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SOIL CHARACTERISTICS UNDER SPONTANEOUS BIOCOVER OF *PHALARIS ARUNDINACEA* L. IN SERBIA

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Abstract
To investigate the quality of soils suitable for the growth of reed canary grass (Phalaris arundinacea) L., soil samples and plant material were taken from six different sites in Serbia where the plant grows spontaneously. The basic fertility of the soil was analysed to determine the soil type on which the reed canary grass grows and the content of essential nutrients in the plant material. The soil samples from sites where reed canary grass grows had different pH values, ranging from strongly acidic to almost neutral. The soils differed in humus content, ranging from slightly humic to very humic. The nitrogen content and the available forms of phosphorus in the soil samples were low, while the content of available forms of potassium was medium to well supplied. The lowest nitrogen content and the highest content of potassium and phosphorus were measured in the stalks of reed canary grass.

Key words: *nitrogen, phosphorus, potassium, reed canary grass (RCG), soil fertility*

INTRODUCTION

Reed canary grass (*Phalaris arundinacea* L.) is a fast-growing, perennial, heterogamous C3 plant (Kinmonth-Schult and Kim, 2011). It is native to Eurasia and North America and has a broad climatic range (Tuck et al., 2006) and is one of the most productive cold-season grasses (Wrobel et al., 2009). RCG is a perennial grass that lives 8-10 years. It is a marginal marsh plant that tolerates flooding in poorly drained, heavy and compacted soils where drought is possible (Lewandowski et al., 2003; Guretzky et al., 2018). Reed canary grass is very widespread in Serbia and grows well on both well-drained and lighter soils.

This species can survive under various environmental stress conditions such as drought, flooding, grazing, frost, contaminated sites and degraded soils (Pandey et al., 2020). This plant can also be used for phytoremediation of soils contaminated by various heavy metals (Kacprzak et al., 2014; Vymazal, 2016; Abdelsalam et al., 2019; Korzeniowska and Stanislawska-Glubiak, 2019).

Reed canary grass belongs to the group of important grasses with medium forage value (Ocokoljić et al., 1983; Ordakowski-Burk et al., 2006). It is characterized by early growth, rapid vegetative spread, high potential for stem elongation, broad physiological tolerance, high structural plasticity and longevity (Lavergne and Molofsky, 2004). This plant tolerates frost well, but has problems with drought. Well-rooted plants can withstand drought and excessive rainfall (Mišković, 1986). The rhizome root system is quite deep, broad with strong, long, scaly and greenish segmented rhizomes close to the soil, which allow RCG to spread (Zhang et al., 2013). The characteristics of this plant include: Mulching of seeds of competing plants, reduction of grazing due to a high alkaloid content (Ordakowski-Burk et al., 2006), tolerance to phytotoxic metals and a low number of known diseases (Lewandowski et al., 2003; Wrobel et al., 2009). Therefore, this perennial plant is very suitable for establishment on non-agricultural (marginal) land and under unfavourable soil conditions. According to Lindvall (2014), fertilisation measures have no influence on the yield of above-ground biomass, while Larsen et al. (2016) are of the opinion that relatively high and constant yields require nitrogen fertilisation.

According to Lord (2015), RCG proven to be the best variety in problematic soil conditions such as those found on non-crop land, outperforming other species in terms of ease of establishment, cost, time to maturity, yield level and level of contamination. The ease and low cost of establishment and cultivation of this plant suggests that RCG can play a role as a secondary energy crop, although it is currently underutilised for commercial cultivation (mainly in Northern Europe) as an energy crop (Robbins et al., 2012; Scordia and Cosentino, 2019; Usťak et al., 2019). The thermal value of RCG straw is 16–18 MJ/kg dry matter (Usťak et al., 2019). One of the problems is the significantly higher ash content (≥8% on a dry matter basis) compared to other second-generation lignocellulosic energy crops, which can impair combustion and other thermochemical processes (Wrobel et al., 2009; Robbins et al., 2012; Nazli et al., 2020). However, despite lower yields, the greenhouse gas intensity calculated for RCG is lower (-2 to 20 kg CO2-equivalents per tonne of biomass) than, for example, the intensity for switchgrass (8-60 kg CO2-equivalents per tonne of biomass), which is due to lower N2O emissions (Wile et al., 2014).

