. 2007; Kabata-Pendias 2011).

Based on the results presented in the studies conducted by Pavlova and Karadjova (2012) and Zlatić et al. (2017), it can be concluded that *Teucrium montanum*, which accumulate a higher amounts of heavy metals (Ni, Fe, Mn, Cu), is not a hyperaccumulator of these elements. The investigated species possess physiological mechanisms that allow adaptation of various stress conditions, caused by increased concentrations of heavy metals in the soil, as well as specific conditions of the habitat on the serpentine substrate.

4.4.2 Plant-Soil Correlation

Correlations between elements in the above-plant parts of *Teucrium montanum* and elements in the serpentine soils from the territory of Serbia were calculated as average values (Zlatić et al. 2017). Significant positive correlations at level $p < 0.01$ were observed between Ca and Zn in *Teucrium montanum* samples and Ca in the soils for Serbian serpentine sites. Negative correlation coefficients were found for Ni and Mg plant samples and Ca in the serpentine soils. Also, the synergistic effect between elements Fe, Ni, Cr, Mn in serpentine soils were found. It has been established that the contents of Mn and Cu in the soil and those of Mn and Fe in the plants correlate. The studies confirmed the antagonistic and synergistic effects of Mn in the process of absorption of Pb and Cd; antagonistic effects of Mn in the process of N, Na and K absorption and the substantial impact of Zn on the low absorption of Mn (Kabata-Pendias 2011). Other scientists confirmed the existence of correlations between certain metals (Yan et al. 2012; Gonneau et al. 2014).

4.4.3 Bioaccumulation Factors

Concentration and the absorption of elements in plant tissues depended on the concentration in the substrate and the ability of the plant to absorb certain amounts of the same. Absorption of certain macro- and microelements is regulated by special mechanisms in plants. Bioaccumulation factor (BAF) is the ratio between the total content of metals in the plants and the content of metals in the soils, and it is used to determine the total amount of metal that the plant accumulated from the soil through the root system into organs (Pandy and Tripathi 2010). The BAF results for *Teucrium montanum* on calcareous and serpentine habitats in most cases had values below 1 (Zlatić et al. 2017). BAF values higher than 1 were observed for K from calcareous and serpentine populations of *Teucrium montanum* while values higher than 1 for Ca were observed for populations of the species of serpentine habitat (Zlatić et al. 2017). The bioaccumulation trends in the species are as follow: Zn > C u > Cr > Mg > Mn > Ni > Pb > Fe.

Factors affecting the mobility, accessibility, and dynamics of soil elements for plants were the pH value, the content of organic matter and clay in the soil, the mechanical composition and humidity of the soil, the content and the presence of hydrated Al and Fe oxides. Elements, such as Zn and Cd, are very mobile in the soil and could be easily accumulated by plants. Elements such as Cu, Mn, Ni, are moderately mobile in the soil and could be rapidly absorbed by plants, while Pb is relatively strongly related to soil particulates and could not be transported to the above-ground parts of plants (Kabata-Pendias 2011).

Zinc is a very mobile in most types of soil. Zinc has an essential metabolic function in the plants, and in the soil solution, it is in the form of free ions or ionic complexes. Plants absorb Zn mainly from the soil in the form of divalent cation (Zn^{2+}). It has been showed that plants absorb Zn in the form of hydrated zinc (Zn^{2+}), in the form of ionic complexes and Zn organic chelates (Kabata-Pendias 2011). The copper is quite immobile in the soil and its mobility was influenced by the organic matter of the soil, the dissolved organic matter, the pH and the content of Cu in the soil. The bioavailability of the soluble Cu forms depended on the molecular weight of the Cu complex, and on their amounts in the soil. Copper is an essential element for plants. Soil pH value play an important role in the availability of Cu and its toxicity to plants. The plants can absorb small quantities of Cu, mainly in the form of Cu^{2+} ions and chelates. Copper concentration is influenced by its concentration and the presence of other ions in soil such as heavy metals, like Zn, Mn, Fe (Kastori 1993). Chromium is a crucial element for plants, which stimulate the growth and development of some plant species. Higher concentrations of Cr showed a toxic effect on plants. The effect of Cr in plants depends on its concentration in soil, but also on the chemical and physical properties of the soil (Kastori 1993). Plants could absorb Mg in the form of Mg^{+2} from the soil solution. The availability of Mg is affected by the parent material, climatic factors, as well as the accumulation capacity of the soil. In the conditions of the acidic soil reaction, the availability of Mg was reduced by its competence with Al or Mn, while the presence of carbonates and increased concentrations of Ca, K and Na could reduce the absorption of Mg in alkaline conditions (Sigel and Sigel 1990). Manganese could be present in soils in several forms (Kabata-Pendias 2011). The plants absorb Mn in acidic soil where it is in the form of Mn^{2+} ions. Absorption of Mn is metabolically controlled while higher concentrations of this element depend on the level of Mn concentration in the soil. The accumulation of Ni had a significant influence on the content of other elements in the substrate, while it had no effect on the absorption and metabolism of Fe (Kastori 1993). Dynamics of Pb in the soil is influenced by numerous factors, like pH value and the presence of organic matter. Lead is mainly occurred in the soil, in the form of Pb^{2+}. Plants poorly can absorb and move inorganic forms of Pb into the above-ground parts, while the adsorption and transport of organic forms are relatively strong. Lead have very low bioavailability, so the roots have a great ability to accumulate Pb, which is one aspect of protecting the above-ground parts of plants. The content of easily soluble Fe fractions is limited compared to the total content in the soil. Iron is a slow-moving element in the soil and showed the tendency to form organic complexes and chelates. Iron ions are not absorbed directly from the plant, but from the complexes with organic compounds. The high level of Fe compound, its precipitation on carbonates and the competence of other cations with Fe^{2+}, are responsible for the low absorption of Fe (Kabata-Pendias 2011).

