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ASSESSMENT OF HEAVY METALS AND ARSENIC CONTENT IN GRASSLANDS OF BULGARKA NATURE PARK

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Summary: The content of lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) in the aboveground biomass of grasslands in Bulgarka Nature Park was investigated. Among the species studied, the content of Pb was highest in *Festuca valida* (20.83 mg kg^{-1} DW) from the grassland in Malusha site, thus exceeding Pb content in the rest grass species between 2 and 85 times. In addition, the exceedance was 2.1 times the maximum permitted level. Pb content in the mixed sample was high (8.47 mg kg^{-1} DW) due to the increased proportion of *Festuca valida* in the mixed sample (80% cover). The content of As in all investigated plants from the grasslands in both Malusha site and Mount Ispolin exceeded 1.2-2.8 fold the mean value (0.21 mg kg^{-1} DW). Cd and Hg were found in low concentrations or even below detection limit. The increased content of Pb and As could be due to elavated natural geochemical background. It could be recommended to analyze the concentrations of heavy metals in animal products from the region and if needed to isolate definite parts of the grasslands for grazing.

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Keywords: Arsenic; cadmium; lead; mercury; aboveground biomass; grassland; nature park.

INTRODUCTION

Bulgarka Nature Park was established in 2002 by a Directive RD-775 from 09.08.2002 of the Ministry of Environment and Waters of Bulgaria with the main purpose for preservation, recreation and maintenance of the natural beech ecosystems and landscapes in the central part of the Balkan mountains as well as the flora and fauna, historical and cultural heritage of the region. The quality of the environment in the park has been investigated with the aim to assess the impact of local industrial sources of pollution on soils and some plant species (Malinova, 2010; Malinova et al., 2010; Bezlova et al., 2012). The impact of the industrial area of Gabrovo and the auto transport on the soil and vegetation in the Park is not significant. In some sites, the soil and beech leaves had enhanced content of As. In addition, a number of medicinal plants form grassland landscapes contained high levels of Pb, Cd and As exceeding significantly the maximum

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permitted levels. No investigations have been carried out on plants used for grazing.

Contamination of plants with heavy metals has been studied mainly in regions located nearby industrial sources of emissions. Specific for grazing GIS has been developed in some European countries as in Germany, named "VIOLETEA", specialized for grasslands located on soils with enhanced levels of heavy metals. The investigations have been focused on the effects of pollution on grassland biodiversity and heavy metal content in plants (Becker, 2012). The data available in literature have shown either a positive correlation between the content of Pb, Cd and As in soils and pasture grasses (Kadovic et al., 2011, Madejón et al., 2012) or a lack of correlation (Doncheva, 2000; Becker and Brändel, 2007). A number of investigations have revealed enhanced content of heavy metals in grass species, hay and straw. Hernández and Pastor (2008) reported that although in single plants Cd was under the detection limit according to the analytical method used, its content in the mixed hay sample was high.

The Long-Range Transboundary Air Pollution (LRTAP) Convention has developed the critical loads approach based on established critical limits of heavy metals in soil solution (UBA, 2004). Although the area of exceedance of the critical loads for metals Cr, Ni, Cu, Zn, As and Se is small, even small exceedances may result in effects in the future due to the accumulative nature of heavy metals in soils. These results support the focus of the 1998 Aarhus Protocol to the 1979 Convention on Long-Range Transboundary Air Pollution concerning Cd, Pb and Hg, which are highly toxic. All these elements have the ability for bioaccumulation and could lead to deterioration of the sanitary characteristics of plant production.

A number of criteria for assessment of heavy metal content in fodder have been developed in terms of protection of animals and human health. According to the German legislation and the norms developed by the Association of German Engineers (Verein Deutscher Ingenieure, VDI) the upper critical level (UCL) of Pb (VDI, 1998) in livestock feed is 10 mg kg^{-1} DW whereas for Cd this value should not exceed 0.5 mg kg^{-1} DW (VDI, 2008). According to De Vries et al. (2005) the average content of Pb, Cd and Hg in the biomass of grasslands is 1-3 mg kg^{-1} DW, 0.05-0.25 mg kg^{-1} DW and 0.01-0.1 mg kg^{-1} DW, respectively.