The aim of our research was to investigate the characteristics of the soil on which RCG occurs spontaneously at different locations in Serbia in order to determine the range of agroecological conditions suitable for this plant species.

MATERIALS AND METHODS

<u>Collection of soil and plant material samples</u>

The reed canary grass usually blooms for a few weeks from May to July, depending on the area (Christian et al., 2006; Usťak et al., 2019). Soil samples and plant material were collected from 6 different locations in Serbia (Table 1) in late July and early August, after the end of the RCG flowering period. The soil samples and plant material were collected from 6 different locations in Serbia (Table 1). The soil samples were taken with a drill at 3 depths: 5-15 cm (surface mineral layer of the soil), 35-45 cm (subsurface soil layer) and 65-75 cm (deep soil layer). At the Homolje site, the soil profile is shallow, up to 20 cm deep, so only the surface layer was sampled for analysis.

When soil samples were taken, the remains of grass leaves, flattened shoots, moss etc. were removed. The samples of soil material were dried, crushed and sieved through sieves with an opening of < 2 mm.

The plant material samples were collected by digging up whole RCG sods, cleaning them from the soil and separating them from roots, rhizomes, stems and leaves (Figure 2). The collected plant material samples were then dried and ground by crushing.

Table 1. Locations and geographical coordinates (GPS) of the sites where the soil and plant material samples were collected

No.	Locations	GPS
1	Veliki Crljeni (fly ash deposit a coal-burning thermal power plant)	44° 43' 23" N, 20° 23' 58" E
2	Homolje	44° 11' 35" N, 21° 56' 42" E
3	Četereže	44° 22' 24" N, 21° 15' 42" E
4	Kruševac	43° 34' 55" N, 21° 12' 12" E
5	Veliki Crljeni	44° 28' 48" N, 20° 19' 13" E
6	Čenta	45° 09' 07" N, 20° 23' 22" E

<u>Analysis methods and procedures</u>

The soil pH was determined at a solid/liquid ratio of 1:2.5 (w/v) (ISO 10390:1994 method – Determination of soil pH). To determine the active acidity (pHa), 10 g of dry soil was weighed and 25 mL of distilled water was added and stirred. After 30 minutes, the pH value was measured directly in a suspension using a pH metre (Iskra MA 5730). The exchangeable acidity (pHe) was determined in a 1M KCl solution.

The total organic C content of the soil was determined by sulfochromic oxidation (SRPS ISO 14235:2005). The total N content of the soil samples was determined using the semi-micro Kjeldahl method (SRPS ISO 11261:2005).

Available phosphorus (P_2O_5) and potassium (K_2O) in the soil were extracted according to Egner et al. (1960) by extraction with AL solution (mixture of 0.1M ammonium lactate and 0.4M acetic acid). P_2O_5 was determined by the molybdenum blue method using a spectrophotometer (580 nm, Shimadzu UV-1900i). K2O concentrations were determined with the Shimadzu AA-7000 flame emission spectrophotometer using a calibration curve generated after measuring a standard of known concentration.

The N concentration in the plant material was determined volumetrically using the Kjeldahl method (Bremner, 1996). The sample of plant material was decomposed by heating with concentrated sulphuric acid in the presence of a catalyst (30% H_2O_2). The resulting solution was then quantitatively transferred to distillation flasks, treated with 40% NaOH solution and subjected to vapour distillation. The N concentration was calculated after titration of the distillate.

The concentrations of P and K in the samples of plant material were determined according to the method described by Jones and Case (1990) by decomposition with HNO_3 at 125°C with the addition of 30% H_2O_2 to decolourise the solution. P was analysed spectrophotometrically with the molybdenum blue method using a spectrophotometer (580 nm, Shimadzu UV-1900i). The concentrations of K were determined using the Shimadzu AA-7000 flame emission spectrophotometer.