4.4.4 Species Adaptations to Metalliferous Soils: Quantity of Secondary Metabolites

Secondary metabolites are compounds that plays a role in the interaction of plants with the environment and have an influence on plant adaptation on different abiotic and biotic stresses (Kliebenstein and Osbourn 2012). Biosynthetic pathways of secondary metabolites are to some extent influenced by environmental factors such as the presence of heavy metals in the substrate, altitude, water regime, radiation, etc. (Endt et al. 2002; Politycka and Adamska 2003). The quantity of secondary metabolites is different in plant parts and depends on the development phase, season and several ecological factors (Filippini et al. 2010). Secondary metabolites participate in the process of adaptation of plant organisms to the ecological environmental conditions, and their quantity varies in plant organs depending on the abiotic and biotic factors (Ramakrishna and Ravishankar 2011). The presence of heavy metals in the substrate caused increased synthesis of secondary metabolites. It has been determined experimentally that certain plants increase the synthesis of phenolic compounds due to the presence of heavy metals. Due to the increase in the number of phenolic compounds, the activity of enzymes involved in their biosynthesis is also enhanced (Michalak 2006).

Phenolic compounds represent a large group of secondary metabolites, which have one or more aromatic rings with one or more hydroxyl groups in the structure. Within the group of phenolic compounds, two subgroups are distinguished: flavonoids and phenolic acids (Quideau et al. 2011). Flavonoids are present in all plant organs, while their amount varies according to the ecological and genetic factors (Stanković et al. 2010, 2012). The role of phenolic acids in plants is various and it reflected in the process of absorption of necessary materials from the substrate and the adaptation of the plant to abiotic and biotic stresses (Mandal et al. 2010). The particularly important role of phenolic compounds is the antioxidant activity. Phenolic compounds act as an reducing agents, donors of hydrogen, and have the properties of metal healing. The antioxidant activity of these compounds is primarily the result of their role as a donor of hydrogen, after which there are less reactive phenoxyl radicals (Quideau et al. 2011).

Zlatić et al. (2017) indicated that the total amount of phenolic compounds in plant extracts of *Teucrium montanum* was in the range from 143.42 to 190.2 mg GA g^{-1} of extract, while the quantity of flavonoids ranges from 46.5 to 54.19 mg Ru g^{-1} of the extract. For *Teucrium montanum*, the concentration of phenolic compounds and flavonoids was higher in extracts from sample originated from serpentine sites than in samples from the calcareous substrate. Also, authors indicated that the total amount of phenolic compounds in plant extracts of *Teucrium montanum* was in the range from 37.11 to 66.52 mg GA g^{-1} of the extract, while the quantity of flavonoids was in range from 15.38 to 20.9 mg Ru g^{-1} of the extract. The concentration of phenolic compounds as well as flavonoids of *Teucrium montanum* was higher in extracts from samples originated from the habitat with serpentine geological substrate compared to samples from the limestone substrate (Zlatić et al. 2017).

There are differences in the quantitative composition of secondary metabolites of plants that were sampled on calcareous and serpentine substrates (Pavlova 2009). The presence of heavy metals in the soil caused increase of secondary metabolites synthesis. It had been determined that certain plants enhance the synthesis of phenolic compounds due to the presence of heavy metals (Michalak 2006; Stanković et al. 2011b).

The increase of phenolic compounds concentration in the plant extracts from *Teucrium montanum* occurred in the response of plants to stress conditions, caused by heavy metals (Lavid et al. 2001). The total amount of phenolic compounds was higher due to the increased concentration of heavy metals (Hamid et al. 2010). The antioxidant system of *Aeluropus littoralis*, under the influence of stress caused by heavy metals, goes through biochemical changes, which are reflected in increase the concentration of phenolic compounds involved in chelation (Rastgoo and Almzadeh 2011). Rusak et al. (2005) indicated that flavonoids have multiple protective functions, such as antioxidant activity. Phenols and flavonoids can be oxidized by peroxidase and react in H_2O_2 phenolic systems, which regulate the input of heavy metals in plants (Michalak 2006). Some heavy metals can cause a decrease in volume of reducted glutathione (GSH) and insufficient activity of antioxidant enzymes, especially glutathione reductase (GR) (Schützendübel and Polle 2002). Based on this knowledge, alternative antioxidants, such as flavonoids, could be produced by plants. Flavonoids build complexes with heavy metals, making them the main systems in the adaptation of plants to stress conditions caused by increasing the concentrations of heavy metals (Michalak 2006; Korkina 2007).

The qualitative and quantitative analysis of the composition of *Teucrium montanum* secondary metabolites confirmed the presence of gentisic acid which is the most represented phenolic acid in the extracts. Also, the phenolic acids, that are present in lower concentrations, are chlorogenic, coumaric, syringic, gallic, vanillin, caffeic, and ferulic acid (Tumbas et al. 2004; Čanadanović-Brunet et al. 2006). A qualitative analysis of the essential oil showed that the main components of the volatile compounds are: β-cadenine, β-selinene, α-calacorene, β-caryophyllene, β-pinene and germacrene (Vuković et al. 2008; Bežić et al. 2011).

The phenolics contribute to the antioxidant potential of plants by neutralization of free radicals (Stanković et al. 2010). Populations of plants sampled from the serpentine localities are differentiated by the increased quantity of total phenolic compounds, as well as intense antioxidant activity in relation to plant populations sampled from calcareous localities. Higher concentration of total phenolic compounds are in correlation with the intense antioxidant activity of *Teucrium montanum* extracts and have a crucial role in the adaptation of this plant to habitats with the higher concentration of heavy metals. Differences in the concentration of secondary metabolites and expressed antioxidant activity could be attributed to a different type of habitat as well as the abiotic factors on which the populations are represented. There are differences in the quantity of phenolic compounds and antioxidant activity among plant populations from serpentine soil. This difference was induced by a different amount of metal at a specific locality, a difference in thermal and water regime, as well as the altitude at which the locality is located (Zlatić et al. 2017).

