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HEAVY METAL UPTAKE BY GRASSLANDS DEVELOPED IN A DEGRADED SOIL IN CENTRAL BALKANS

SUMMARY

Extensively managed natural grasslands are predominant in Central Balkan countries (Montenegro, Bosnia and Herzegovina, and Serbia) and the productivity of these communities is extremely low in general. Beside main limitations in grassland productivity, such as strongly acidic soils, organic matter fluctuations, high variability in phosphorus content, some soils were developed on bedrocks with potentially high heavy metal content. The researches were conducted in 2016 and 2017 on representative grasslands in three countries, mainly on *Agrostietum capillaris* type of community. There were five study sites in Montenegro, two in Bosnia and Herzegovina, and six in Serbia in mountainous region.

We analysed nutritive status of the topsoil samples collected in summer in each study site, as well as possible presence of heavy metals (Ni, Cd, Pb and Cr) in the soil. The experimental fields were cut once in the time of inflorescences formation of the dominant grasses and the total concentrations of heavy metals (Pb, Ni, Cd and Cr) in the samples of plant material were determined. In all study sites soil pH was acidic, with low P content, except in a certain site in Bosnia. Generally, the soils were low productive, but according to Regulation of tolerant amount of hazardous and toxic materials in soil, there were not surpassed maximum permissible concentrations of Ni, Cd, Pb and Cr in Montenegro and Bosnia, while in some sites in Serbia very high concentrations of Ni and Cr were observed. Although some elements exceeded maximum permissible amount for soil and water, the ability of plants collected from the *Agrostietum capillaris* communities to accumulate heavy metals was generally low. It could be explained by the physiology of dominant plant species (grasses), which influenced relatively low uptake and generally low accumulation of micronutrients.

Keywords: *Agrostietum capillaris*, Central Balkans, degraded soil, heavy metals.

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INTRODUCTION

Mountain soils have performed vital services on the ecosystem for a long time that help to ensure food security and nutrition to 900 million mountain people around the world and benefit billions more living downstream (FAO, 2015).

The Balkans are a mountainous region. Most of the Balkans have a harsh, continental climate with hot, dry summers and cold winters. The peninsula was once covered with many forests. However, over the centuries, they have been cut down to make room for settlements, agriculture and especially, for pastures and meadows as a forage source for animal husbandry.

Agricultural land covers about 38% of Montenegro. Meadows and pasture cover 33.1% of the territory or 88.25% of agricultural land. Only 10% of the territory is below 200 m above sea level, 35% from 200 to 1 000 m, 40% from 1 000 to 1 500 m, while about 15% is above 1 500 m (Radojičić, 2002). On the basis of the influence of agro-ecological factors, primarily the climate and soil, as well as the way and level of exploitation, Montenegro is provisionally divided into five production regions, where the Northern-mountain and Polimlje-Ibar regions are more suitable than others for fodder production and animal husbandry (Dubljević, 2009). Different forms of flysch are present in the coastal area and on the southern slope of Durmitor mountain and surrounding massifs ('Durmitor flysch'). Natural grasslands, especially pasture, cover poorer land, unsuited to intensive exploitation. They are mostly on steep slopes, shallow soils and with many large stones. Meadows are on deeper soil, flatter and fertile, especially in river basins and plateaux of hilly-mountain areas.

Forty-five percent of agricultural land in Bosnia is hilly (300– 700 m), of medium quality and well suited to semi-intensive livestock production (Alibegović-Grbić, 2009). Mountain areas (> 700 m) account for a further 35% of agricultural land but high altitude, steep slopes and lower soil fertility limit the use of this land to grazing in spring and summer.

