

FERTILITY AND CHEMICAL COMPOSITION OF FOREST SOILS COVERED WITH *Allium ursinum* L. IN SERBIA

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ABSTRACT

A. ursinum is a wild growing species, which is often found on various localities throughout Serbia, primarily within forest and occasionally on meadow. Fresh picked leaves and bulbs are used for preparing a salad or meals. For this research we have collected and analyzed soil and plant material from 12 different locations in Serbia. The analyses of basic fertility of soils have shown that *A. ursinum* grows mainly on acidic and soils containing high percentage of humus, with high total nitrogen content, low to medium supplies in available phosphorus and high supplies in available potassium. In those soils there is a very strong correlation between the total N content and total organic C (0.931). There is a relatively wide range of concentrations of overall Cd, Co, Cr, Cu, Ni, Pb and Zn in soils on which *A. ursinum* grows. The translocation factor for *A. ursinum* is $TF \geq 1$ has been calculated for Cd, Pb and Zn.

KEYWORDS:

Wild garlic, soil fertility, trace elements, heavy metals

INTRODUCTION

Green leafy vegetables are an important ingredient in human nutrition, because they contain essential nutrients like vitamins, minerals, fibres and antioxidants. Among 920 onion species of the *Allium* genus there are many species appropriate for human nutrition like wild growing *Allium ursinum* L. [1]. It can be found in broadleaved forests or on forest glades, with dense stands, in moderate climatic conditions throughout Europe and in the north of Asia [2]. It is a perennial plant from which the above-ground part (leaves) is most frequently used by picking in the spring (Figure 1). New bulbs are also edible, and together with leaves are used for making salads. Because of its characteristic taste and nutrition value, *A. ursinum* has found its place in the cuisines of many nations as a seasoning or salad [3]. As a me-

dicinal herb it is used in ethnomedicine [4, 5]; extracts and pure ingredients of *A. ursinum* represent a source of bioactive compounds [3, 4]. These compounds, in laboratory conditions, show antimicrobe and antioxidative activity [6].

The number of plant species, the structure and composition of plant communities depend on agrochemical characteristics of soil and managing the same [7]. The characteristics of soil substrate, on which *A. ursinum* grows, have not been specially analyzed, although some researchers consider that specific soil properties can have an effect on nectar characteristics in *A. ursinum* [8]. The goals of our research are (I) to establish the range of soil conditions on which this species grows in Serbia through basic analyses of soil fertility condition and (II) assess the impact of soil conditions on the concentrations of some heavy metals in individual *A. ursinum* organs.



FIGURE 1
Wild garlic (*Allium ursinum* L.)

TABLE 1
Basic soil fertility of locations covered by *Allium ursinum* in Serbia

Location	GPS		Elevation (m.s.l.)	pH _{H2O}	pH _{KCl}	N _{tot} (%)	Available (mg/100 g of soil)		Total organic C (%)	C/N
	N	E					P ₂ O ₅	K ₂ O		
Taor	44,1094	19,8052	976	6.26	5.72	0.3523	5.12	20.14	5.435	15.43
Boždarevac	44,5508	20,3991	143	6.32	5.13	0.2732	3.41	29.23	2.099	7.68
Svileuva	44,4950	19,8110	181	6.02	4.72	0.3429	2.97	20.25	3.614	10.54
Bigrenica	44,0071	21,4704	282	6.97	5.99	0.4554	40.18	47.47	5.248	11.52
Petnica	44,2481	19,9551	272	6.62	5.54	0.3288	5.37	30.52	3.541	10.77
Bojčinska šuma	44,7456	20,1472	84	5.77	4.53	0.3081	8.75	38.11	3.228	10.48
Rudine	43,7150	19,7731	1029	6.89	6.25	0.2727	37.84	64.50	2.452	8.99
Kosmaj	44,4706	20,5748	509	5.71	4.97	0.2775	4.23	21.86	3.005	10.83
Rudnik	44,1288	20,5464	1105	5.46	4.42	0.4490	11.87	27.36	4.308	9.59
Ovčar	43,8982	20,1858	293	6.75	6.08	0.5780	4.45	21.32	7.526	13.02
Grabovica/Ždreban	44,0243	20,4871	497	6.25	5.20	0.2911	2.98	27.60	2.409	8.28
Lipovička šuma	44,6367	20,4122	277	5.34	4.04	0.1718	2.46	16.84	1.310	7.63
Average:			471	6.20	5.22	0.3417	10.80	30.43	3.681	10.40
SD:			364	0.55	0.71	0.1073	13.46	13.75	1.723	2.26