4.4.5 Species Adaptations to Metalliferous Soils: Morphology

Serpentine soils represent a special substrate for the development of flora and vegetation (Brooks 1987). Plants, which are present on the serpentine soil, possesses numerous structural and functional adaptations, which are different from the plants represented on other types of soil. Serpentine plants are characterized by "serpentine syndrome", which is a result of the disturbed relationship between Mg and Ca, heavy metal toxicity and other pedological physicochemical characteristics (Kruckeberg 1984; Brooks 1987). Additionally, to the low water potential of the soil, the plants on the serpentine have great tolerance to the increase of concentration of heavy metals from the substrate (Proctor 1999). Numerous studies have showed that populations represented in different serpentine soils have similar characteristics (Proctor and Woodell 1975; Kruckeberg 1984; Proctor and Nagy 1992). Facultative serpentinophytes showed a "serpentine syndrome" such as a complex of morpho-functional adaptations that differentiate the plant populations present on serpentine soils. These adaptations include smooth-surface leaves with waxy coatings, plants with high developed roots, plagiotropism and nanism (Kruckeberg 1984; Proctor and Woodel 1975; Brady et al. 2005).

The habitats of *Teucrium montanum* are calcareous rocks, pine forests on calcareous soil, pastures on serpentine substrate. The species occupy different type of habitats from 30 to 2000 m above sea level. Species is represented in the communities of deciduous, mixed and coniferous forests, in the communities of serpentine rocky and continental calcareous pastures, gorge and canyons, in the communities of the submediterranean region, the communities of the xerophilous type, and the slopes of the mountainous and mountain regions. In the territory of Serbia, the species *Teucrium montanum* is the most represented on the calcareous and serpentine substrate, rocky meadows and dry pastures around the pine forests (Stanković and Zlatić 2019).

Previous morpho-anatomical studies of *Teucrium montanum* from different habitats indicated that species possess phenotypic plasticity manifested through more or less xeromorphic properties. Studies of *Teucrium montanum* from calcareous and serpentine localities showed physiological and ecological differences between populations, presented on the serpentine and calcareous substrate, with specific features in the morpho-anatomical structure of serpentine plants designated as serpentinomorphoses (Stevanović and Stevanović 1985). Morpho-anatomic differences were observed in plants from different populations as a response to the adaptive abilities of the species to certain environmental conditions. In plant populations from calcareous habitats, xeromorphic characteristics were observed, which are in accordance with the general habitat conditions determined by the hygrothermal soil regime.

Teucrium montanum on these habitats is compact and densely branched bushes. The leaves are small, covered with hairs and moderately curved to the abaxial side. At the cross-section, the leaves are thicker with a distinct cuticle compared to the serpentine population. They possess a large number of vascular bundles and a more developed mesophyll. On the serpentine habitats, *Teucrium montanum* is represented in the bushy habitus form with low density. On the shoots, there are fewer leaves than on the plants from calcareous habitats. Leaves are smaller and ticker in relation to leaves from calcareous populations, while the edges of the leaves are strongly curved to the abaxial side. At the cross-section, the leaves are thin with a well-developed cuticle. Cells of mesophyll are smaller with poorly expressed intercellular space. In plants with serpentine habitats, xeromorphic characteristics are strongly expressed in connection with the serpentine substrate and the effect of physiological drought (Stevanović and Stevanović 1985).

4.5 Conclusions

This chapter is a review of the ecological, physical, chemical, morphological and geographical distribution of two selected species of the genus *Teucrium*. *Teucrium chamaedrys* and *T. montanum* demonstrate a wide ecological tolerance as they could be found in different habitats, like rocky calcareous and serpentine terrains, open grasslands, pastures, and pine forests. Both species showed the ability to absorb a large number of elements from soil both essential (Cu, Fe, Ni, Mn, Zn) and non-essential (Co, Cr, Pb) for its biological functions. Plant populations from serpentine habitats possess a higher content of heavy metals, like Mn, Ni, Cr, than calcareous one. Among the selected species *Teucrium montanum* can accumulate more metals (Cr, Fe, Ni) compared to *Teucrium chamaedrys*. Based on the difference between the ability of absorption and accumulation of heavy metals, described plant species could not be classified as metal-hyperaccumulating plants. *Teucrium chamaedrys* and *T. montanum* are classified as excluders, containing relatively low metal concentrations in their aerial parts, despite the high element concentrations in the soil. The presence of heavy metals and other environmental conditions from serpentine possess significantly higher values of secondary metabolites in plant populations, compared to non-serpentine ones. The significant morpho-anatomic differences between populations are related to the differences in edaphic conditions of the serpentine and non-serpentine sites. The serpentine populations are characterized by their reduced size – lower stems, smaller leaves, and shorter internodes, compared to the non-serpentine populations. Selected plant species collected from naturally metalliferous (serpentine) soils should be avoided because of the increased concentration of heavy metals in plant tissues. Traditional medicinal products, which contains heavy metals in concentrations hazardous to human health. Usage of these plants should be limited. Plants collected from serpentine soils require strict control. Medicinal herbs should be gathered and cultivated only from non-metalliferous sites.

References

Adamidis GC, Aloupi M, Kazakou E, Dimitrakopoulos PG (2014) Intra-specific variation in Ni tolerance, accumulation and translocation patterns in the Ni-hyperaccumulator *Alyssum lesbiacum*. Chemosphere 95:496–502

Akula R, Ravishankar GA (2011) Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav 6:1720–1731

Antonovics J, Bradshaw AD, Turner RG (1971) Heavy metal tolerance in plants. Adv Ecol Res 7:1–85

Ater M, Lefebvre C, Gruber W, Meerts P (2000) A phytogeochemical survey of the flora of ultramafic and adjacent normal soils in North Morocco. Plant Soil 218:127–135

Azmat R, Haider S, Nasreen H, Aziz F, Riaz M (2009) A variable alternative mechanism in adapting the plants to heavy metal environment. Pak J Bot 41:2729–2738

Babalonas D, Karataglis S, Kabassakalis V (1984) The ecology of plant populations growing on serpentine soils. III. Some plant species from North Greece in relation to the serpentine problem. Phyton 24:225–238