Soils in Serbia, and especially in western Serbia, are poor in available P, while K content is variable and depends on location. Grassland dry matter yield was between 2.03 to 5.70 t ha⁻¹, confirmed that pastures and meadows need to be maintained through fertilizer application (Simić *et al.*, 2016). Zlatibor is a vast rolling plateau that is geographically defined by the territory between rivers Sušica and Uvac, the eastern slopes of mountain Tara and the western slopes of Murtenica. The mountain Zlatibor in western Serbia is largely built of Upper Jurassic ultramafics. Geologic bedrock at mountain Zlatibor is composed of serpentinite, peridotites and serpentinitized peridotites. In the northern, part of the region limestone prevails, while, in contrast to the limestone, the main rock type, away from the northern and eastern fringes of the plateau, is green serpentinite and this forms the largest serpentinite massif in Serbia.

Natural grasslands, meadows and pasture are the most important sources of roughage in Balkan countries, especially in hilly-mountain areas where they provide the only feed for cattle. In winter, cattle are mainly fed hay collected

from natural meadows and in summer, cattle graze the pastures and the meadows after mowing.

Mountain pastures, which make up the majority of the natural grasslands, are of great importance for production of animal feed and protection against soil erosion on steep and rocky terrain. Better pastures at altitudes of 1 000 to 1 500 m on deeper and more fertile soil are used for both mowing and grazing, but shallow and eroded sites are only grazed. At higher altitudes, there is a zone of montane pastures which are less used due to inaccessibility and distance unless they are near larger summer settlements (Dubljević, 2005; 2007). Although the mountain region can be seen as a unique area of mountain and high mountain pastures, there are substantial differences between localities. These are characterized by varied floristic composition and grass cover influenced by differences in climate, relief and soil. The best pastures are on flat, less rocky terrain with permanent mountain settlements and higher altitude summer settlements.

Beside natural conditions, human activities have had a big influence on the floristic composition and productivity of these pastures, by manuring (moving sheepfold) and more intensive exploitation. These are the main source of animal feed for both summer and winter. The land base for agriculture is thus very limited in both quantity and quality. Excessive deforestation, inappropriate conversion of grassland to arable and uncontrolled cultivation of sloping terrain are degrading the land, even in the valleys and lowland regions.

Meadows of *Agrostietum capillaris* are of secondary anthropogenic origin, as they are the result of two anthropogenic factors: reduced area under forest on the one hand, and mowing, on the other hand. The association *Agrostietum capillaris* covers a huge area in the hilly region of the Balkan Peninsula (Vučković *et al.*, 2010). The association *Agrostietum capillaris* prospers on low-nutrient acidic soils of this region. On southeast part of Bosnia, western Serbia and northern part of Montenegro, this community is the dominant meadow type and it is widely distributed. In this area, it develops on quite different sites.

In large concentrations, many of the trace elements/metals may be toxic to plants and/or animals, or may affect the quality of foodstuffs for human consumption. These potentially toxic elements include As, B, Cd, Cu, F, Pb, Hg, Mo, Ni, Se and Zn. The relationship between trace elements in plants and amounts absorbed and utilized by the animal is again complex and depends on factors such as selectivity in grazing, the degree of dependence of the animal on grass as a source of trace element dietary intake, digestibility of the diet and form and 'availability' of the ingested trace elements (Thornton and Alloway, 1974).

Therefore, our research was directed to ascertain the content of heavy metals in the specific soils of the mountains in Balkan countries (Serbia, Bosnia & Herzegovina and Montenegro), covered by grasslands, in order to estimate element concentration in the produced forage, because deficiency or excess of dietary mineral elements may cause animal health concerns.

MATERIAL AND METHODS

The experiments were carried out in 2016 and 2017 in the mountainous region of Montenegro, Serbia and Bosnia & Herzegovina. Pasture areas of the study sites are mainly occupied by plant community *Agrostietum capillaris*. For the purpose of determining the concentrations of heavy metals in soil and plants, samples were collected from permanent grasslands in diverse ecological conditions from thirteen sites in three countries (Figure 1).

