TOTAL PHENOLICS AND PHENOLIC ACIDS CONTENT IN LEAVES, RHIZOMES AND RHIZOSPHERE SOIL UNDER *Ceterach officinarum* D.C., *Asplenium trichomanes* L. AND *A. adiantum nigrum* L. IN THE GORGE OF SIĆEVO (SERBIA)

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Abstract

Đurđević L., Mitrović M., Pavlović P., Bojović S., Jarić S., Oberan L., Gajić G., Kostić O.: Total phenolics and phenolic acids content in leaves, rhizomes and rhizosphere soil under *Ceterach officinarum* D.C., *Asplenium trichomanes* L. and *A. adiantum nigrum* L. in the Gorge of Sićevo (Serbia). Ekológia (Bratislava), Vol. 26, No. 2, p. 164–173, 2007.

Ceterach officinarum D.C., *Asplenium trichomanes* L. and *A. adiantum nigrum* L. occur in the fissures of precipitous calcareous rocks in the Gorge of Sićevo. Here, humus rhizosphere soil, i.e. Lithosol, mainly consisting of the leaf and root remains of the ferns in different degradation phases gets formed. The present study was concentrated on determination of total phenolics in the fern leaves, rhizomes and rhizosphere soil and on the analyses of phenolic acids. In the leaves of all three ferns total free phenolics were dominant and exceeded that of their bound forms 1.28–6.95 times. The highest amounts of phenolics were found in the leaves (free of 11 744.00 μg g^{-1} and bound of 9 135.00 μg g^{-1}, respectively) and rhizomes (free of 2 811.11 μg g^{-1} and bound 1 962.74 μg g^{-1}, respectively) of *Ceterach officinarum*. The humus of rhizosphere soil under the ferns was characterized by higher amounts of total bound phenolics (up to 3 873.34 μg g^{-1}). It contained low amounts of free phenolic acids (0.687–9.115 μg g^{-1}). The content of bound phenolic acids exceeded that of their free forms up to 280 times. Since ferns as pioneer species grow at first in the rock fissures either containing no soil or soil in the stage of formation, produces phenolic compounds as secondary metabolites and thus play the significant role in soil and humus formation.

Key words: biodiversity, ferns, humus, Lithosol, phenolics, rhizosphere soil

Introduction

Ceterach officinarum D.C., *Asplenium trichomanes* L. and *A. adiantum nigrum* L. (fam. *Aspleniaceae*) belong to the class of small size ferns, from the group that appeared in the

Tertiary period. They are usually sparse within ecosystems and because of that they have not attracted the attention of scientists. As typical chasmophytes, they occur in relict poly-dominant communities of the vegetation in the rock fissures together with a Tertiary relict and endemic species *Ramonda serbica* P a n č. in the Gorge of Sićevo and in the ravines situated on the northern slopes of the Šara Mt. (Pančić, 1874; Petrović, 1882; Grebenščikov, 1950; Jovanović-Dunjić, 1952; Janković, Stevanović, 1981).

According to early investigations of soil formation on limestone rocks, the first stage begins with formation of tiny soil on the rocks under moss. In the further development somewhat more black humous soil is formed which still remains on stone or is being accumulated into limestone fissures with ferns (Pavićević, 1953).

The ferns are characterized by a number of secondary metabolites, especially those from the group of phenolics that serve as a taxonomically characteristic in determination of their systematic appertainance (Saunders, McClure, 1976; Swain, 1980; Iwashina, Matsumoto, 1993). Some fern species are dominant in a phytocoenosis because toxic phenolic compounds excreted through the leaves and roots, or appearing in the soil upon the microbial degradation of dead fern parts (plant litter) are known to act inhibiting seed germination and growth of other plant species, thus keeping them back (Stewart, 1975; Glass, 1976; Gliessman, Muller, 1978; Humphrey, Swaine, 1997).

The data on phenolics as secondary metabolites of ferns and their role and significance in soil formation of plant communities representing rock vegetation are rather sparse in the available literature. This prompted us to perform qualitative and quantitative analyses of phenolic acids as the components of the soil humus in the rhizosphere soil under *Ceterach officinarum*, *Asplenium trichomanes* and *A. adiantum nigrum* in the Gorge of Sićevo. In addition, the content of total phenolics (free and bound forms) in the fern leaves and rhizomes were determined. The aim of this study was to enhance our understanding of how fern secondary metabolites influence humus rhizosphere soil, i.e. Lithosol formation.