Bani A, Imeri A, Echevarria G, Pavlova D, Reeves R, Morel J-L, Sulçe S (2013) Nickel hyperaccumulation in the serpentine flora of Albania. Fresenius Environ Bull 22:1792–1801

Bazan G, Galizia G (2018) Geographical and ecological outline of metal(loid) accumulating plants in Italian vascular flora. Ecocycles 4:47–64

Bentham G (1833) Labiatarum genera et species. Ridgeway Sons, London

Berni R, Luyckx M, Xu X, Legay S, Sergeant K, Hausman J-F, Lutts S, Cai G, Guerriero G (2018) Reactive oxygen species and heavy metal stress in plants: impact on the cell wall and secondary metabolism. Environ Exp Bot 161:98–106

Bezić N, Vuko E, Dunkić V, Ruščić M, Blažević I, Burčul F (2011) Antiphytoviral activity of sesquiterpene-rich essential oils from four Croatian *Teucrium* species. Molecules 16:8119–8129

Bini-Maleci L, Servettaz O (1991) Morphology and distribution of trichomes in Italian species of *Teucrium* sect. *Chamaedrys* (Labiatae): a taxonomical evaluation. Plant Syst Evol 174:83–91

Blake L, Goulding KWT (2002) Effects of atmospheric deposition, soil pH and acidification on heavy metal content in soil and vegetation of semi-natural ecosystems at Rothamsted experimental station. Plant Soil 240:235–251

Boissier PE (1879) Lamiaceae. In: Flora Orientalis 4. apud H. Georg, Geneva/Basel, pp 805–822

Boyd R, Wall M, Santos S, Davis M (2009) Variation of morphology and elemental concentrations in the California nickel hyperaccumulator *Streptanthus polygaloides* (Brassicareae), soil and biota of serpentine: a world view. Northeast Nat 16:21–39

Brady K, Kruckeberg A, Bradshaw H (2005) Evolutionary ecology of plant adaptation to serpentine soils. Ann Rev Ecol Evol S 36:243–266

Branković S, Cupara S, Glisić R, Djelić G, Grbović F, Kojicić K, Milovanović O (2017) Phytoaccumulation in plants of mountain Goč in Serbia. Studia Universitatis “Vasile Goldiş”, Seria Ştiinţele Vieţii 27:196–201

Bratteler M, Baltisberger M, Widmer A (2006) QTL analysis of intraspecific differences between two *Silene vulgaris* ecotypes. Ann Bot 98:411–419

Broadhurst CL, Chaney RL, Angle JS, Maugel TK, Erbe EF, Murphy CA (2004) Simultaneous hyperaccumulation of nickel, manganese and calcium in *Alyssum* leaf trichomes. Environ Sci Technol 38:5797–5802

Brooks RR (1987) Serpentine and its vegetation: a multidisciplinary approach (ecology, phytogeography and physiology). Dioscorides Press, Portland

Brooks RR, Yang XH (1984) Elemental levels and relationships in the endemic serpentine flora of the central Dyke, Zimbabwe and their significance as controlling factors for the flora. Taxon 33:392–399

Brunetti G, Soler-Rovira P, Farrag K, Senesi N (2009) Tolerance and accumulation of heavy metals by wild plant species grown in contaminated soils in Apulia region – Southern Italy. Plant Soil 318:285–298

Cakmak I, Kirkby EA (2008) Role of magnesium in carbon partitioning and alleviating photooxidative damage. Physiol Plant 133:692–704

Čanadanović-Brunet J, Djilas MS, Ćetković SG, Tumbas TV, Mandić IA, Čanadanović MV (2006) Antioxidant activities of different *Teucrium montanum* L. extracts. Int J Food Sci Technol 41:667–673

Chaney RL, Angle JS, Broadhurst CL, Peters CA, Tappero RV, Sparks DL (2007) Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J Environ Qual 36:1429–1443

Conner JK, Sterling A (1996) Selection for independence of floral and vegetative traits: evidence from correlation patterns in five species. Can J Bot 74:642–644

Cornara L, Roccotiello E, Minganti V, Drava G, De Pellegrini R, Mariotti MG (2007) Level of trace elements in plants growing on serpentine and metalliferous soils. J Plant Nutr Soil Sci 170:781–787

Di Toppi LS, Gabrrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Diklić N (1974) Teucrium. In: Jakovljević S (ed) Flore de la Republique Socialiste de Serbie VI, 1st edn. Serbian Academy of Sciences and Arts, Belgrade, pp 349–357

Dudić B, Rakić T, Sinzar J, Atanacković V, Stevanović B (2007) Differences of metal concentrations and morpho-anatomical adaptations between obligate and facultative serpentinophytes from Western Serbia. Arch Biol Sci 59:341–349

Ekim T (1982) *Teucrium* L. In: Davis PH (ed) Flora of Turkey and the East Aegean Islands VII. Edinburgh University Press, Edinburgh, pp 53–75

Endt DV, Kijne JW, Memelink J (2002) Transcription factors controlling plant secondary metabolism: what regulates the regulators? Phytochemistry 61:107–114

Evstatieva L, Hardalova R, Stoyanova K (2007) Medicinal plants in Bulgaria: diversity, legislation, conservation and trade. Phytol Balcan 13:415–427

Ewald J (2003) The calcareous riddle: why are there so many calciphilous species in the Central European flora. Folia Geobot 38:357–366

Farago ME (1994) Plants and chemical elements biochemistry, uptake, tolerance and toxicity. Wiley-VCH, Weinheim

Filippini R, Piovan A, Borsarini A, Caniato R (2010) Study of dynamic accumulation of secondary metabolites in three subspecies of *Hypericum perforatum*. Fitoterapia 81:115–119

Franco A, Rufo L, Zuluaga J, Fuente V (2013) Metal uptake and distribution in cultured seedlings of *Nerium oleander* L. (Apocynaceae) from Rio Tinto (Huelva, Spain). Biol Trace Elem Res 155:82–92