The data presented in the tables represent arithmetic means and standard deviations (SD) of the results of three analyses performed for each parameter studied. The correlation coefficients between the analysed parameters and the statistical significance of the correlation coefficients were determined using Microsoft Excel 2010 for Windows.

RESULTS AND DISCUSSION

Reed canary grass can survive in a wide pH range from 4.9 to 8.2 (Carlson et al., 1996). According to Mišković (1986), however, an alkaline pH value is not tolerated, nor is an acidic value below 5. According to Usťak et al. (2019), the optimum pH value is around 5.0, while according to Perdereau et al. (2017), a neutral pH value applies.

The data from Table 2 show that plants grow on soil substrates with a wide range of pH values, from extremely low (Homolje site pH in KCl: 3.01) to neutral. Moreover, the differences between active and substitutional acidity (ΔpH values according to Baize, 1993) are within the typical range for natural, uncontaminated soils (from 0.5 to 1.5).

Regarding the concentration of organic C and organic matter (Table 2), RCG predominantly on slightly humic substrates. The organic C at the Veliki Crljeni site is residue from incompletely burnt coal in a thermal power plant, which is not considered organic matter in the soil.

Table 2. Soil pH and organic carbon concentration in soils where RCG grows spontaneously

Location	Depth (cm)	pH in H_2O	pH in KCl	ΔpH	Organic carbon (%)	Organic matter (%)
Veliki Crljeni	5-15	7.09	6.32	0.77	3.42 ± 0.40	-
	35-45	7.61	6.92	0.69	1.78 ± 0.14	-
	65-75	7.64	7.01	0.63	1.05 ± 0.08	-
Železnik	5-15	6.07	5.48	0.59	1.95 ± 0.04	3.37
	35-45	6.17	5.10	1.07	1.36 ± 0.04	2.35
	65-75	6.16	4.97	1.19	0.76 ± 0.05	1.32
Homolje	5-15	4.08	3.01	1.07	2.90 ± 0.07	4.99
Četereže	5-15	4.94	3.92	1.02	1.44 ± 0.57	2.49
	35-45	5.04	3.88	1.16	1.20 ± 0.03	2.08
	65-75	5.17	3.95	1.22	0.79 ± 0.43	1.22
Kruševac	5-15	5.98	5.32	0.66	1.41 ± 0.00	2.43
	35-45	6.63	5.59	1.04	1.35 ± 0.27	2.33
	65-75	6.50	5.43	1.07	1.28 ± 0.05	2.20
Čenta	5-15	7.00	5.56	1.44	1.88 ± 0.10	3.24
	35-45	6.73	5.51	1.22	1.58 ± 0.52	2.72
	65-75	7.56	6.15	1.41	0.80 ± 0.00	1.38
Range of values (min-max)	5-15	4.08-7.09	3.01-6.32	0.59-1.07	1.41-3.42	2.43-4.99
	35-45	5.04-7.61	3.88-6.92	0.69-1.22	1.21-1.78	2.08-2.72
	65-75	5.17-7.64	3.92-7.01	0.63-1.41	0.78-1.28	1.22-2.20

The total nitrogen content in the soil samples collected from areas covered with RCG is low (< 0.07%, Table 3). The total nitrogen content in the analysed soil samples generally decreases with depth.

Regarding the available P_2O_5 content in soils where RCG occurs naturally, they are low in P_2O_5 (Table 3). However, in terms of available K_2O content, these soils show a wide range of values from moderately to well supplied with K_2O. Interestingly, there is no clear pattern for the decrease in available P_2O_5 and K_2O content with depth in the soils analysed.