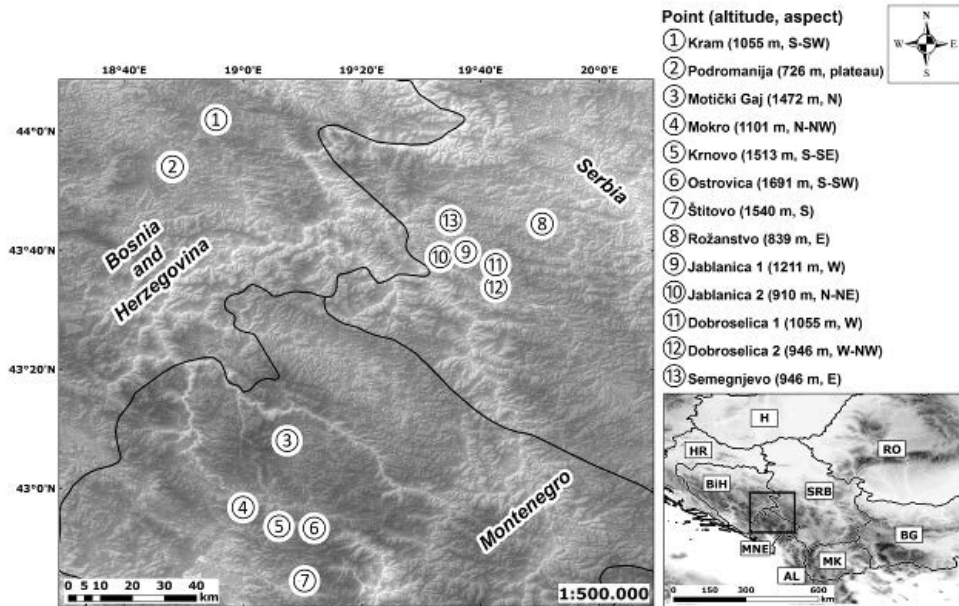


Figure 1. Study locations in three countries

Based on morphological and basic physico-chemical soil characteristics after IUSS (2014) classification, three types of pasture soils were determined: Dystric cambisol (Dystric leptosol), Eutric Cambisol (on limestone) ie. Mollic Leptosol and soils composed of serpentine, peridotites and serpentinized peridotites. Brown acid soils (Dystric cambisols), covering an area of 395 000 ha, are the second major type of soil in Montenegro, and are especially characteristic of the north-east of Montenegro. Brown eutric soils (Eutric cambisol), cover an area of 118 000 ha, and occupy the lowest land of river basins (old river terraces), ravines and karst fields. Serpentine (ophiolitic, ultramafic) rocks represent a group of siliceous rocks which are characterized by calcium deficiency, high concentrations of aluminium, iron, magnesium, nickel, cobalt and chromium, and a few plant nutrients. In contrast to other acid siliceous rocks, the pH values of the serpentine substrate vary from basic to ultrabasic (pH 5.5–8). Serpentine flora and vegetation differ from those occurring on other types of siliceous substrates or limestone. Open serpentine habitats are characterized by pronounced thermophilous character and xeric conditions. The xerothermic

character of serpentine plants is also enhanced by the specific chemical composition of the serpentine substrate (Kabaš *et al.*, 2013). The study fields were mowed once in the time of inflorescences formation of the dominant grass – *Agrostis capillaris* (late June – early July). Nutritive status of the topsoil samples (0-10 cm) collected in summer in each study site was determined by followed methods: pH values in deionized water and 1M KCl (1:2.5 w v⁻¹); total organic C by dichromate redox titration method; total N by semi-micro Kjeldahl method; and available P and K by the AL-method (Pansu and Gautheyrou, 2006).

The total concentrations of heavy metals (Pb, Ni, Cd and Cr) in the samples of plant material were determined, according to procedure described by Jones and Case (1990), by the decomposition of HNO₃ at 125°C. After cooling down by 30% H₂O₂ was added and digesting was continued until the digest was clear. In order to determine the content of available microelements in the soil, dried and sieved soil was mixed continuously for 2 h in 1 M ammonium acetate and 0.01 M EDTA mixture (pH 7), according to the Standard NF X 31-120.22. The concentrations of the microelements and heavy metals were determined by atomic absorption spectrophotometry (Shimadzu AA 7000). The obtained results were processed by calculating average value and standard deviation for each sample.