Gabbrielli R, Pandolfini T (1984) Effect of Mg^{+2} and Ca^{+2} on the response to nickel toxicity in a serpentine and nickel accumulating species. Physiol Plant 62:540–544

Golubović T, Blagojević B (2013) Concentration of heavy metals in medicinal plants in Serbia – potential health risk. Paper presented at the International science conference “Reporting for sustainability”, Becici, Montenegro, 7–10 May 2013

Gonneau C, Genevois N, Frérot H, Sirguey C, Sterckeman T (2014) Variation of trace metal accumulation, major nutrient uptake and growth parameters and their correlations in 22 populations of *Noccaea caerulescens*. Plant Soil 384:271–287

Graham RD, Welch RM, Grunes DL, Cary EE, Norvel WA (1987) Effects of zinc deficiency on the accumulation of boron and other mineral nutrients in barley. Soil Sci Soc Am J 51:652–657

Greuter W, Burdet HM, Long G (1986) Med-checklist III, Conservatoire et Jardin Botaniques ville de Genève, Genève

Grubešić RJ, Vladimir-Knezevic S, Kremer D, Kalodera Z, Vukovic J (2007) Trichome micromorphology in *Teucrium* (Lamiaceae) species growing in Croatia. Biologia (Bratislava) 62:148–156

Grubešić R, Kremer D, Vladimir-Knežević S, Vuković Rodríguez J (2012) Analysis of polyphenols, phytosterols, and bitter principles in *Teucrium* L. species. Cent Eur J Biol 7:542–550

Haby VA, Russelle M, Skogley P, Earl O (1990) Soil testing for potassium, calcium and magnesium. In: Westerman RL (ed) Soil testing and plant analysis, 3rd edn. Soil Science Society of America, Madison, pp 181–228

Hamid N, Bukhari N, Jawaid F (2010) Physiological responses of *Phaseolus vulgaris* to different lead concentrations. Pak J Bot 42:239–246

Harborne JB, Tomás-Barberán FA, Williams CA, Gil MI (1986) A study of flavonoids from European *Teucrium* species. Phytochemistry 25:2811–2816

Hossain MR (2007) Flora of some heavy hyperaccumulators recorded in Penjwin and Mawat in Iraqi Kurdistan. Dissertation, Sulaimani University Iraq

Ibrahim MH, Kong YC, Mohd Zain NA (2017) Effect of cadmium and copper exposure on growth, secondary metabolites and antioxidant activity in the medicinal plant sambung nyawa (*Gynura procumbens* (Lour.) Merr). Molecules 22:1–16

Ivancheva S, Stancheva B (2001) Ethnobotany in Bulgaria. In: Ozhatay N (ed) Proceedings of the 2nd Balkan Botanical Congress, Istanbul

Jain A, Ranade R, Pritam P, Joshi N, Vavilala SL, Jain A (2014) A comparative study of antioxidant activity, total phenolic and flavonoid contents in different parts of *Helicteres isora* L. AJLS 2:292–302

Jenny H (1980) The soil resource: origin and behaviour. Springer, New York

Johnston WR, Proctor J (1977) Metal concentrations in plants and soils from two British serpentine sites. Plant Soil 46:275–278

Juranović Cindrić I, Zeiner M, Glamuzina E, Stingeder G (2013) Elemental characterisation of the medical herbs *Salvia officinalis* L. and *Teucrium montanum* L. grown in Croatia. Microchem J 107:185–189

Jurišić R, Kalodera Z, Grgić J, Grgić Z, Ćavar S (2001) Determination of copper, cobalt and nickel in *Teucrium* species growing in Croatia. Acta Pharma 51:75–80

Kabata-Pendias A (2011) Trace elements in soils and plants, 4th edn. Taylor & Francis Group, London

Kabata-Pendias A, Pendias H (1984) Trace elements in soils and plants. Taylor & Francis Group, London

Karataglis S, Babalonas D, Kabasakalis B (1982) The ecology of plant populations growing on serpentine soils. II Ca/Mg ratio and the Cr, Fe, Ni, Co concentrations as development factors of *Buxus sempervirens* L. Phyton (Austria) 22:317–327

Karley AJ, White PJ (2009) Moving cationic minerals to edible tissues: potassium, magnesium, calcium. Curr Opin Plant Biol 12:291–298

Kashem MA, Singh BR (2002) The effect of fertilizer additions on the solubility and plant-availability of Cd, Ni and Zn in soil. Nutr Cycl Agroecosyst 62:287–296

Kassim J, Rahim B (2014) Some heavy metals content in plants grown on serpentinitic soil from Penjwin and Mawat area at Kurdistan region – Iraq. Int J Agric Sci Nat Res 1:31–39

Kastori R (1993) Heavy metals and pesticides in soil – heavy metals and pesticides in the soil of Vojvodina (in Serbian). Faculty of Agriculture, Novi Sad

Kataeva MN, Alexeeva-Popova NV, Drozdova IV, Beljaeva AI (2004) Chemical composition of soils and plant species in the Polar Urals as influence by rock type. Geoderma 122:257–268

Kay K, Ward K, Watt L, Schrmske D (2011) Plant speciation. In: Harisson S, Rajakaruna N (eds) Serpentine: the evolution and ecology of a model system. University of California Press, Berkely/Los Angeles/London, pp 71–95

Kazakou E, Dimitrakopoulos PG, Baker AJM, Reeves RD, Troumbis AY (2008) Hypotheses, mechanisms and trade-off s of tolerance and adaptation to serpentine soils: from species to ecosystem level. Biol Rev 83:495–508

Kazakou E, Adamidis G, Baker A, Reeves R, Gogino M, Dimitrakopoulos P (2010) Species adaptation in serpentine soils in Lesbos Island (Greece): metal hyperaccumulation and tolerance. Plant Soil 332:369–385

Kliebenstein DJ, Osbourn A (2012) Making new molecules – evolution of pathways for novel metabolites in plants. Curr Opin Plant Biol 15:415–423

Konstantinou M, Babalonas D (1996) Metal uptake by Caryophyllaceae species from metalliferous soils in northern Greece. Plant Syst Evol 203:1–10