Arsenic is known to be hardly accessible to plants. According to Kabata-Pendias and Pendias (1989) the mean content of As in the above-ground grass biomass varied within the range 0.28-0.33 mg kg^{-1} DW whereas Reinds et al. (2006) reported an average value of 0.21 mg kg^{-1} DW. Kabata-Pendias and Pendias (1989) reported high As concentrations concerning wild grass species, the highest values found in roots and leaves of *Mentha aquatica* (540 and 216 mg kg^{-1} DW, respectively) as well as in roots of *Phragmites australis* (688 mg kg^{-1} DW) (Baronia et al., 2004). Recently published data have shown high content of As in medicinal plants in Bulgarka Nature Park (Bezlova et al., 2012).

Assessment criteria for heavy metal contamination of grass species from grasslands in terms of possible impact on animals have been developed in Bulgaria. Todorov & Dardzhonov (1995)

determined standards for nutrition of sheep and goats based on the average values for many macro- and microelements in fodder including grass from grasslands however, neither of the elements Pb, Cd, As and Hg was analyzed in the study. The maximum permitted levels of Cd and Pb in different crops were established in a joint research of the "N. Poushkarov" Institute and the Institute of Hygiene and Occupational Diseases (Bozhinova et al., 1996).

The aim of the present study was to analyze and assess the content of Pb, Cd, As and Hg in the above-ground phytomass in grassland landscapes of Bulgarka Nature Park taking into account the Park Directorate's intention to restore grazing in the high-mountain areas of the Park.

MATERIALS AND METHODS

Sampling sites and plant material

Samples for analysis were collected from the above-ground phytomass in grassland landscapes of Bulgarka Nature Park. The grasslands are located in the flat sections of the ridge part of the park – Malusha site and Mount Ispolin, occupying altitudinal range between 1300 m and 1500 m. Malusha site occupies an area of 37 ha, 14 ha being included within the park. Mount Ispolin occupies 300 ha including 100 ha rocks and an area outside the park. The areas near the villages Potok and Ezeroto as well as Voditsi holiday village are with limited grazing due to the steep slopes of the Balkan Mountains and the small area of the grassland.

Sampling was carried out in late June 2011 and 2012. Samples of mixed species composition were used for chemical analyses (20-30 grams of fresh material). After the analysis of the quantitative proportion of the species, samples from the dominating grass species were collected. Each sample, either mixed or individual included plant material collected from 5 sample sites located in the transect with a total length of 400 m in east-west direction. A total of 20 samples were collected.

Sample analysis

Dry weight was measured after oven drying at 40°C until constant weight. For decomposition of the samples closed microwave digestion with HNO_3 + H_2O_2 was used. The content of Cd and Pb was determined by Graphite furnace atomic absorption spectrophotometry (GFAAS) whereas a hydride system was applied for As and Hg. Analyses were conducted in the licensed Central Laboratory for Chemical Testing and Control of the Ministry of Agriculture and Food in compliance with Bulgarian State Standards EN 14084:2003 and EN 13806:2003. The results were calculated on a dry weight (DW) basis.

Data analysis

Data analysis included descriptive statistics and One-sample t-tests for revealing the differences between the measured concentrations of the elements in the studied plants and the upper critical level or the natural concentration range.