RESULTS AND DISCUSSION

Environmental conditions

The basic agrochemical properties of the meadows are presented in Table 1. The soil reaction (pH in H₂O and 1M KCl) ranged from acid to very acid, while the contents of total organic C and N where high (Džamić *et al.*, 1996).

Table 1. Chemical properties of the soil

Location	pH		Total organic C %	OM %	Total N %	AL-P ₂ O ₅ mg 100 g ⁻¹	AL-K ₂ O mg 100 g ⁻¹
	in H ₂ O	in KCl					
Podromanija	5.11	3.98	3.95	6.80	0.324	23.7	34.9
Han Kram	5.33	4.42	5.89	10.2	0.408	3.22	22.7
Motički Gaj	5.82	5.11	7.27	12.5	0.146	1.9	27.7
Mokro	5.40	4.16	4.92	8.48	0.313	1.01	21.9
Krnovo	5.52	4.52	5.27	9.08	0.445	1.01	21.2
Ostrovica	5.03	4.18	9.74	16.8	0.818	3.13	33.3
Štitovo	5.26	4.16	10.5	18.1	0.844	1.37	45.5
Rožanstvo	5.98	4.88	4.92	8.49	0.323	0.21	19.8
Jablanica 1	6.56	5.55	6.28	10.8	0.465	4.57	22.3
Jablanica 2	5.80	4.22	4.15	7.15	0.298	2.38	9.89
Dobroselica 1	5.98	4.88	4.92	8.49	0.327	0.21	19.8
Dobroselica 2	6.16	4.58	5.46	9.40	0.374	5.94	31.8
Semegnjevo	6.67	5.74	9.83	17.0	0.689	9.59	61.0

Relatively large amount of rainfall and low temperatures during the year, favour the slow pace of the process of mineralization of organic substances in these mountain soil, and which result in higher amounts C and N. The amount of precipitation is relatively high in central Balkan mountainous region, more than 1000 mm.

Although the amount of precipitation has a great effect on the permanent grassland productivity, especially that during vegetation, it is frequently not well disturbed. This happens because a rainy period is frequently followed by a marked fall of temperature, which retards growth and development of grasses, and hence reduces the productivity of the biomass (Dubljević, 2007; Vučković *et al.*, 2010). Analysed soil substrate had low P content (except one location in Bosnia), and well-supplied with available K (Džamić *et al.*, 1996).

Heavy metals content in plants

Mountain Zlatibor was situated in serpentine areas of Serbia. Serpentine soils are known for elevated heavy metal load. Therefore they may pose a threat that can compromise mineral content of grassland plant's organs. Serpentine soils are loaded with certain heavy metals, and are known to host and give rise to metal hyperaccumulating plants (Reeves *et al.*, 1999). Heavy metal analyses were made of various plants shoots growing on soils with elevated Ni and Cr, covered mainly by *Agrostietum capillaris* communities (Table 2). According to Regulation of tolerant amount of hazardous and toxic materials in soil (Official gazette of RS, 1994), in eight tested soil samples Ni content and in six soils samples Cr surpassed the maximum permissible concentrations (Table 2).

Table 2. Total and available heavy metal content of topsoil (0-10 cm) and herbage in permanent grasslands (mg kg⁻¹ dry weight)

Available heavy metals content in topsoil				
Location	Ni	Pb	Cr	Cd
Podromanija	2.9±0.14	10.9±0.9	<0.1±0	1.3±0.08
Han Kram	1.4±0.08	6.9±0.16	<0.1±0	0.1±0.01
Motički Gaj	0.6±0.00	7.5±0.57	<0.1±0	0.4±0.01
Mokro	3.1±0.04	3.5±0.22	<0.1±0	0.1±0.0
Krnovo	1.3±0.07	5.4±0.25	<0.1±0	0.2±0.0
Ostrovica	7.9±0.35	9.9±0.02	<0.1±0	0.6±0.0
Štitovo	2.0±0.02	19.2±0.7	<0.1±0	0.7±0.01
Rožanstvo	5.06±0.14	5.8±0.02	0.43±0.0	1.14±0.01
Jablanica 1	28.7±0.85	4.6±0.0	1.69±0.23	0.06±0.0
Jablanica 2	116±1.0	2.8±0.4	4.14±0.37	0.09±0.0
Dobroselica 1	10.9±0.2	2.19±0.29	1.07±0.06	0.28±0.01
Dobroselica 2	119±7.0	2.26±0.38	3.56±0.15	0.12±0.01
Semegnjevo	319±2.9	7.19±0.03	5.3±0.63	0.17±0.02