FIGURE 2
Underground (roots and bulb) and aboveground part (leaf) *A. ursinum* L.

MATERIALS AND METHODS

For this study we collected *A. ursinum* and soils from 12 locations in Serbia, Southeast Europe (Table 1). Sampling was carried out during April and May 2019. During the soil sample collection (from 0-20 cm depth), remnants of forest litter, fallen leaves, flattened grass, tree and shrub branches, mosses, etc., were removed. All research results relate to the features of the so-called fine soil, so that they are comparable with other authors' results. For the determination of basic soil fertility: pH, organic C content, total N (N_{tot}), available P₂O₅ and K₂O; and the analyses of total contents of Ca, Cd, Co, Cr, Cu, Mg, Ni, Pb and Zn, soil samples were air dried, ground in ceramic mortar and pestle and sieved through a 2 mm-sieve.

The plant material was washed with tap water, then rinsed with distilled water and air dried. The

plant material (Figure 2) was then separated on the underground (roots and bulb) and aboveground part (leaf). The plant material was the ground for the analyses of total Cd, Co, Cr, Cu, Ni, Pb and Zn concentrations.

pH values were determined in water and 1M KCl, 1:2.5 w/v (ISO 10390:1994). Total organic C in the soil was determined by soil sample mineralization with boiling dichromate and sulphuric acid mixture and subsequent titration of excessive dichromate with Mohr salt solution [9]. N_{tot} in soil and plant samples was determined by semi-micro Kjeldahl method (ISO 11261:1995).

Available phosphorus (P₂O₅) and potassium (K₂O) were extracted according to Egner et al. [10], by extraction with AL solution (mixture of 0.1M ammonium lactate and 0.4M of acetic acid). P₂O₅ was determined by molybdenum blue method via spectrophotometer (580 nm, Shimadzu UV-1900i). The

K₂O concentrations have been determined by flame emission spectrophotometry on 766.5 nm, i.e. on the atomic absorption spectrophotometer Shimadzu AA-7000, according to calibration curve obtained after measuring the standard of known concentration.

The concentrations of metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in plant samples were determined by atomic absorption spectrophotometry (using a Shimadzu AA-7000 instrument), and their absorption values were compared with those of known standards. The translocation factor (TF) [11] has been calculated as follows:

$$TF = \frac{\text{Concentration of metals in leaves}}{\text{Concentration of metals in roots}} \quad (1)$$

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

RESULTS AND DISCUSSION

The soils on which *A. ursinum* grows are mostly acidic (Table 1), range of values from 5.34 to 6.97; 6.20 on the average; and from 4.04 to 6.25, average 5.22 for pH_{H2O} and pH_{KCl} respectively. The availability of metals in the soil has an increasing tendency at lower pH and decreasing at higher pH values [12].

These soils are characterized by relatively high N_{tot} content, 0.3417% on the average (Table 1). Due to relatively high N_{tot} content, soils with natural meadows and forests are characterized by relatively high intensities of mineralization and high indexes of N availability [13, 14].

The content of available K₂O is also relatively high, average 30.43 mg/100 g of soil (Table 1).