Table 3. Content of total nitrogen (N) and available P_2O_5 and K_2O in the analysed soils (±SD)

Location	Depth (cm)	Total N (%)	Available P_2O_5 (mg/100 g soil)	Available K_2O (mg/100 g soil)
Veliki Crljeni	5-15	0.026 ± 0.003	4.91 ± 0.48	17.57 ± 4.29
	35-45	0.014 ± 0.008	6.46 ± 0.57	14.23 ± 10.75
	65-75	0.008 ± 0.001	5.71 ± 0.25	7.65 ± 1.48
Železnik	5-15	0.042 ± 0.006	9.07 ± 0.77	39.08 ± 0.30
	35-45	0.046 ± 0.003	3.22 ± 0.62	21.47 ± 2.90
	65-75	0.041 ± 0.000	2.23 ± 0.15	20.83 ± 1.69
Homolje	5-15	0.054 ± 0.000	0.67 ± 0.14	11.23 ± 0.82
Četereže	5-15	0.051 ± 0.008	4.36 ± 0.02	34.31 ± 0.62
	35-45	0.039 ± 0.005	4.42 ± 0.22	17.85 ± 6.08
	65-75	0.032 ± 0.001	9.71 ± 0.09	29.57 ± 2.27
Kruševac	5-15	0.060 ± 0.005	8.04 ± 0.50	17.52 ± 0.61
	35-45	0.054 ± 0.001	2.12 ± 0.51	13.48 ± 0.77
	65-75	0.058 ± 0.001	3.61 ± 0.45	11.56 ± 1.03
Čenta	5-15	0.038 ± 0.000	1.77 ± 0.48	12.32 ± 0.98
	35-45	0.068 ± 0.007	1.44 ± 0.03	18.26 ± 0.24
	65-75	0.028 ± 0.002	1.26 ± 0.06	6.88 ± 0.21
Range of values (min-max)	5-15	0.026 - 0.060	0.67 - 9.07	11.23 – 39.08
	35-45	0.014 - 0.068	1.44 - 6.46	13.48 – 21.47
	65-75	0.008 - 0.058	1.26 - 9.71	6.88 – 29.57

The potential use of this perennial grass in the remediation of polluted sites is discussed with the potential for biofuel production given some of its other characteristics, for example, high tolerance to site conditions and potential ability to accumulate certain trace metals (Lavergne and Molofsky, 2004). Most macronutrients are accumulated in the aboveground biomass, which makes RCG suitable for phytoextraction of nutrients from the water and bottom sediments of eutrophic lakes and rivers (Polechońska and Klink, 2014).

According to Usťak et al. (2019), the nitrogen (N) content in the above-ground biomass of cultivated RCG is between 0.90-1.36% from spring to autumn. We found significantly lower nitrogen concentrations in spontaneously growing RCG in Serbia (Table 4). In the RCG stem, nitrogen concentrations are on average twice as low as in the roots and upper parts of the plant. In addition, the nitrogen content in the roots and rhizomes of RCG is relatively constant (0.537 ± 0.067%), in contrast to the aerial part, where the differences in content between locations are more pronounced. Root activity and morphology allow *P. arundinacea* to accumulate N and P during senescence (Huang et al., 2020). According to Usťak et al. (2019), the phosphorus (P) content in the above-ground biomass of cultivated RCG is between 0.14-0.23% from spring to autumn. Again, we found significantly lower phosphorus concentrations in spontaneously growing RCG in Serbia (Table 4). On average, the highest phosphorus concentrations are found in the stem, slightly lower in the roots and the lowest in the upper parts of the RCG. In addition, the phosphorus concentrations in the different organs of the RCG are quite different from site to site.