RESULTS AND DISCUSSION

Among the grass species tested, the content of Pb was highest in *Festuca valida* (20.8 mg kg^{-1} DW) from the grassland in Malusha site, thus exceeding both Pb content in the rest grass species (2-85 times) and the upper critical level (Fig. 1). The exceedance was 2.1 times the limit accepted for our country - 10

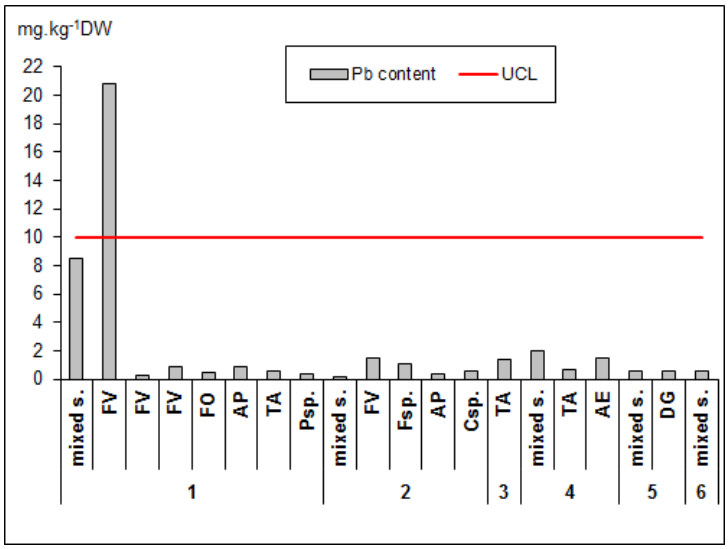

Figure 1. Pb content in mixed samples and samples of different grass species.
Legend: Sample plot: 1 – Malusha; 2 – Ispolin; 3 – Uzana; 4 – Ezero; 5 – Voditsi; 6 – Potok.
FV – (*Festuca valida*); FO – (*Festuca valesiaca*); AP – (*Alopecurus pratensis*); TA – (*Trifolium alpestre*); AE – (*Arrhenatherum elatius*); DG – (*Dactylis glomerata*); Psp. – (*Poa* sp.); Fsp. – (*Festuca* sp.); Csp. – (*Carex* sp.).

mg kg^{-1} DW (Bozhinova et al., 1996) and coincided with the upper critical level for animal protection in Germany (VDI, 2008). The content of Pb in the rest grass species tested (*Festuca valesiaca*, *Alopecurus pratensis*, *Trifolium alpestre* and *Poa* sp.) varied within the range 0.25 – 0.87 mg kg^{-1} DW, which was significantly lower than the limits mentioned above and even lower than the mean values for grass species in grasslands and meadows, being 1-3 mg kg^{-1} DW according to De Vries et al. (2005).

The analysis of the species composition of the mixed sample collected from the eastern part of the grassland in Malusha site showed that *Festuca valida* was the dominating grass species (80% cover). The high Pb content found in the mixed sample (8.47 mg kg^{-1} DW) was due to the enhanced accumulation of Pb in *Festuca valida* (20.83 mg kg^{-1} DW). For comparison, the natural Pb content in pasture grasses in the region of the Lead-zinc smelter - Kardzhali varied within the range 0.80-2.80 mg kg^{-1} DW and could be increased up to 74 mg kg^{-1} DW due to industrial pollution (Bozhinova et al., 1994).

It is worth noting that *Festuca valida* samples collected from other areas of Malusha site contained much less Pb (0.25 – 0.85 mg kg^{-1} DW). The mean value and the standard deviation for Pb content in different species from the grassland (3.47±7.66 mg kg^{-1} DW) showed data variability which was indicative for the diverse chemical composition of the soils on a limited area and the absence of any aerosol contribution of either local or regional sources to the measured high Pb concentrations. It has been reported that permanent grazing on one and the same place is safe if Pb content is below 5 mg

kg^{-1} DW, suggesting that avoiding certain areas of the grassland could reduce the risk to animals (Bozhinova et al., 1994).