However, although, according to content of available P₂O₅, these soils are medium supplied (average 10.80 mg/100 g of soil), they are mostly poor in P₂O₅. The contents of easily available P₂O₅ and K₂O in forest soils, are according to Nešić et al. [15], most frequently on optimal supply level.

The surface soil layer on which *A. ursinum* grows has predominantly a high percentage of humus, and contains 3.681% of organic C on the average (Table 1). This is considerably above the range of average values of the organic C content in forest soils in Serbia, 1.1-2.3% is noted by Nešić et al. [15]. Thereby, O-horizon in oak and beech forest soils, on which *A. ursinum* most often emerges, are characterized, relative to other types of forest trees, by medium content of soil organic C and N_{tot} and lower pH values [16]. According to Bodo et al. [8], the content of soil organic C negatively correlates with total quantity of nectar in *A. ursinum*.

The C/N ratio in analysed soils is in a wide range of values, from 7.63 to 15.43; average 10.40 (Table 1). Namely, in Serbia, according to Dželetović et al. [13], in forest soils, the values of C/N ratio are in the range of values from 10.31–12.79, and in natural meadow soils they are in the range from 9.04–10.52 [14]. Thereby, although the contents of total C and N in forest and meadow soils are in a relatively wider range of values, the correlation between these two parameters, according to Dželetović et al. [13] Dželetović and Mihailović [14], is very strong. Also, the results of our research confirm a very strong correlation (r = 0.931) between the total organic C and N_{tot} content in soils covered with *A. ursinum*.

The range of Ca and Mg concentrations is wide in the analysed soils, whereby average Mg concentrations are 10 times higher on the average from the average concentrations of Ca (Table 2). In most natural soils, the Ca concentration is usually higher relative to Mg [17]. According to Bodo et al. [8], the magnesium content in the soil positively correlates with total nectar quantity in *A. ursinum*.

TABLE 2
Concentrations of Ca, Cd, Co, Cr, Cu, Mg, Ni, Pb and Zn in soils covered by *A. ursinum* in Serbia (mg/kg)

Location	Ca	Cd	Co	Cr	Cu	Mg	Ni	Pb	Zn
Taor	906.55	0.15	12.02	45.55	18.66	7285.23	46.72	16.04	67.15
Boždarevac	257.88	0.16	8.91	23.38	12.69	4511.72	34.78	24.84	59.65
Svileuva	28.53	0.20	6.39	13.88	9.33	3917.43	13.65	14.36	54.72
Bigrenica	3229.89	0.09	10.76	25.53	22.15	7472.10	32.01	9.89	82.48
Petnica	262.44	0.20	8.29	20.71	11.80	5203.49	27.46	24.33	59.77
Bojčinska šuma	34.05	0.15	5.54	12.74	9.14	3973.32	13.71	22.04	47.78
Rudine	416.89	0.16	6.11	19.48	14.29	4585.24	25.08	21.91	62.11
Kosmaj	385.50	0.16	11.37	45.65	14.45	6375.57	49.51	52.40	70.52
Rudnik	22.14	0.33	12.52	26.15	20.62	6981.27	55.03	124.03	167.08
Ovčar	499.42	0.39	15.89	73.97	21.10	4889.58	208.90	35.05	69.11
Grabovica/Ždrebán	43.92	0.18	8.17	11.33	6.14	4359.76	7.78	33.49	49.51
Lipovička šuma	18.37	0.04	7.49	12.85	8.08	3872.15	16.12	19.70	46.27
average	508.80	0.19	9.46	27.60	14.04	5285.57	44.23	33.17	69.68
SD	897.44	0.09	3.10	18.65	5.49	1366.64	54.05	30.75	32.43

TABLE 3
Strength of correlations between the analyzed soil parameters (r): (a) very strong (0.901 - 1.000); (b) strong (0.751 - 0.900); and (c) medium and weak (≤ 0.750)