Total heavy metals content in topsoil				
Podromanija	31.8±1.9	26.1±4.8	25.4±1.1	2.18±0.02
Han Kram	17.5±0.1	15.2±0.3	23.0±0.1	0.17±0.00
Motički Gaj	22.3±0.1	30.8±2.1	1.62±0.0	1.41±0.05
Mokro	112±2.2	28.2±1.6	92.3±2.5	<0.05±0.0
Krnovo	50.7±3.8	11.9±0.6	27.4±4.2	0.45±0.00
Ostrovica	58.9±2.8	14.7±0.1	37.7±2.6	0.73±0.01
Štitovo	33.0±1.7	24.0±0.4	29.5±1.2	1.02±0.05
Rožanstvo	543±34	36.6±2.7	140±8.3	1.48±0.05
Jablanica 1	415±4.3	21.9±0.7	113±0.4	0.20±0.0
Jablanica 2	1105±40.5	17.0±0.1	382±11.5	0.26±0.04
Dobroselica 1	260±10.3	17.0±1.1	128±5.5	1.21±0.01
Dobroselica 2	2687±7	16.0±1.0	544±11.5	0.0±0.0
Semegnjevo	2694±19	31.4±0.08	508±8.6	0.28±0.03
MPL§	50	100	100	3
Heavy metals concentrations in plants				
Podromanija	9.57±0.17	6.66±3.19	<0.1±0.0	0.31±0.0
Han Kram	7.22±0.61	11.1±3.56	<0.1±0.0	0.46±0.11
Motički Gaj	5.97±0.43	12.1±2.79	0.31±4.1	0.16±0.0
Mokro	9.53±1.15	6.17±1.92	<0.1±0.0	<0.05±0.0
Krnovo	3.79±0.29	1.26±1.18	<0.1±0.0	0.50±0.32
Ostrovica	7.08±0.22	4.62±2.09	<0.1±0.0	0.28±0.0
Štitovo	4.74±0.71	3.14±3.94	<0.1±0.0	0.69±0.0
Rožanstvo	2.85±0.46	5.70±1.76	2.21±0.15	0.42±0.03
Jablanica 1	7.70±1.25	1.62±0.43	0.73±1.03	0.13±0.02
Jablanica 2	6.56±1.06	2.45±2.0	2.02±0.05	0.12±0.05
Dobroselica 1	4.37±0.35	7.49±1.18	2.19±1.10	0.21±0.02
Dobroselica 2	7.63±0.36	9.25±1.50	3.60±0.42	0.12±0.09
Semegnjevo	12.4±0.51	2.79±1.12	1.59±0.06	0.41±0.50
NC*	0.1-5	1-5	<0.1-1	<0.1-1
TC**	30	20	2	10
MTLF***	50	40	-	1

§ MPL- maximum permissible levels of dangerous and hazardous matters according to Official Gazette of RS, 1994; *Normal concentrations in plants according to Chaney (1983); **TC - Toxic concentrations in plants according to Kabata-Pendias (2010); ***MTLF - Maximum tolerant level for fodder in plants according to NRC (2005); Official Gazette of RS (2009)

Some plant species, mainly from families *Boraginaceae*, *Cruciferae*, *Myrtaceae*, *Leguminosae* and *Caryophyllaceae* are accumulators of Ni. In most cases, Ni is accumulated in the roots. Almost all measured concentrations of Ni

in plants from meadows were below the critical concentration for normal plant growth except in three locations in Serbia, where locations

Jablanica 2, Dobroselica 2, and especially Semegnjevo, all situated at the mountain Zlatibor, surpassed maximum tolerant level for fodder in plants (NRC, 2005). It was effect of very high total amount of Ni registered in the soil (1105, 2687, 2694 mg kg⁻¹ dry weight, respectively). It is fair to assume a risk that well adapted plants in mountain grasslands could be capable of holding increased amounts of serpentine soil-defining metals (Ni and Cr).