Korkina LG (2007) Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. Cell Mol Biol 53:15–25

Krause W (1958) Andere Bodenspezialisten. In: Michael G (ed) Handbuch der Pflanzenphysiologie, vol 4. Springer, Berlin, pp 758–806

Kruckeberg AR (1969) The implications of ecology for plant systematics. Taxon 18:92–120

Kruckeberg AR (1984) California serpentines: flora, vegetation, geology, soils, and management problems. University of California Press, Berkeley

Kruckeberg AR (1992) Plant life of western North American ultramafics. In: Roberts B, Proctor J (eds) The ecology of areas with serpentinized rocks: a world view. Kluwer Academic Publishers, Dordrecht, pp 31–73

Kukier U, Peters CA, Chaney RL, Angle JS, Roseberg RJ (2004) The effect of pH on metal accumulation in two *Alyssum* species. J Environ Qual 33:2090–2102

Lambers H, Stuart Chapin F III, Pons TL (2008) Plant physiological ecology, 2nd edn. Springer, New York

Lavid N, Schwartz A, Yarden O, Tel-Or E (2001) The involvement of polyphenols and peroxidase activities in heavy metal accumulation by epidermal glands of the waterlily (Nymphaeaceae). Planta Med 212:323–331

Lieth H, Markert B (1988) The establishment of element concentration cadasters for ecosystems (ECCE) in the different vegetation zones of the earth. Biol Int 16:7–11

Lombini A, Dinelli E, Ferrari C, Simoni A (1998) Plant-soil relationships in the serpentinite screes of Mt Prinzera (Northern Apennines, Italy). J Geochem Explor 64:19–33

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158

Maleš Ž, Pilepić KH, Bojić M, Tatalović Z (2015) Determination of the content of total polyphenols, non-tannin polyphenols and tannins in five species of the genus *Teucrium* L. Period Biol 117:453–455

Mandal MS, Chakraborty D, Dey S (2010) Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav 5:359–368

Markova M (1992) *Teucrium* L. In: Kozuharov S (ed) Opredelitel na vishite rastenia v Balgaria (in Bulgarian). Nauka i Izkustvo, Sofia, pp 491–492

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, Waltham

Mayer MS, Soltis PS (1994) The evolution of endemics: a chloroplasts DNA phylogeny of the *Streptanthus glandulosus* complex (Cruciferae). Syst Bot 19:537–574

Mengel K, Kirkby EA (1987) Principles of plant nutrition, 4th edn. International Potash Institute, Worblaufen-Bern

Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Pol J Environ Stud 15:523–530

Milošević-Djordjević O, Stošić I, Stanković M, Grujičić D (2013) Comparative study of genotoxicity and antimutagenicity of methanolic extracts from *Teucrium chamaedrys* and *Teucrium montanum* in human lymphocytes using micronucleus assay. Cytotechnology 65:863–869

Nasim SA, Dhir B (2010) Heavy metals alter the potency of medicinal plants. Rev Environ Contam Toxicol 203:139–149

Navarro T (2010) *Teucrium* L. In: Castroviejo S et al (eds) Flora Iberica, VerbenaceaeLabiataeCallitrichaceae, vol 7. Real Jardín Botánico, CSIC, Madrid, pp 30–166

Navarro T, El Oualidi J (2000) Synopsis of *Teucrium* L. (Labiatae) in the Medditerranean region and surrounding areas. Flora Medit 10:349–363

Nikolov S (2006) Encyclopedia of medicinal plants in Bulgaria (in Bulgarian). Publishing House Trud, Sofia

O'Dell RE, Rajakaruna N (2011) Intraspecific variation, adaptation, and evolution. In: Harrison S, Rajakaruna N (eds) Serpentine: evolution and ecology in a model system. University of California Press, Berkeley, pp 97–137

Obratov-Petković D, Popović I, Belanović S, Kadović R (2006) Ecobiological study of medicinal plants in some regions of Serbia. Plant Soil Environ 52:459–467

Obratov-Petković D, Bjedov I, Belanović S (2008) The relationship between heavy metal contents and bedrock in some species of genus *Teucrium* L. in Serbia. In: Ruzichkova G (ed) Proceedings of the 5th conference on medicinal and aromatic plants of Southeast European countries, Brno

Pacifico S, D'Abrosca B, Pascarella MT, Letizia M, Uzzo P, Piscopo V, Fiorentino A (2009) Antioxidant efficacy of iridoid and phenylethanoid glycosides from the medicinal plant *Teucrium chamaedris* in cell-free systems. Bioorg Med Chem 17:6173–6179

Pandy P, Tripathi K (2010) Bioaccumulation of heavy metal in soil and different plant parts of *Albizia procera* (Roxb.) seedling. Bioscan 5:263–266

Pavlova D (2009) Morphological variation in *Teucrium chamaedrys* from serpentine and non-serpentine populations, soil and biota of serpentine: a world view. Northeast Nat 16:39–55

Pavlova D (2010) A survey of the serpentine flora in the West frontier Bulgarian Mountains (Vlahina and Ogražden). Phytol Balcan 16:97–107

Pavlova D, Alexandrov S (2003) Metal uptake in some plants growing on serpentine areas in the Eastern Rhodopes Mountains (Bulgaria). Ot Sist Bot Dirgisi 10:13–31

Pavlova D, Karadjova I (2012) Chemical analysis of *Teucrium* species (Lamiaceae) growing on serpentine soils in Bulgaria. J Plant Nutr Soil Sci 175:891–899

Pavlova D, Karadjova I (2013) Toxic elements profiles in selected medicinal plants growing on serpentines in Bulgaria. Biol Trace Elem Res 156:288–297

Pavlova D, Kozuharova E, Dimitrov D (2003) A floristic catalogue of the serpentine areas in the Eastern Rhodope Mountains (Bulgaria). Polish Bot J 48:21–41

Peev D (1989) *Teucrium* L. In: Velchev V, Kuzmanov B (eds) Flora Reipublicae Popularis Bulgaricae, vol 9. Bulgarian Academy of Sciences, Sofia, pp 241–249