Parameters	pH _{KCl}	N _{tot}	P ₂ O ₅	K ₂ O	Opr. C	Ca	Cd	Co	Cr	Cu	Mg	Ni	Pb
N _{tot}	0.453 ^c												
P ₂ O ₅	0.543 ^c	0.183 ^c											
K ₂ O	0.519 ^c	-0.011 ^c	0.884 ^b										
Organic C	0.523 ^c	0.931 ^a	0.107 ^c	-0.113 ^c									
Ca	0.528 ^c	0.393 ^c	0.687 ^c	0.378 ^c	0.426 ^c								
Cd	0.196 ^c	0.750 ^c	-0.209 ^c	-0.182 ^c	0.623 ^c	-0.274 ^c							
Co	0.317 ^c	0.741 ^c	-0.105 ^c	-0.364 ^c	0.771 ^b	0.275 ^c	0.609 ^c						
Cr	0.469 ^c	0.652 ^c	-0.123 ^c	-0.281 ^c	0.776 ^b	0.171 ^c	0.577 ^c	0.879 ^b					
Cu	0.508 ^c	0.771 ^b	0.442 ^c	0.152 ^c	0.775 ^b	0.602 ^c	0.432 ^c	0.784 ^b	0.679 ^c				
Mg	0.309 ^c	0.446 ^c	0.345 ^c	0.041 ^c	0.504 ^c	0.628 ^c	0.089 ^c	0.619 ^c	0.406 ^c	0.793 ^b			
Ni	0.400 ^c	0.755 ^b	-0.122 ^c	-0.232 ^c	0.772 ^b	0.053 ^c	0.742 ^c	0.812 ^b	0.900 ^b	0.595 ^c	0.141 ^c		
Pb	-0.345 ^c	0.281 ^c	-0.119 ^c	-0.165 ^c	0.087 ^c	-0.299 ^c	0.574 ^c	0.413 ^c	0.134 ^c	0.323 ^c	0.353 ^c	0.141 ^c	
Zn	-0.116 ^c	0.490 ^c	0.194 ^c	0.011 ^c	0.313 ^c	0.095 ^c	0.511 ^c	0.499 ^c	0.173 ^c	0.633 ^c	0.624 ^c	0.193 ^c	0.884 ^b

TABLE 4
Average concentrations of Cd, Co, Cr, Cu, Ni, Pb and Zn in soils and in organs of *A. ursinum* (mg/kg \pm SD) and translocation factors (TF) from all locations

	Cd	Co	Cr	Cu	Ni	Pb	Zn
soil	0.19 \pm 0.09	9.46 \pm 3.10	27.60 \pm 18.65	14.04 \pm 5.49	44.23 \pm 54.05	33.17 \pm 30.75	69.68 \pm 32.43
root	0.30 \pm 0.38	1.05 \pm 1.17	11.02 \pm 7.83	9.12 \pm 10.01	5.91 \pm 3.59	3.18 \pm 3.30	12.64 \pm 6.82
bulb	0.09 \pm 0.21	0.59 \pm 0.54	3.35 \pm 2.74	0.00 \pm 0.00	2.55 \pm 1.77	1.42 \pm 2.55	8.75 \pm 2.63
leaf	0.32 \pm 0.49	0.41 \pm 0.58	3.23 \pm 5.11	1.92 \pm 2.50	3.51 \pm 2.19	4.68 \pm 7.34	12.17 \pm 5.96
TF	1.07	0.40	0.29	0.21	0.59	1.47	0.96

Due to natural and anthropogenic causes, very diverse concentrations of trace metals appear in various habitats, from scarce to toxic [18]. A greater quantity of heavy metals in a soil can be manifested by increased accumulation in plants [19]. Various factors have an impact on biological accessibility, mobility, acceptance and toxicity of heavy metals in soil (soil pH, presence and nature of sorbents, inorganic and organic matter, root exudates and nutrients) [20]. Although the cadmium content in soil is low on all locations, 0.19 mg/kg on the average, it accumulated in plant material, i.e. in all parts. Cavanagh et al. [21] have established that the soil Cd and pH content correlate considerably with the Cd concentration in the onion, explaining the 38% variation, while the inclusion of the location as an additional variable, explained the 50% variation. The results of our research show slight correlation of Cd concentrations in soil with pH values ($r = 0.196$; Table 3). It is interesting that none of the analysed soil parameters correlate with pH value (Table 3). Although Cd concentrations in the root and leaf of *A. ursinum* are not phytotoxic, they are higher than in the soil, which resulted that TF is >1.0 (Table 4).