On the other hand, Ni content in Montenegro on three locations (table 2), was higher than maximum permissible amount in soil, but available Ni concentrations were between 0.6-7.9 mg kg⁻¹. Concentrations of Ni in plants collected from all sites were below 10 mg kg⁻¹. The elements Ni and Cr, are presumed essential for ruminants, needs for Ni is from 60-70 mg kg⁻¹ (Miranda *et al.*, 2009).

The Cr concentration was <0.1 mg kg⁻¹ in the soils of Bosnia and Montenegro, and, consequently, the Cr concentration in plant tissue samples was low. All locations in Serbia had much higher concentration Cr in the soil than limit concentration (>100 mg kg⁻¹). Also, concentration in plant tissue samples was close to toxic concentrations in plants according to Kabata-Pendias (2010), which could be potentially detrimental for the plant growth. Cr is an essential element for organisms as its important for normal metabolism of glucose. It is not a toxic element, and negative effects on the function of the organism halves at concentration greater than 50 mg kg⁻¹ (Đorđević *et al.*, 2009).

The highest amounts of Ni and Cr were found in locations Jablanica 2, Dobroselica 2, and Semegnjevo, but it seems that harmful amounts of these metals were effectively prevented from being accumulated in the stem or the photosynthetic leaf tissue. It is in a line with results Vicić *et al.* (2013) obtained in populations of *Teucrium montanum*, sampled in the study, and confirm that the mechanism employed is metal exclusion, even if the amounts of Cr and Ni were somewhat higher in the roots.

Although Pb occurs naturally in all plants, it has not been shown to play any essential role in their metabolism. The Pb concentration in forage crops is ranged from 2.1 (grasses) to 2.5 mg kg⁻¹ (clovers) and Pb is considered as metal with the lowest biological accessibility and highest bioaccumulation in the roots (Kabata-Pendias, 2010). It was not confirmed by results from investigated meadows, where some the examined plant tissues accumulated Pb content > 5 mg kg⁻¹, which could be explained by the vicinity of road and traffic-related air pollution. The measured Cd content in soil was low and Cd was neither readily soluble nor easily phytoavailable.

Our results suggest that Ni, Cr, Pb, and Cd content in the forage produced on the permanent grassland in the three Balkan countries can be considered safe for usage in afore-mentioned ways. However, mineral composition and soil conditions that govern metal mobility vary greatly in serpentine soils, therefore their accumulation still has a potential of reaching potentially hazardous levels.

Therefore, it is necessary to evaluate the actual heavy metal content, especially so in populations growing on metal-loaded serpentine soil.

CONCLUSIONS

Our goals were to survey selected metal content of permanent grasslands from Central Balkans. We aimed to define if the metal levels determined within their tissues are close to or exceed the thresholds that would put their nutritive properties in question. All four elements reported in this survey (Ni, Cr, Pb, Cd) represent elements, which can be toxic, if their levels surpass the usual toxicity thresholds.

We assessed the heavy metal status of extensive meadows in Serbia, Bosnia and Montenegro, in relation to the abundance of those chemical elements of the soil. Dominant species in *Agrostietum capillaris* are grasses and the level of all studied elements in the plant biomass collected from investigated meadows were within the allowed limits.

In general, it can be concluded that from the results of this study, the mineral element concentrations of analyzed herbage samples from all sites do not exceed maximal tolerance levels for fodder. Precautionary measures of metal content scanning are suggested for beneficial properties of these grasslands to remain safe and uncompromised.

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