Pichi-Sermolli R (1948) Flore e vegetazione delle serpentine e delle alti ofioliti dell'alta valle del Tevere (Toscana). Webbia 6(1):380

Politycka B, Adamska D (2003) Release of phenolic compounds from apple residues decomposing in soil and the influence of temperature on their degradation. Pol J Environ Stud 12:95–98

Pollard AJ, Powell KD, Harper FA, Smith JAC (2002) The genetic basis of metal hyperaccumulation in plants. Crit Rev Plant Sci 21:539–566

Proctor J (1999) Toxins, nutrient shortages and droughts: the serpentine challenge. Trees 14:334–335

Proctor J, Nagy L (1992) Ultramafic rocks and their vegetation: an overview. In: Baker AJM, Proctor J, Reeves RD (eds) The vegetation of ultramafic (serpentine) soils. Intercept Limited, Andover, pp 469–494

Proctor J, Woodell SRJ (1975) The ecology of serpentine soils. Adv Ecol Res 9:256–366

Quideau S, Deffieux D, Douat-Casassus C, Pouysegu L (2011) Plant polyphenols: chemical properties, biological activities, and synthesis. Angew Chem Int Ed 50:586–621

Rajaharuna N, Bohm B (1999) The edaphic factor and patterns of variation in *Lasthenia californica* (Asteraceae). Am J Bot 86:1576–1596

Rajakaruna N (2004) The edaphic factor in the origin of plant species. Int Geol Rev 46:471–478

Rajakaruna N, Boyd R (2008) Edaphic factor. In: Jørgensen SE, Fath BD (eds) General ecology. Encyclopedia of ecology, vol 2. Elsevier, Amsterdam, pp 1201–1207

Ramakrishna A, Ravishankar GA (2011) Influence of biotic stress signaling on secondary metabolites in plants. Plant Signal Behav 6:1720–1731

Rašić S, Dogo S, Slanković L (2006) Inorganic analysis of herbal drugs. Part II. Plant and soil analysis – diverse bioavailability and uptake of essential and toxic elements. J Serb Chem Soc 71:1095–1105

Rastgoo L, Alemzadeh A (2011) Biochemical responses of gouan (*Aeluropus littoralis*) to heavy metals stress. Aust J Crop Sci 5:375–383

Redzić S (2010) Wild medicinal plants and their usage in traditional human therapy (Southern Bosnia and Herzegovina, W. Balkan). J Med Plant Res 4:1003–1027

Reeves R (2006) Hyperaccumulation of trace elements by plants. In: Morel JL, Echevarria G, Goncharova N (eds) Phytoremediation of metal-contaminated soils, NATO science series, vol 68. Springer, Dordrecht

Reeves RD (2017) A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytol 218:407–411

Reeves RD, Baker AJM, Borhidi A, Berazain R (1999) Nickel hyperaccumulation in the serpentine flora of Cuba. Ann Bot 83:29–38

Reeves RD, Baker AJM, Becquer T, Echevarria G, Miranda ZJG (2007) The flora and biogeochemistry of the ultramafic soils of Goiás state. Brazil. Plant Soil 293:107–119

Reichinger HK (1941) Monographische stiudie über *Teucrium* Sect. *Chamaedrys*. Bot Arch 42:335–420

Ritter-Studnička H (1968) Die serpentinomorphosen der flora Bosniens. Bot Jahrb 88:443–465

Roberts BA, Proctor J (1992) The ecology of areas with serpentinized rocks: a world view. Kluwer Academic Publishers, Dordrecht

Rusak G, Gutzeit H, Ludwig-Müller J (2005) Structurally related flavonoids with antioxidative properties differentially affect cell cycle progression and apoptosis of human acute leukemia cells. Nutr Res 25:143–155

Salmerón-Sar̃nchez E, Fuertes-Aguilar J, Španiel S, Per̃rez-García FJ, Merlo E, Garrido-Becerra JA, Mota J (2018) Plant evolution in alkaline magnesium-rich soils: a phylogenetic study of the Mediterranean genus *Hormathophylla* (Cruciferae: Alysseae) based on nuclear and plastid sequences. PLoS One 13(12):e0208307. https://doi.org/10.1371/journal.pone.0208307

Sawidis T, Halley J, Llupo S, Bellos D, Veros D, Symeonidis L (2014) Nickel and iron concentrations in plants from mining area Pogradec, Albania. Environ Eng Manag J 13:861–871

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Sen Gupta A, Berkowitz GA, Pier PA (1989) Maintenance of photosynthesis at low leaf water potential in wheat. Plant Physiol 89:1358–1365

Shallari S, Schwartz C, Hasko A, Morel J-L (1998) Heavy metal in soils and plants of serpentine and industrial sites of Albania. Sci Total Environ 209:133–142

Sharma P, Dubey R (2006) Cadmium uptake and its toxicity in higher plants. In: Khan NA, Samiullah (eds) Cadmium toxicity and tolerance in plants. Alpha Science International Ltd, Oxford, pp 63–86

Shaul O (2002) Magnesium transport and function in plants: the tip of the iceberg. Biometals 15:309–323

Sigel H, Sigel A (1990) Compendium on magnesium and its role in biology, nutrition, and physiology, 1st edn. CRC Press, New York

Skinner WM, Martin RR, Naftel SJ, Macfie S, Séquin CF (2005) Multi-technique studies of the distribution of metals between the soil, rhizosphere and roots of *Populus tremuloides* growing in forest soil. In: Abstract of the 8th international conference on the biogeochemistry of trace elements, Adelaide

Stanković M, Zlatić N (2019) Ethnobotany of *Teucrium* species. In: Martinez JL, Muñoz-Acevedo A, Rai M (eds) Ethnobotany: local knowledge and tradition. CRC Press, Taylor & Francis Group Ltd, Oxford, pp 214–231