Table 4. NPK content in organs RCG (% of dry matter ±SD)

Location	Part of plant	N	P	K
Veliki Crljeni	Root	0.528 ± 0.014	0.055 ± 0.001	0.462 ± 0.050
	Stem	0.237 ± 0.047	0.032 ± 0.005	0.710 ± 0.024
	Stem top	0.557 ± 0.018	0.090 ± 0.006	0.461 ± 0.018
Železnik	Root	0.511 ± 0.013	0.162 ± 0.015	0.594 ± 0.040
	Stem	0.182 ± 0.012	0.174 ± 0.016	1.424 ± 0.099
	Stem top	0.373 ± 0.007	0.117 ± 0.014	0.658 ± 0.031
Homolje	Root	0.565 ± 0.018	0.087 ± 0.033	0.372 ± 0.018
	Stem	0.371 ± 0.022	0.045 ± 0.003	0.687 ± 0.072
	Stem top	0.937 ± 0.128	0.061 ± 0.009	0.335 ± 0.160
Četereže	Root	0.500 ± 0.002	0.117 ± 0.001	0.447 ± 0.047
	Stem	0.285 ± 0.023	0.135 ± 0.005	0.646 ± 0.020
	Stem top	0.539 ± 0.021	0.022 ± 0.002	0.519 ± 0.038
Kruševac	Root	0.655 ± 0.001	0.061 ± 0.007	0.545 ± 0.070
	Stem	0.292 ± 0.013	0.231 ± 0.023	0.498 ± 0.085
	Stem top	0.710 ± 0.017	0.107 ± 0.095	0.694 ± 0.045
Čenta	Root	0.461 ± 0.013	0.079 ± 0.003	0.309 ± 0.017
	Stem	0.177 ± 0.001	0.079 ± 0.004	0.678 ± 0.055
	Stem top	0.383 ± 0.000	0.070 ± 0.005	0.305 ± 0.019
	Root (average)	**0.537** ± 0.067	**0.094** ± 0.040	**0.455** ± 0.106
	Stem (average)	**0.257** ± 0.074	**0.116** ± 0.078	**0.774** ± 0.327
	Stem top (average)	**0.583** ± 0.214	**0.078** ± 0.035	**0.495** ± 0.161

According to Usťak et al. (2019), the potassium (K) content in the above-ground biomass of cultivated RCG is between 1.12-1.23% during the summer. In spontaneously growing RCG in Serbia (Table 4), we found significantly lower potassium concentrations. On average, the highest potassium concentrations are found in the stem, the lowest in the roots and the upper parts of the plant. In addition, the potassium concentrations in the different organs of canary grass vary from site to site.

Lower concentrations of N, P and K as well as the non-uniformity of concentrations in different organs of RCG between locations (relatively high values of standard deviations for the average concentrations) clearly indicate insufficient availability of these macronutrients in spontaneously growing RCG. The correlation between the total N content and the available K_2O in the depth of the soil profile and the concentrations of nitrogen and potassium in the organs of the RCG is weak ($r \leq 0.750$). This means that the nitrogen and potassium concentrations in the substrate have no significant influence on the concentrations in the roots and above-ground parts of the RCG. However, the concentration of available phosphorus (P_2O_5) in the subsurface layer of the analysed soils is strongly correlated with the phosphorus concentration in the RCG roots (r = 0.765), while the concentration of available P_2O_5 in the deep layer correlates with the phosphorus concentration in the RCG stem (r = 0.875).

CONCLUSSION

Reed canary grass grows spontaneously on different soil types. The pH values of the soil range from extremely low to neutral. In Serbia, RCG grows spontaneously mainly on substrates with low humus content. The total nitrogen content and the available forms of phosphorus in the soil samples were low, while the potassium content varied greatly. Lower concentrations of N, P and K as well as the non-uniformity of concentrations in different organs of RCG between locations clearly indicate insufficient availability of these macronutrients in spontaneously growing RCG. We observed weak correlations between total N content and available K_2O in the soil with the concentrations of N and K in the organs of RCG ($r \leq 0.750$). However, the concentrations of available phosphorus (P_2O_5) are strongly correlated with the phosphorus concentrations in the RCG roots (r = 0.765) and in the RCG stem (r = 0.875).

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Figure 1. Sponaneous biovover of reed canary grass on the abandoned field in Železnik, Serbia.

Figure 2. Collection of plant samples.