Material and methods

Sampling of soil and plant material

Soil and plant specimens were collected on June 10, 2002 from the fissures of precipitous limestone rocks at different spots of the Gorge of Sićevo (15 km SE from town Niš), from the St. Petka Monastery to the village of Gradište, above the international road Niš – Sofia. The soil solely occurs locally in rock fissures in which ferns grow. The same quantities (5x500 g) of fern leaves, rhizomes and rhizosphere soil under *Ceterach officinarum*, *Asplenium trichomanes* and *A. adiantum nigrum* were uniformly collected from northern parts of the gorge. The material was air-dried, milled and sieved through the sieve with 0.5 mm diameter holes before the analyses of phenolic compounds.

Analysis of phenolics in the fern leaves, rhizomes and soil

Free forms of phenolics were extracted from dried fern leaves and rhizomes (5x2.0 g) in boiling 80% ethyl alcohol (3x30 ml, 4 h) and ethylacetate (3x30 ml, 4 h). The bound phenolics were extracted in ethylacetate after the pretreatment procedure involving the one hour boiling of dried material in 2 N NaOH.

After the drying at ambient temperature, the soil specimens were sieved through a 0.5 mm pore-size sieve. In this way large rhizome remains were removed. Free forms of phenolics were extracted from 5x3 g *per* sample of dried soil in boiling ethylacetate (3x50 ml), during the 24-hour period. The residual soil was treated with 15 ml of 2 N NaOH and after the boiling for 24 h, conc. HCl was added dropwise to pH 2.0 and the bound phenolics were extracted in ethylacetate (Hennequin, Juste, 1967; Katase, 1981a, b). Both samples of free and bound phenolics were used for immediate HPLC analysis or stored at –20 °C until use. The quantity of total phenolics (free and bound forms) was detected spectrophotometrically, using the Folin-Ciocalteau reagent (Feldman, Hanks, 1968). The calibration curve was constructed using different concentrations of ferulic acid.

Determination of phenolic acids by HPLC analysis

Phenolic acids were detected between 210 and 360 nm using a Hewlett Packard diode array detector (HP 1100 HPLC system). The separation was achieved with a Nucleosil 100-5 C_{18} column, 5 μm, 4.0x250 mm (Agilent Technologies, USA). A step-gradient of acetonitrile in water was used: 15% acetonitrile (5 min, gradient), 30% acetonitrile (20 min, gradient), 40% acetonitrile (25 min, gradient), 60% acetonitrile (30 min, gradient), 60% acetonitrile (35 min, gradient), and 100% acetonitrile (45 min, isocratic). In order to avoid tailing of the phenolic acids, 0.05% *o*-phosphoric acid was added to the solvents. The flow rate was 1 ml/min, the injection volume was 5 μl. The identity of the phenolic acids was determined by comparison of the retention times and maximum of absorption of known peaks with pure standards. *p*-hydroxybenzoic and syringic acids (Acros organics, USA), ferulic, vanillic and *p*-coumaric acids (Serva, Germany), were used as phenolic standards. Content of phenolic aids was expressed in μg g^{-1} dry weight.

Soil analysis

The soil pH was potentiometrically measured in H_2O and 0.01 M $CaCl_2$ solution (1:2.5), respectively. Carbon and nitrogen were determined by the method of Anstat in the modification of Ponomareva and Nikolaeva (Ponomareva, Plotnikova, 1975). The Corg:Ntot ratio was determined by calculation.

Statistical analysis

Statistical evaluation of the differences in total content of phenolics, the composition of phenolic acids and soil pH, Corg, Ntot, Corg:Ntot ratio of rhizosphere soil between: (a) *Asplenium trichomanes/A. adiantum nigrum*; (b) *A. trichomanes/Ceterach officinarum*; (c) *Asplenium adiantum nigrum/Ceterach officinarum* was performed with ANOVA tests. Standard deviation did not exceed 15% of the mean values of phenolics and phenolic acids content.