The Co concentrations in the examined soils range from 5.54 to 15.89 mg/kg, average 9.46 mg/kg (Table 2). According to Kabata-Pendias [22] the Co average value in the soil surface layer is approximately 10 mg/kg. Thereby, the Co concentrations in the examined soils show a strong correlation ($r = 0.771$) with the organic C concentration (Table 3).

The Cr concentrations in the examined soils range from 11.33 to 73.97 mg/kg, average 27.60 mg/kg (Table 2). This is somewhat lower than the

average Cr content in the soil (60 mg/kg), stated by Kabata-Pendias [22]. The Cr concentrations in the examined soils show a strong correlation ($r = 0.879$) with Co (Table 3). The accumulation of this element showed certain regularity, i.e. considerably higher accumulation in the root or in the underground parts of the plant compared with the aboveground part (Table 4).

In soils covered with *A. ursinum* the Cu concentrations range from 6.14 to 22.15 mg/kg, average 14.04 mg/kg (Table 2), which is lower than the usual values for Cu concentrations in soils (14-109 mg/kg) [22]. Thereby, the Cu concentrations in the examined soils correlate with the contents of: N_{tot} ($r = 0.771$); organic C ($r = 0.775$); Co concentrations ($r = 0.784$); and Mg ($r = 0.793$) (Table 3). Copper is a necessary metal for higher plants and algae, especially for photosynthesis process. However, the accumulation of copper in the *A. ursinum* plant parts did not have certain regularity (Table 4).

The Ni concentrations in the examined soils are in a very broad range, from 7.78 to even 208.90 mg/kg (Table 2). It is considered that natural Ni concentration in soils is lower than 100 mg/kg [23]. A high Ni concentration in the soil from Ovčar is probably the result of natural Ni enrichment, because the lands in the area around the mountain Ovčar in Western Serbia are characterized by increased Ni concentrations [24]. Thereby, the Ni concentrations in the examined soils correlate with (Table 3): N_{tot} ($r = 0.755$); organic C ($r = 0.772$); Co concentrations ($r = 0.812$); and Cr ($r = 0.900$) (Table 3).

Nickel was not accumulated significantly in the plant material even on the locations with excess Ni

in the surface. Higher concentrations of Ni in underground parts is observed in almost all samples (Table 4). Plants cannot sustain their life cycle without adequate Ni supply, as it is a constituent part of several enzymes required for nitrogen metabolism in higher plants [25] and Ni supports plants in overcoming numerous biotic and abiotic stresses [26]. However, excessive Ni concentrations may lead to production of reactive oxygen species – ROS, and so affects numerous physiological and biochemical processes, such as photosynthesis, transpiration, as well as mineral nutrition, and it also causes phytotoxicity in plants [23]. Plants growing on metal contaminated surfaces show changed metabolism, reduced growth, and produce lower biomass and accumulate metals [27].