In the grasslands of the other studied sites: Mount Ispolin, Uzana, as well as the areas near to the settlements - the villages Potok, Ezeroto and Voditsi holiday village, Pb content was below the upper critical level (Fig. 2). In most species it was lower than 1 mg kg^{-1} DW, but in some species like *Trifolium alpestre*, *Arrhenatherum elatius* or in the mixed samples the concentrations of Pb were 1.34 mg kg^{-1} DW, 1.52 mg kg^{-1} DW and 0.17-1.97 mg kg^{-1} DW, respectively. The mean Pb content in the studied species from the above sites was 0.95±0.46 mg kg^{-1} DW, which was below the limit pointed as mean by De Vries at al. (2005).

All studied grass species in the park showed low concentrations of Cd. The mean value of 0.075±0.05 mg kg^{-1}DW was below the upper critical level in natural meadows and grasslands in Bulgaria (1 mg kg^{-1} DW) as well as below the upper critical level for animal health preservation accepted in Germany (0.5 mg kg^{-1} DW) (Fig. 2). The mean values for Cd measured in the different grass species in Malusha, Mount Ispolin and Uzana sites were 0.063±0.03 mg kg^{-1} DW, 0.076±0.04 mg kg^{-1} DW and 0.040 mg kg^{-1} DW, respectively. On the other hand, Cd content in the samples from areas near to the villages Potok, Ezeroto and Voditsi holiday village was below detection limit of the method used (<0.025 mg kg^{-1}). For comparison, Cd content in grasslands from other regions in Bulgaria varied within the range 0.08-0.20 mg kg^{-1} DW (Bozhinova et al., 1994).

Our data on As content confirm again that the grassland in Malusha site is a potential source of risk to animals

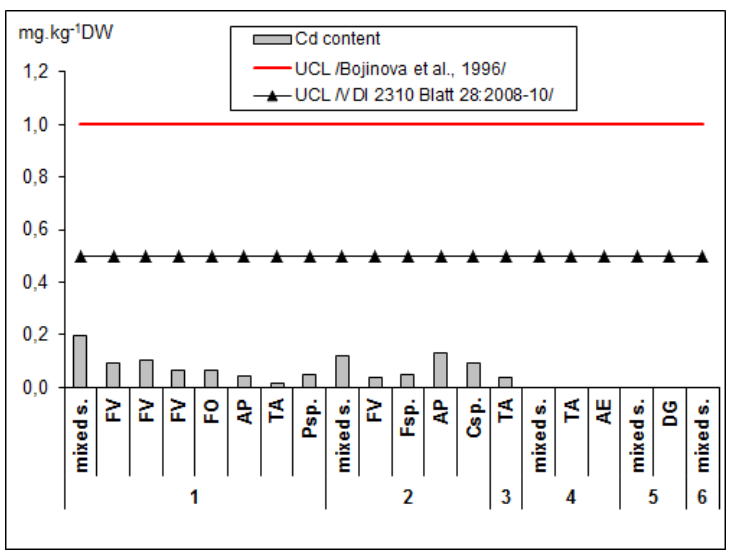

Figure 2. Cd content in mixed samples and samples of different grass species.
Legend: Sample plot: 1 – Malusha; 2 – Ispolin; 3 – Uzana; 4 – Ezero; 5 – Voditsi; 6 – Potok.
FV – (*Festuca valida*); FO – (*Festuca valesiaca*); AP – (*Alopecurus pratensis*); TA – (*Trifolium alpestre*); AE – (*Arrhenatherum elatius*); DG – (*Dactylis glomerata*); Psp. – (*Poa* sp.); Fsp. – (*Festuca* sp.); Csp. – (*Carex* sp.).

(Fig. 3). The mean As content in the studied grass species in Malusha site was 0.362±0.12 mg kg^{-1} DW. Content of As in all investigated plants exceeded 1.2-2.8 fold the mean value of 0.21 mg kg^{-1} DW accepted for grass species (Reinds et al., 2006).