Results

The ferns *Ceterach officinarum*, *Asplenium trichomanes* and *A. adiantum nigrum* grow in the fissures of precipitous calcareous rocks in the Gorge of Sićevo. As pioneer species, they appear at first in the rock fissures with insufficient space for the growth of the rhizomes and containing no soil. In this way, the ferns contribute to the destruction and grinding of limestone rocks. In the later stages, Lithosol-Humous rhizosphere soil in the initial phases of formation gets formed. It occurs locally in rock fissures and consists of dead leaf and rhizome remains in different stages of decomposition.

In the leaves of all the three fern species, the content of free phenolics significantly exceeded that of their bound forms (1.28–6.95 times). The highest content of total free and bound phenolics was recorded in the leaves of *Ceterach officinarum* and the lowest one in the leaves of *Asplenium adiantum nigrum* (Fig. 1). Rhizomes of *Ceterach officinarum* contained much higher amounts of free phenolics in relation to the two other fern species ($p < 0.001$), (Fig. 2).

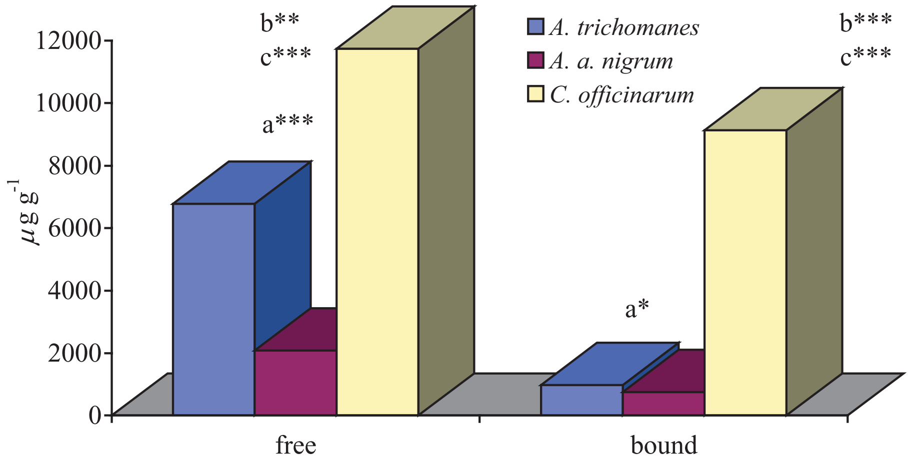

Fig. 1. Total phenolics content in the fern leaves (μg g^{-1}): a – *Asplenium trichomanes/ Asplenium adiantum nigrum*; b – *A. trichomanes/Ceterach officinarum*; c – *A. a. nigrum/C. officinarum* (ANOVA, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $n = 5$).

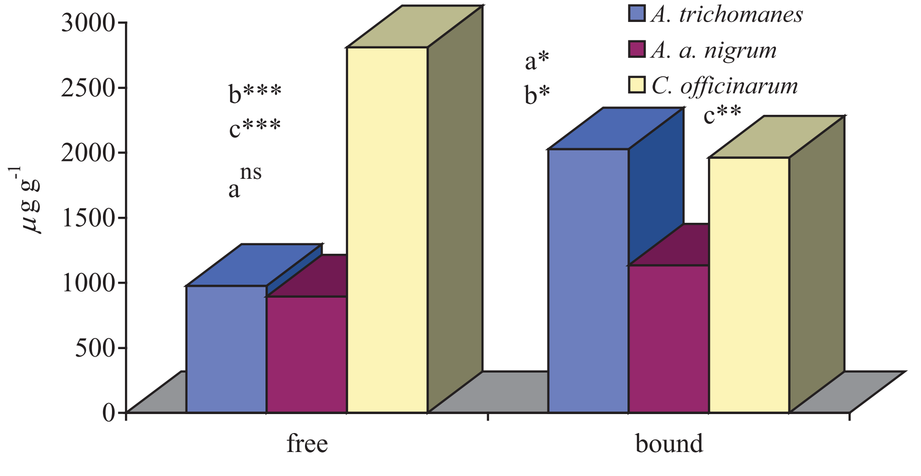

Fig. 2. Content of total phenolics (free and bound forms) in the fern rhizomes (μg g^{-1}): a – *A. trichomanes/A. a. nigrum*; b – *A. trichomanes/Ceterach officinarum;* c – *A. a. nigrum/C. officinarum* (ANOVA, ns – not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; $n = 5$).