Stanković M, Topuzović M, Solujić S, Mihajlović V (2010) Antioxidant activity and concentration of phenols and flavonoids in the whole plant and plant parts of *Teucrium chamaerdys* L. var. *glanduliferum* Haussk. J Med Plant Res 4:2092–2098

Stanković M, Curcić M, Zizić J, Topuzović M, Solujić S, Marković S (2011a) *Teucrium* plant species as natural sources of novel anticancer compounds: antiproliferative, proapoptotic and antioxidant properties. Int J Mol Sci 12:4190–4205

Stanković M, Nicifirović N, Topuzović M, Solujić S (2011b) Total phenolic content, flavonoid concentrations and antioxidant activity of the whole plant and plant parts extracts from *Teucrium montanum* L. var. *montanum*, f. *supinum* (L.) Reichenb. Biotechnol Biotechnol Equip 25:2222–2227

Stanković M, Stefanović O, Čomić L, Topuzović M, Radojević I, Solujić S (2012) Antimicrobial activity, total phenolic content and flavonoid concentrations of *Teucrium* species. Cent Eur J Biol 7:664–671

Stanković SM, Petrović M, Godjevać D, Dajić-Stevanović Z (2015) Screening inland halophytes from the Central Balkan for their antioxidant activity in relation to total phenolic compounds and flavonoids: are there any prospective medicinal plants? J Arid Environ 120:26–32

Štepankova J (1996) Karyological variation in the group of *Myosotis alpestris* (Boraginaceae). Folia Geobot Phytotaxon 31:251–262

Štepankova J (1997) The effect of serpentine on morphological variation in the *Galium pumilum* group (Rubiaceae). Thaiszia – J Bot 7:29–40

Stevanović B, Stevanović V (1985) Morpho-anatomical characteristics of the species *Teucrium montanum* L. from different habitats. Bot Serb 9:73–88

Treutter D (2006) Significance of flavonoids in plant resistance: a review. Environ Chem Lett 4:147–157

Tumbas TV, Mandić IA, Ćetković SG, Djilas MS, Čanadanović-Brunet MJ (2004) *HPLC* analysis of phenolic acids in Mountain germander (*Teucrium montanum* L.) extracts. Acta Period Technol 35:265–273

Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds) (1972) Flobra Europaea III. Cambridge University Press, Cambridge

Vega FA, Covelo EF, Vazques JJ, Abdrade L (2007) Influence of mineral and organic components on copper, lead and zinc sorption by acid soils. J Environ Sci Health A 42:2167–2173

Veličković MJ, Dimitrijević SD, Mitić SS, Mitić NM, Kostić AD (2014) The determination of the phenolic composition, antioxidative activity and heavy metals in the extracts of *Calendula officinalis* L. Adv Technol 3:46–51

Vergnano Gambi O (1992) The distribution and ecology of the vegetation of ultramafic soils in Italy. In: Roberts B, Proctor J (eds) The ecology of areas with serpentinized rocks. A world view. Kluwer Academic Publishers, Dordrecht, pp 217–247

Vergnano Gambi O, Gabbrielli R, Pancaro L (1982) Nickel, chromium, and cobalt in plants from Italian serpentine areas. Acta Oecol 3:291–306

Vicić D, Polavder S, Stojiljkovic M, Jurišic B, Bojat N (2013a) Content and allocation of nickel, chromium, cobalt, copper and zinc in *Teucrium montanum* L. from serpentine habitats in Serbia. Acta Agric Serb 18:101–110

Vicić D, Stoiljković M, Polavder S (2013b) Tissue Mg:Ca ratio in *Teucrium montanum* L. from serpentine soils of Serbia. Soil Plant 62:31–38

Vlase L, Benedec D, Hanganu D, Damian G, Csillag I (2014) Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis*, *Ocimum basilicum* and *Teucrium chamaedrys*. Molecules 19:5490–5507

Vuković N, Milošević T, Sukdolak S, Solujić S (2008) The chemical composition of the essential oil and the antibacterial activities of the essential oil and methanol extract of *Teucrium montanum*. J Serb Chem Soc 73:299–305

Wallace A, Jones M, Alexander GV (1982) Mineral composition of native wood plants growing on a serpentine soil in California. Soil Sci 134:42–44

Westerbergh A (1994) Serpentine and non-serpentine *Silene dioica* plants do not differ in nickel tolerance. Plant Soil 167:297–303

Westerbergh A, Rune O (1996) Genetic relationship among *Silene dioica* (Caryophyllaceae) populations on and off serpentine: a review. Symb Bot Ups 46:277–284

Westerbergh A, Saura A (1992) The effect of serpentine on the population structure of *Silene dioica* (Caryophyllaceae). Evolution 46:1537–1548

Whitea PA, Claxtonb LD (2004) Mutagens in contaminated soil: a review. Mutat Res 567:227–345

Wright J, Stanton M, Scherson R (2006) Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. Evol Ecol Res 8:1–21

Yaman M (2014) *Teucrium* as a novel discovered hyperaccumulator for the phytoextraction of Ni-contaminated soils. Ekol Derg 23:81–89

Yan X, Zhang F, Zeng C, Zhang M, Devcota PL, Yao T (2012) Relationship between heavy metal concentrations in soils and grasses of roadside farmland in Nepal. Int J Environ Res Public Health 9:3209–3226

Zdraveva P, Pavlova D, Krasteva I, Pencheva I (2018) Phytochemical analysis on populations of *Teucrium chamaedrys* from serpentine sites in Bulgaria. CR Acad Bulg Sci 71:185–192

Zhang G, Wu F, Wei K, Dong Q, Dai F, Chen F, Yang J (2006) Cadmium stress in higher plants. In: Samiullah N (ed) Cadmium toxicity and tolerance in plants. Alpha Science International, Oxford, pp 87–103

Zlatić N, Stanković M, Simić Z (2017) Secondary metabolites and metal content dynamics in *Teucrium montanum* L. and *Teucrium chamaedrys* L. from habitats with serpentine and calcareous substrate. Environ Monit Assess 189:110

Zonn SV (1982) Iron in soils. Publishing House, Nauka