The Pb concentrations in the examined soils are within the range of concentrations usual for natural unpolluted soils, from 7.78-55.03 mg/kg. An exception is only the soil from mountain Rudnik, with 124.03 mg/kg. In the vicinity of this location is a Pb/Zn mine, which explains the naturally increased Pb concentrations in soil. Pb is present in a small quantity in almost all cultivated crops, and its concentration has been increasing considerably by growing these crops on soils contaminated with Pb [28]. The Pb concentrations in the examined soils more intensively correlate only with Zn concentrations ($r = 0.884$) (Table 3). Although detrimental effects of Pb are shown on sprouting, establishing stands, growth, uptake of nutrients and assimilation, ultra-structural and oxidative damages, carbon metabolism and enzymatic activities in plants [28], these symptoms have not been noticed in *A. ursinum* on Rudnik location (Figure 3). A high tolerance of some species of *Allium* genus to lead (Pb) is well known, in which the storage organ – bulb has

the main role [29]. In addition, higher Pb concentrations that we have established in the leaf, not in the root and bulb, have resulted in relatively high TF (1.47).

Zinc is naturally present in all soils, most often in the concentrations ranging from 10-100 mg/kg [30]. In the examined soils only the sample of the soil from Rudnik location exceeds this range of concentration values, with 167 mg/kg, which has been expected, because in the environment (surroundings) nearby there is a Pb/Zn mine. Soils that are zinc contaminated with adverse effect on the soil ecosystem, are located around Zn smelting works, near Zn mine and under galvanized structures (e.g. high-voltage power-line poles) [30]. Toxic effects have been identified in overall Zn concentrations ranging from 100 to >1000 mg/kg, and toxicity is decreasing with an increase of exchangeable ion soil capacity [30]. Potentially detrimental Zn levels in soil from the mountain Rudnik (167 mg/kg), according to Kaur and Garg [31] may result in various changes in plants, such as: reduced growth, reduced photosynthesis and respiration, unbalanced mineral nutrition and intensified generation of ROS. These symptoms, as we have stated previously for Pb, have not been noticed. Zinc is quite uniformly accumulated in all the examined parts of the plant, without significant variations, so that TF is approximately 1.0 (Table 4).

In relation to similar research carried out in the region, Vučić et al. [32] have recorded considerably lower Cu concentrations in *A. ursinum*, from 1.56-1.93 mg/kg, Zn: 2.31-2.61 mg/kg, Ni: 0.36-0.39 mg/kg, Cr: 0.07-0.08 mg/kg and Co: 0.02-0.04 mg/kg, while Pb and Cd were below detection limit. On the contrary, Stojković [33] stated considerably higher Zn concentrations in *A. ursinum* (10.5-33.6



FIGURE 3
A. ursinum on location Rudnik.

mg/kg). Increased content of organic matter in the soil reduces the availability of Cd, Pb and Zn. When examining *A. ursinum* in the conditions existing in Slovakia, in neutral ($\text{pH}_{\text{KCl}} = 7.02$) and in soil with high percentage of humus (3.47%), it is recorded somewhat higher metal concentrations: Zn 190 mg/kg, Cd 3.88 mg/kg, Cu 24.8 mg/kg, Ni 39.1 mg/kg, Pb 40.8 mg/kg [34].

CONCLUSION

The analyses of the basic soil fertility have shown that *A. ursinum* grows in Serbia mainly on acidic and soils with high percentage of humus, with high N_{tot} content, having low to medium percentage of available phosphorus, and high percentage of available potassium. In those soils there is a very strong correlation between the N_{tot} content and total organic C ($r = 0.931$). Therefore, *A. ursinum* grows on soils with wider range of concentrations of total Cd, Co, Cr, Cu, Ni, Pb and Zn. Potentially low detrimental Pb and Zn levels in the soil have not shown visible symptoms of toxicity in plants. In *A. ursinum* plants the translocation factor (TF) ≥ 1 for Cd, Pb and Zn has been calculated.

ACKNOWLEDGEMENTS

We acknowledge Ministry of Education, Science and Technological Advancement of Republic of Serbia, Agreement No: 451-03-9/2021-14/200019 (Ž. Dželetović and G. Andrejić); 451-03-9/2021-14/200116 (A. Simić); and 451-03-9/2021-14/200217 (J. Marković and S. Babić).

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Received: 14.12.2021

Accepted: 08.02.2022

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