In contrast to vegetative fern parts with the dominance of free phenolic compounds, rhizosphere soil is characterized by much higher content of their bound forms. So, the amount of bound phenolics in the rhizosphere soil under *C. officinarum* exceeded that of the free forms 26.21 times. The higher amount of bound phenolics was measured in the rhizosphere soil under *C. officinarum* in comparison to two other ferns ($p > 0.01$), (Fig. 3). In contrast to vegetative fern parts large reduction of total free phenolics in soil (93.57–98.98%) whereas considerable lower reduction of bound phenolics (up to 65.10%) was observed. The reason is leaching of phenolics because of low amount of soil in rock fissures and high permeability of calcareous rock.

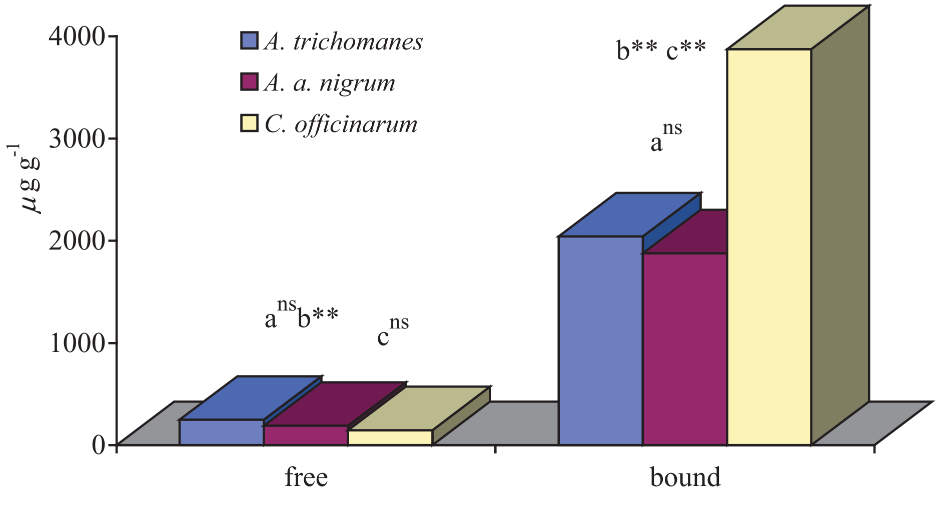

Fig. 3. Amount of total phenolic compounds (free and bound forms) in the rhizosphere soil (μg g^{-1}): a – *A. trichomanes/A. a. nigrum*; b – *A. trichomanes/C. officinarum*; c – *A. a. nigrum/C. officinarum* (ANOVA, ns – not significant; ** $p < 0.01$; $n = 5$).

In the rhizosphere soil under the ferns, four free phenolic acids (*p*-coumaric, ferulic, *p*-hydroxybenzoic and vanillic acid) in low amounts were detected (Fig. 4). As far as bound forms of phenolic compounds are concerned, in addition to the above four phenolic acids, syringic acid was also identified (Fig. 5). The amount of bound forms exceeded that of the free forms for 10 to 280 times. Rhizosphere soil under *C. officinarum* contained the highest amounts of bound phenolic acids. Derivatives of benzoic acid (*p*-hydroxybenzoic and vanillic acid) as free forms making 6–11% of total free phenolics were dominant in all soils under the examined ferns. On the other hand, bound forms of cinnamic acid derivatives (*p*-coumaric and ferulic acid) representing 13– 21% of total bound forms of phenolics were the most abundant.

