Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 79 (9) S135–S136 (2014) *Supplementary material*

SUPPLEMENTARY MATERIAL TO

Dynamics of soil chemistry in different serpentine habitats of Serbia

DRAŽEN D. VICIĆ[1]*, MILOVAN M. STOILJKOVIĆ[2], JORDANA M. NINKOV[3], NENAD Č. BOJAT[4], MARKO S. SABOVLJEVIĆ[5] and BRANKA M. STEVANOVIĆ[5]

[1]Faculty of Ecology and Environmental Protection, Union – "Nikola Tesla" University, cara Dušana 62–64, 11000 Belgrade, Serbia, [2]University of Belgrade, "Vinča" Institute of Nuclear Sciences, Department of Physical Chemistry, P. O. Box 522, 11001 Belgrade, Serbia, [3]Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia, [4]Faculty of Economics and Engineering Management, Cvećarska 2, 21000 Novi Sad, Serbia and [5]Institute of Botany and Botanical Gardens, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

J. Serb. Chem. Soc. 79 (9) (2014) 1185–1198

EXPERIMENTAL DETAILS

I. Brđani Gorge (BR) is a steppe-like serpentine rocky ground with rich vegetation cover, exceptions being frequent barren exposures of large ultramafic boulders. The site has a pronounced xeric character, as it is a SW oriented steep hill near the ridge top, almost entirely exposed to sunlight during the day. In that locality, *H. sendtneri* grows in a very dense tufted form, and shares the habitat with *C. marantae* and *S. rigidum*. Đetinja River Gorge (DJ) is a steep E–SE exposed hill with a mobile crushed serpentine rock substratum, which hosts impoverished vegetation only. The River Đetinja that runs about 20 meters below ensures that the air is constantly moist. There, *C. marantae* grows in dense, but dispersed tufts. Ravnik (RA) sampling locality is a S–SW exposed terrain, not directly above a river or a stream. The shallow surface of soil is abundant with organic matter, pine needles primarily. In this Scots Pine forest, the sunlight exposure on the ground floor is in part decreased by adult trees reaching above. Less dense *H. sendtneri* tufts of a more evenly dispersed population are found on the fully vegetated forest ground floor. A vertical cliff on one side and a busy road on the other border the limestone habitat of Ovčar Banja (OB). It is very steep, with scarce vegetation but with *S. rigidum* growing scattered at the bottom of the cliff.

II. Sequential extraction into seven fractions was performed according to Zeien and Brümmer (1989).[15,17,38-40] Air-dried samples finer than 2 mm were weighed to 1 g (±0.0001) and transferred to acid-washed 50 mL PP tubes. To obtain the F1 fraction, 25 mL of 1 M NH_4NO_3 (*p.a.*, Acros Organic) were added to the soil and the acute-angled tubes shaken for 24 h on a horizontal shaker. The mixture was then centrifuged at 2500 rpm for 15 min and supernatant filtered through a 0.45 μm cellulose acetate syringe filter (hereinafter centrifuging and filtering was done identically throughout the extraction). To stabilize the solution, 0.25

* Corresponding author. E-mail: vicicdra@gmail.com

mL of 65 % HNO_3 (*p.a.*, Carlo Erba) was added. For the extraction of the F2 fraction, 25 mL of 1 M CH_3COONH_4 (*p.a.*, Fisher Scientific; pH 6 adjusted with conc. acetic acid) were added to the remaining soil and shaken for another 24 h, centrifuged, filtered, and the solution stabilized with 0.25 mL of 65 % HNO_3 (*p.a.*, Carlo Erba). Another 12.5 mL of 1 M NH_4NO_3 were added to the remaining soil and shaken for 10 min, centrifuged, filtered and added to the first part of the F2 extract. The F3 fraction was gained first by adding 25 mL of 0. 1M NH_2OH-HCl (*p.a.*, Acros Organic) + 1 M CH_3COONH_4 (pH 6 adjusted with 37 % HCl) to the remaining solid phase. After 30 min of shaking, the mixture was centrifuged, filtered and the solution stabilized with 0.25 mL 37 % HCl (*p.a.*, VWR Prolabo). Next, 12.5 mL of 1 M CH_3COONH_4 was added and shaken for 10 min with the residual soil, then centrifuged and filtered into the first part of the F3 extract. This step was repeated once more. To obtain the fourth (F4) fraction, 25 mL of 0.025 M NH_4-EDTA (*p.a.*, Fisher Scientific; pH 4.6 adjusted with 28 % NH_4OH, *p.a.*, J. T. Baker) was added to the soil from the previous fraction and shaken for 90 min, centrifuged and filtered. Another 12.5 mL of 1 M CH_3COONH_4 was added to the soil residue, shaken for 10 min, then centrifuged, filtered and added to the first part of the extract. The fifth fraction (F5) was extracted with 25 mL of 0.2 M NH_4 oxalate buffer (consisting of 28.422 g L^{-1} ammonium oxalate, *p.a.*, Fisher Scientific; 25.214 g L^{-1} oxalic acid dehydrate, *p.a.*, Fisher Scientific; pH 3.25 adjusted with 28 % NH_4OH) by shaking for 4 h in the dark. After the content had been centrifuged and filtered, the previous step was repeated with 12.5 mL of the same solution and 10 min of shaking in the dark. Fraction F6 was extracted with 25 mL of 0.1 M ascorbic acid + 0.2 M NH_4-oxalate buffer (all: *p.a.*, Fisher Scientific; pH 3.25 adjusted with 28 % NH_4OH), which was added to the remaining solid phase and digested for 30 min in a water bath at 96 °C, then centrifuged and filtered. Another 12.5 mL of 0.2 M NH_4-oxalate buffer was added to the remaining soil and shaken for 10 min in the dark, then centrifuged, filtered and added to the first part of the F6 extract. The seventh fraction (F7) was the difference after the sum of first six fractions (F1 to F6) had been subtracted from the total amounts determined in hotplate *aqua regia* extraction.

The first fraction represented mobile metals – water soluble and exchangeable, and easily soluble organic complexes. The second fraction incorporated easily soluble, specifically adsorbed metals, metal bound to carbonates, and organo–metal complexes. The third fraction contained easily reducible metals bound to Mn-oxides. The fourth was the final plant-available fraction, and represented EDTA-extractable metals bound to organic matter. Fractions F5, F6 and F7 were unavailable for plants: F5 representing a moderately reducible fraction bound to amorphous and poorly crystalline Fe-oxides, and F6 a strongly reducible fraction bound to crystalline Fe-oxides. Solutions of hotplate-digested soil and sequential extracts were processed with an Inductively-Coupled Plasma Optical Emission Spectrometer (Spectroflame P, 27.12 MHz, 2.5 kW) for the following elements: Ag, Al, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sc, Sr, Ti and Zn.