The concentration of As in the studied grass species in Malusha site was enhanced. In some samples As content was found to be even beyond the range of mean values for grass species (0.28-0.33 mg kg^{-1} DW) pointed by Kabata-Pendias and Pendias (1989). In the mixed sample, *Festuca valida* and *Trifolium alpestre* the upper range limit was exceeded 3.9-fold, 1.1-1.8-fold and 1.4-fold, respectively.

In the grassland of Mount Ispolin the mean As content was 0.282±0.05 mg kg^{-1} DW. In all studied grass species the measured As content exceeded up to 1.7-fold the mean value according to Reinds et al. (2006). Only in *Festuca* sp. the upper range limit pointed by Kabata-Pendias and Pendias (1989) was insignificantly exceeded (1.1-fold). The measured As value of the mixed sample did not exceed that of *Festuca* sp. as the proportion of the latter in the sample was low. Some plants in the studied site like *Alopecurus pratensis* and *Carex* sp. accumulated less As.

In the two largest grasslands of Malusha site and Mount Ispolin the concentration of As in the grass species varied strongly, which was indicative for the absence of any aerosol contribution to the measured values as was established for Pb.

Lower content of As was measured in the grass species from the grasslands near to the villages Potok, Ezeroto and Voditsi holiday village compared with Malusha site. The measured mean value was

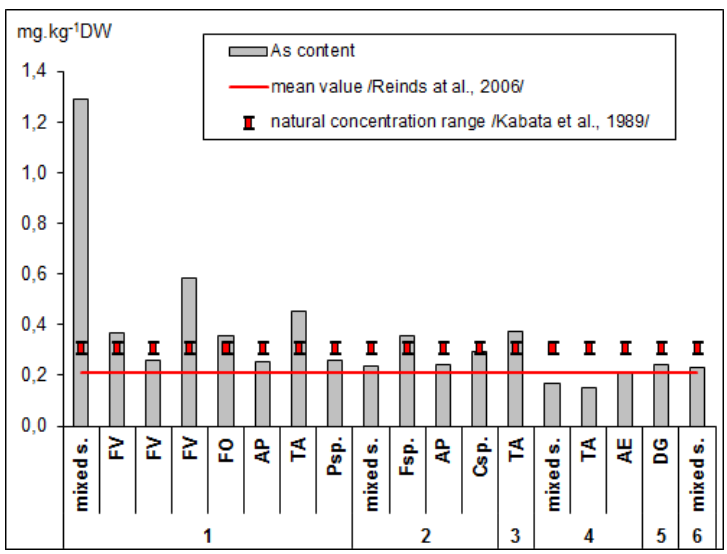

Figure 3. As content in mixed samples and samples of different grass species. Legend: Sample plot: 1 – Malusha; 2 – Ispolin; 3 – Uzana; 4 – Ezero; 5 – Voditsi; 6 – Potok.
FV – *(Festuca valida);* FO – *(Festuca valesiaca);* AP – *(Alopecurus pratensis),* TA – *(Trifolium alpestre);* AE – *(Arrhenatherum elatius);* DG – *(Dactylis glomerata);* Psp. – *(Poa* sp.*);* Fsp. – *(Festuca* sp.*);* Csp. – *(Carex* sp.*).*

0.201±0.05 mg kg^{-1} DW. Only *Trifolium alpestre* from Uzana site showed higher levels of As (0.372 mg kg^{-1} DW), which exceeded 1.8-fold the accepted mean value of 0.21 mg kg^{-1} DW (Reinds et al., 2006).

One-sample t-test was conducted in order to analyze the statistically significant differences between the measured concentrations of the elements in the studied plants and the upper critical level or the natural concentration range of the elements (Table 1). Our results showed statistically significant differences concerning the upper critical level for the accumulation of Pb and Cd, which can be accounted for by only one very high value exceeding the upper critical level for Pb (10 mg kg^{-1} DW) and predominating low concentrations of Pb and Cd. The results on Cd accumulation differed from those considered by De Vries et al. (2005) as range limits of Cd natural concentration in the grazing biomass (0.05-0.25 mg kg^{-1} DW). On the other hand, the concentrations of Pb (1-3 mg kg^{-1}DW) and As (0.05-0.025 mg kg^{-1} DW) did not differ significantly from their natural content reported by De Vries et al. (2005).