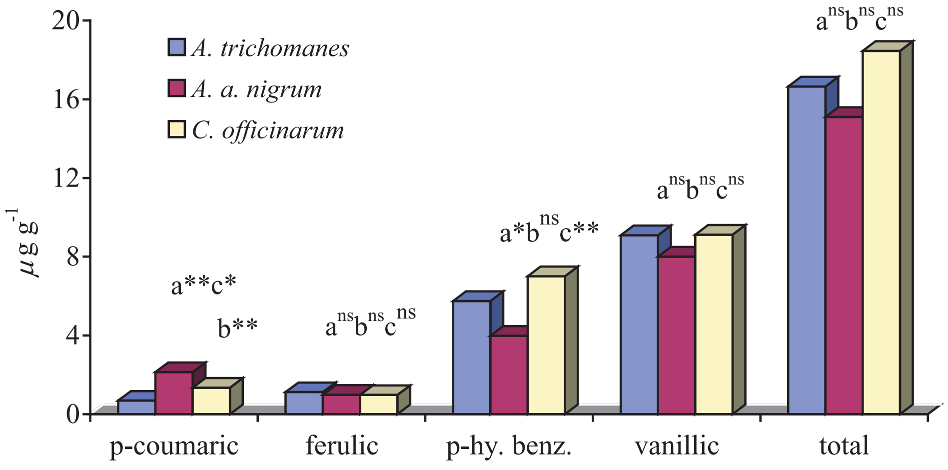

Fig. 4. Amount of free phenolic acids in the rhizosphere soil (µg g^{-1}): a – *A. trichomanes/A. a. nigrum*; b – *A. trichomanes/C. officinarum*; c – *A. a. nigrum/C. officinarum* (ANOVA, ns – not significant; * $p < 0.05$; ** $p < 0.01$; $n = 5$).

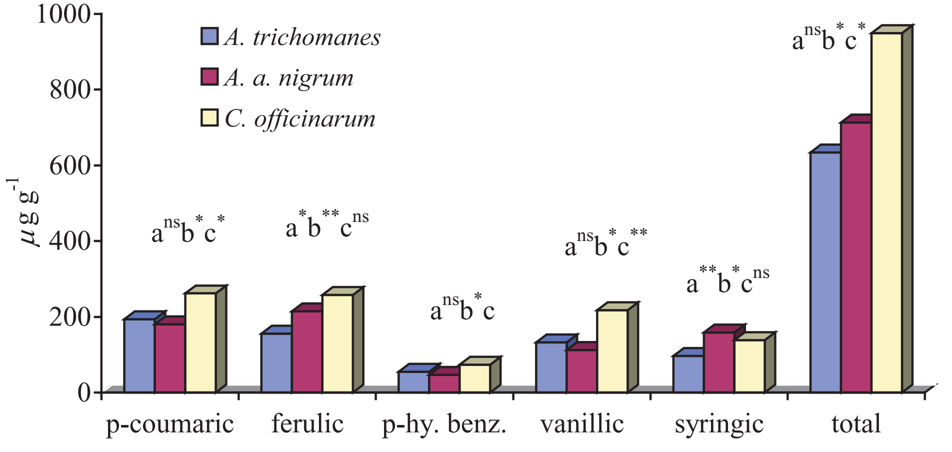

Fig. 5. Amount of bound phenolic acids in the rhizosphere soil under the three fern species (µg g^{-1}): a – *A. trichomanes/A. a. nigrum*; b – *A. trichomanes/C. officinarum*; c – *A. a. nigrum/C. officinarum* (ANOVA, ns – not significant; * $p < 0.05$; ** $p < 0.01$; $n = 5$).

Soil pH and C:N ratio of ferns rhizosphere soil

Influence of stone to the first stage of soil formation is apparently very great and it is affected by neutral media reaction. Soil pH under ferns was between 7.10–7.40 in H_2O. The highest concentrations of C and N were measured in rhizosphere soil under *C. officinarum*, and the lowest under *Asplenium adiantum nigrum* (Table 1). High percentage of C and N is a consequence of participation of degraded plant parts of ferns (rhizomes and leaves especially). The C:N ratio was high (8.97–9.76), but did not differ significantly between three ferns.

T a b l e 1. Soil pH, Corg, Ntot, Corg:Ntot ratio of rhizosphere soil under three ferns.

	Corg %	Ntot %	Corg:Ntot	pH	
				H_2O	$CaCl_2$
Asplenium trichomanes	18.00 ± 0.44 a**	1.86 ± 0.21 a^{ns}	9.76 ± 0.97 a^{ns} b^{ns} c^{ns}	7.40 ± 0.16 a* b^{ns}	6.70 ± 1.22 a^{ns} b^{ns} c^{ns}
Asplenium adiantum nigrum	16.28 ± 0.73	1.78 ± 0.27	9.28 ± 1.18	7.14 ± 0.17	6.44 ± 0.28
Ceterach officinarum	20.62 ± 1.11 b** c***	2.30 ± 0.12 b** c*	8.97 ± 0.59	7.40 ± 0.14 c*	6.70 ± 0.19

Compared are: a – *A. trichomanes/A. a. nigrum*; b – *A. trichomanes/C. officinarum*; c – *A. a. nigrum/C. officinarum*. (ANOVA, ns – not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n = 5).

Discussion

The ferns were reported to be characterized by a high number of different secondary metabolites, especially those from the group of phenolic compounds (Akabori, 1978; Cooper-Driver, 1980; Giannasi, 1980; Smith, 1980; Star, 1980). In the leaves of ferns from the *Aspleniaceae* family, several phenolic compounds, mainly representing the derivatives of luteoline, apigenine, quercetin and kaempferol were identified (Iwashina, Matsumoto, 1993, 1995). Our earlier studies demonstrated the dominance of cinnamic acid derivatives in relation to benzoic acid derivatives in the soil under these three fern species at Maljen Mt. (W Serbia), (Đurđević et al., 2000). Rhizosphere soil under *Ceterach officinarum, Asplenium trichomanes* and *A. adiantum nigrum* in the Gorge of Sićevo contained 4.6 times higher amount of total phenolic compounds and 6-fold higher amounts of individual phenolic acids in comparison with the soil under the ferns in plant communities of Maljen Mt. (Đurđević et al., 2000). This could be explained by the fact that relict hasmophyte fern community in the Gorge of Sićevo is older than that of the Maljen Mt. The soil under *Pteridium aquilinum* was reported to contain *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxycinnamic and ferulic acid in the concentrations ranging from 0.4x10-5 to 4.9x 10-5 mol. During the vegetation period the leaves of this fern species contain high concentrations of these phenolic acids

(Glass, Bohm, 1969). Our studies presented here demonstrated that the rhizosphere soil under the ferns in the Gorge of Sićevo contains far higher amounts of phenolic acids and the content of *p*-hydroxybenzoic acid ranged from $3.40x10^{-4}$ to $5.33x10^{-4}$ mol and that of *p*-coumaric acid from $1.18x10^{-3}$ to $1.59x10^{-3}$ mol. Earlier studies showed that the soil in the Gorge of Sićevo under the phytocoenosis *Cetereto-Ramondietum serbicae* (with dominance of fern *Ceterach officinarum*), results from the degradation of dead plant parts in the rock fissures. This soil is very young and shallow (2–4 cm deep) and belongs to humous black soil (16.4% humus and 0.95% nitrogen) (Jovanović-Dunjić, 1952; Tatić, Stefanović, 1976). In this first stage of soil formation percentage of organic matter is very high and amounts up to 72 percent, as well as the high percent of humus which amounts up to 35 percent (Pavićević, 1953).

Similar to our results Gallet et al. (1999) was reported that phenolics released by *Empetrum hermaphroditum* (dominant dwarf-shrub of boreal forests) foliage and litter have important effects on a range of properties of the forest ecosystem including below ground processes. Phenolic acids were identified in *E. hermaphroditum* soil water extracts, with vanillic, protocatechuic and ferulic acids acid being the most abundant. All of the six phenolic acids present in *E. hermaphroditum* leaves were identified in humus produced under a dense cover of *E. hermaphroditum* but they could also be produced by litter from other plants, especially pine needles. Contrary to Gallet et al. (1999) findings where the influence of other species is possible, we consider the role of ferns as pioneer species in humus rhizosphere soil formation in fissures of precipitous calcareous rocks as crucial.