The content of Hg in the studied grass species was below detection limit of the method used. The only exception was *Festuca valida* in the grasslands of Malusha site and Mount Ispolin where the measured values for Hg content were 0.020 mg kg^{-1} DW and 0.018 mg kg^{-1} DW, respectively (Fig. 4).

Table 1. Results of the "Test of means against reference constant" (one-sample t-test).

Element	UCL value	N	t-value	p
Pb	10.00	20	-7.383	5.4E-07*
Cd	0.50	14	-32.901	6.6E-14*
	1.00	14	-71.929	2.7E-18*
Element	**Natural concentration range**	**N**	**t-value**	**p**
Pb	0.00	20	2.063	0.053
	1.00	20	1.119	0.277
	3.00	20	-0.771	0.450
Cd	0.00	14	6.127	3.6E-05*
	0.05	14	2.225	0.044*
	0.25	14	-13.387	5.6E-09*
As	0.00	17	5.733	3.1E-05*
	0.28	17	1.301	0.212
	0.33	17	0.509	0.617

*$p < 0.05$ – statistically significant differences.

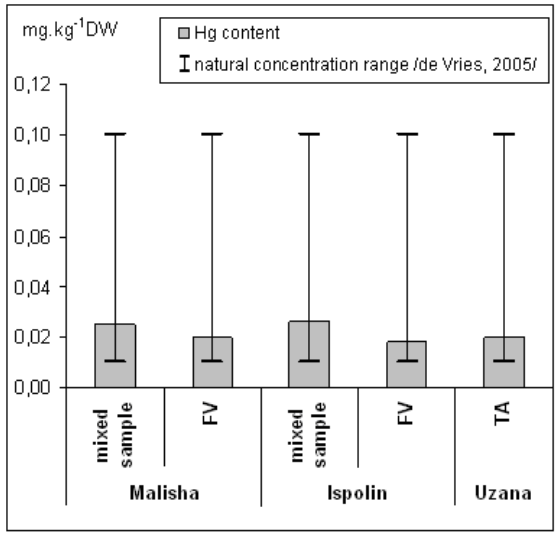

Figure 4. Hg content in mixed samples and samples of different grass species. Legend: FV – (*Festuca valida*); TA – (*Trifolium alpestre*).

The content of Hg in the mixed samples of both grasslands was 0.025-0.026 mg kg^{-1} DW. The measured values did not exceed the range limits of natural Hg concentration in the grazing biomass as reported by De Vries et al. (2005).

Our results showing high concentrations of Pb and As together with predominating low concentrations of Cd in the studied grass species from the grasslands of Malusha site and Mount Ispolin complement previously reported data on exceedances of these elements detected in medicinal plants and beech leaves from the same sites (Malinova et al., 2010; Bezlova et al., 2012).

CONCLUSIONS

The present investigation showed elevated content of Pb and As in the above-ground biomass of grasslands in certain areas of Bulgarka Nature Park (Malusha site and Mount Ispolin). Among the species studied, *Festuca valida* was found to be a highly accumulating species contributing to the exceedance above the upper critical level of the mixed samples as well. The data variability on Pb and As content in the studied grass species from the high-mountain grasslands was indicative for the absence of aerosol deposition of these elements. The detected increased concentrations could be due to elavated natural geochemical background. In grazing biomass near to settlements the content of Pb an As was not increased. The existing criteria for assessment of heavy metal content and As in the above-ground biomass of grasslands refer to each element separately. However, assessment criteria for the combined effects of two or more heavy metals on plants and humans have not been developed, yet. Thus, it could be recommended to analyze the concentrations of heavy metals in animal products from the region and if needed to isolate definite parts of the grasslands for grazing.

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