The humus layer of forest soils under several trees was contained water-soluble phenolic compounds. Among the monomeric compounds, ferulic, *p*-coumaric, vanillic, protocatechuic, syringic and benzoic acid dominated the phenol spectra. The amount of individual phenolic acids was low (up to 15 $\mu g\ g^{-1}$). There were 50 $\mu g\ g^{-1}$ of total free and 70–100 $\mu g\ g^{-1}$ of bound phenolics. The phenolic acids composition of the soil was partly dependent on the tree species (Kuiters, Denneman, 1987). In comparison to completely formed forest soil the rhizosphere soil under ferns contained 5 times more of free and 38.7 times of bound phenolics. Similar to the results of Kuiters and Denneman (1987) among the bound phenolic acids, coumaric, ferulic, vanillic and syringic acid dominated the phenol spectra. Likewise, the amount of free phenolic acids of rhizosphere soil is not dependent on fern species whereas the amount of the bound phenolic acids depends in less degree.

In such conditions the ferns *Ceterach officinarum*, *Asplenium trichomanes* and *A. adiantum nigrum* produce secondary metabolites that returned to the rhizosphere soil and cause high concentrations of phenolic compounds in soil humus. Humus matters are formed during the degradation process of organic remains in the soil due to the activity of soil organisms. Phenolic compounds of plant origin such as flavones, flavonols, catechins, phenolic acids etc., phenolics synthesized by microorganisms or resulting from lignin decomposition represent important structural units for the synthesis of humic and fulvic acids in the soil (Flaig, 1971; Haworth, 1971; Martin, Haider, 1971; Alexander, 1977).

In summary, the results obtained throughout the present study provide a better insight into the role the ferns *Ceterach officinarum*, *Asplenium trichomanes* and *A. adiantum nigrum* as pioneer species play in degradation of the parent limestone rock and in soil formation. They also point to the significance of their phenolic compounds as the secondary metabolites in soil humus formation.

Translated by the authors

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References

Alexander, M., 1977: Lignin decomposition. Introduction to soil microbiology II. John Wiley and Sons Inc., New York, Santa Barbara, London, Toronto, p. 174–187.

Akabori, J., 1978: Flavonoid pattern in the *Pteridaceae* IV. Seasonal variation of the flavonoids in the fronds of *Adiantum monochlamys*. Bot. Mag. Tokyo, *91*: 137–139.

Cooper-Driver, G.A., 1980: The role of flavonoids and related compounds in fern systematics. Bull. Torr. Bot. Club, *107*: 116–127.

Đurđević, L., Mitrović, M., Pavlović, P., 2000: Analysis of phenolic acids in rhizosphere soil under fern *Asplenium trichomanes* L., *A. adiantum nigrum* L. and *Ceterach officinarum* D.C. var. crenatum M o o r e at Maljen Mountain (Serbia). 2nd Balkan Bot. Cong., Istanbul, Turkey, May 14–18, 2000, Abstract 129.

Feldman, A.W., Hanks, R.W., 1968: Phenolic content in the roots and leaves of tolerant and susceptible citrus cultivars attacked by *Rodopholus similis*. Phytochemistry, *7*: 5–12.

Flaig, W., 1971: Organic compounds in soil. Soil Sci., *111*, p. 19–33.

Gallet, C., Nilsson, M.C., Zackrisson, O., 1999: Phenolic metabolites of ecological significance in *Empetrum hermaphroditum* leaves and associated humus. Plant and Soil, *210*: 1–9.

Giannasi, D.E., 1980: Flavonoid evidence for generic lines in selected gymno grammoid ferns. Bull. Torr. Bot. Club, *107*: 128–133.

Glass, A.D.M., Bohm, B.A., 1969: The accumulation of cinnamic and benzoic acid derivatives in *Pteridium aquilinum* and *Athyrium filix-femina*. Phytochemistry, *8*: 371–377.

Glass, A.D.M., 1976: The allelopathic potential of phenolic acids associated with the rhizosphere of *Pteridium aquilinum*. Can. J. Bot., *54*: 2440–2444.

Gliessman, S.R., Muller, C.H., 1978: The allelopathic mechanisms of dominance in bracken (*Pteridium aquilinum*) in southern California. J. Chem. Ecol., *4*: 337–362.

Grebenščikov, O., 1950: La végétation de la Gorge de Sićevo. Bulletin du Muséum d,Histoire Naturelle du Pays Serbe, Série B, Sci. Biol., 3–4: 175–194.

Haworth, R.D., 1971: The chemical nature of humic acid. Soil Sci., *111*: 71–79.

Hennequin, J.R., Juste, C., 1967: Présence d'acides phenols libre dans le sol: Etude de leur influence sur la germination et la croissance des vegétaux. Ann. Agron., *18*: 545–569.

Humphrey, J.W., Swaine, M.D., 1997: Factors affecting the natural regeneration of *Quercus* in Scottish oakwoods. I. Competition from *Pteridium aquilinum*. J. Appl. Ecol., *34*: 577–584.

Iwashina, T., Matsumoto, S., 1993: The flavonoids from three Pakistani *Asplenium* subgenera *Ceterach* and *Ceterachopsis* species and their chemotaxonomic consideration. In Nakike, T., Malik, S. (eds): Cryptogamic flora of Pakistan. Nat. Sci. Museum, Tokyo, *2*: 301–315.

Iwashina, T., Matsumoto, S., 1995: Flavonoid characters of five *Adiantum* species in Pakistan. In Watanabe, M., Hagiwara, H. (eds), Cryptogams of the Himalayas. Nepal and Pakistan Dept. Bot., Nat. Sci. Museum, Tsukuba, Japan, *3*: 179–191.

Janković, M.M., Stevanović, V., 1981: Contribution to study of *Ramonda serbica* Panč. phytocoenosis in the gorges of the Šara mountain northern slopes (in Serbian). Ecology (Belgrade), *16*: 1–34.

Jovanović-Dunjić, R., 1952: Les phytocénoses des espéces Ramondia dans la Serbie. Annales de l,Institut Biologique á Sarajevo. Traïté de Mémorie á Karl Maly, 5, 1–2: 256–270.

Katase, T., 1981a: The different forms in which *p*-coumaric acid exists in a peat soil. Soil Sci., *131*: 271–275.

Katase, T., 1981b: The different forms in which *p*-hydroxybenzoic, vanillic, and ferulic acids exist in a peat soil. Soil Sci., *132*: 436–443.

Kuiters, A.T., Denneman, C.A.J., 1987: Water-soluble phenolic substances in soils under several coniferous and deciduous tree species. Soil Biol. Biochem., *19*: 765–769.

Martin, J.P., Haider, K., 1971: Microbial activity in relation to soil humus formation. Soil Sci., *111*: 54–70.

Pančić, J., 1874: Flora of principality of Serbia. National Printing-House, Belgrade, 798 pp.

Pavićević, N., 1953: Soil types of the Suva Planina Mt. (in Serbian). Soil and Plant (Belgrade), *1*: 1–48.

Petrović, S., 1882: Flora of Niš surroundings. Royal-Serbian State Printing Firm, Belgrade, 950 pp.

Ponomareva, V.V., Plotnikova, T.A., 1975: Simultaneous determination of overall C and N content in peat soils using the Anstet method, modification Ponomareva and Nikolaeva. In Methods of determination of humus content and composition in soils (mineral and peat) in Russia. Federal Academy of Agricultural Sciences V. I. Lenin. Central Pedology Museum V.V. Dokucaev, Leningrad, p. 79–83.

Saunders, J.A., McClure, J.W., 1976: The distribution of flavonoids in chloroplasts of twenty five species of vascular plants. Phytochemistry, *15*: 809–810.

Smith, D.M., 1980: Flavonoid analysis of the *Pityrogramma triangularis* complex. Bull. Torr. Bot. Club, *107*: 134–145.

Star, A.E., 1980: Frond exudate flavonoids as allelopathic agents in *Pityrogramma*. Bull. Torr. Bot. Club, *107*: 146–153.

Stewart, R.E., 1975: Allelopathic potential of western bracken. J. Chem. Ecol., *1*: 161–169.

Swain, T., 1980: The importance of flavonoids and related compounds in fern taxonomy and ecology: An overview of the symposium. Bull. Torr. Bot. Club, *107*: 113–115.

Tatić, B., Stefanović, M., 1976: Chemical analysis of stones and soil of the habitats of *Ramonda* species in Yugoslavia (in Serbian). Bull. Inst. Jard. Bot. Univ. Belgrade, *9*: 127–130.

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