

COBALT AND ARSENIC CONCENTRATION IN HERBS GROWING IN FIELD POND AREAS IN POLAND

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Abstract. This paper deals with cobalt and arsenic accumulation in above-ground parts of herbs growing in intermittent field ponds with no outlets in Poland. Those ponds were located in different surroundings. In the experiment described here the following herbs were analysed: *Potentilla anserina* L., *Mentha arvensis*, *Achillea millefolium* L., *Comarum palustre* L., *Lysimachia vulgaris* L. and *Lycopus europaeus* L. Both plant and soil samples were collected from three moisture sections: wet, periodically wet and dry. Cobalt and arsenic content in plants and soil was determined with inductively coupled plasma-atomic emission spectrometry, or the ICP–AES method, after dry mineralization of the material. Cobalt and arsenic content was significantly dependent on the herb species. The highest content of cobalt was found in *Mentha arvensis*, and arsenic in *Lysimachia vulgaris* L. and *Comarum palustre* L., with *Achillea millefolium* having the lowest content of arsenic. The highest content of those metals was in the bottom sediment and in the soil from the depression with arable land around. Arsenic had a higher accumulation coefficient in herbs than cobalt. Cobalt content in herb biomass did not exceed the limits, but in the case of arsenic it was too high in all herbs apart from *Achillea millefolium* L.

Keywords: *selected heavy metals, plants, soil, in-field ponds*

Introduction

Arsenic and cobalt belong to elements very common in the environment, and in higher levels they are poisonous to humans and animals (Bowell, 1994; Hamilton, 1994; Kabata-Pendias and Pendias, 1999). Arsenic compounds are used as herbicides and defoliant in agriculture and forestry mainly. The amount of arsenic in soil depends on its content in parent rock, the type of soil formation and on anthropogenic factors. Anthropogenic pollution is mainly of industrial origin but there are other sources like using communal and industrial waste or plant protecting products in agriculture (Krysiak and Karczewska, 2007). In soil, arsenic is adsorbed by iron oxides, mainly by iron hydroxides of different degrees of crystallisation (Bowell, 1994; Voigt et al., 1996; Wenzel et al., 2001). Because of that this metal is not very mobile and not easily available to plants. A change of soil pH or soil aeration can cause a partial destruction of iron oxides, and adsorbed arsenic is released (Masscheleyn et al., 1991; Marin et al., 1993).

Gál et al. (2008) indicate that there are different kinds of cobalt ions in soil depending on soil pH, which, together with reduction potential, determines bioavailability of the metal. Suttle et al. (2003) and Bakkaus et al. (2008) provide

different degrees of cobalt bioavailability, from 3 to 13% and from 1.5 to 37%, respectively. In agricultural areas free from industrial pollution, cobalt can get into the soil together with phosphorus fertilisers.

Of course, its presence, like presence of other heavy metals in the soil-plant environment, can be harmful to people and animals. Plants growing in natural habitats, even if not directly exposed to pollution, can contain amounts of heavy metals which are too high (Ražić et al., 2008; Malinowska and Jankowski, 2016, 2017; Bolan et al., 2017).

The aim of the experiment was to determine cobalt and arsenic content in herbs used in the pharmaceutical, cosmetic and food industries. They were sampled from different moisture sections of three in-field ponds.

Materials and methods

The plant material was sampled in 2014, from mid June till the end of July. Six herb species growing in the areas of three field ponds on the Siedlce Plateau were sampled: *Potentilla anserina* L., *Mentha arvensis*, *Achillea millefolium* L., *Comarum palustre* L., *Lysimachia vulgaris* L., *Lycopus europaeus* L. Three experimental areas with three ponds were marked with capital letters: A, B and C (Fig. 1).

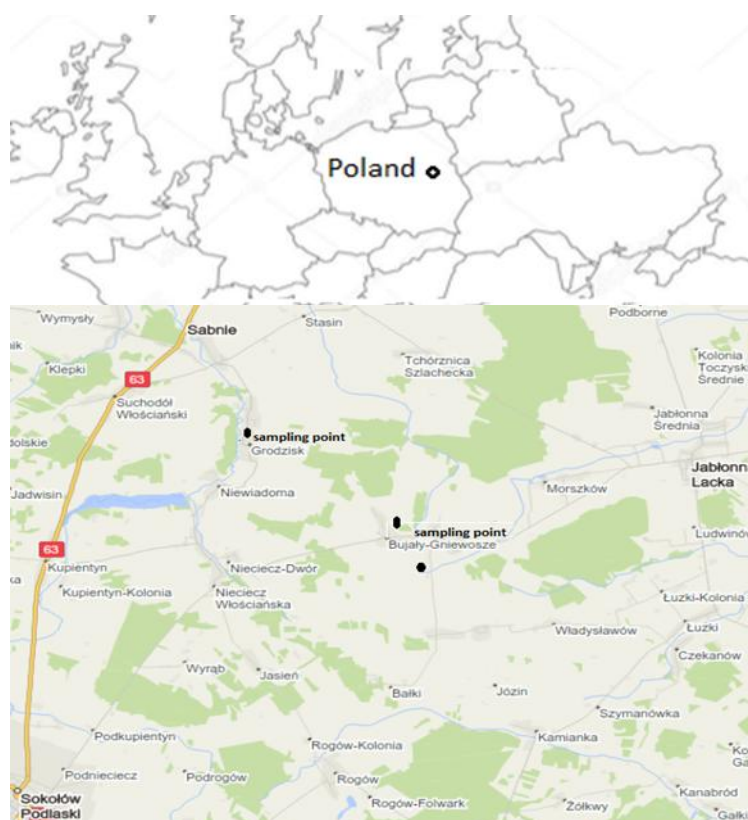


Figure 1. A map of the sampling site in an urban area, Poland

Pond A was surrounded by arable land, B by permanent grassland and pond C was overgrown with bushes, with cultivated fields 100 m away from it. What decided about the choice of the ponds was the fact that they were surrounded by fields, but also the

diversity of vegetation and the pond sizes, ranging from 15000 m² to 850 m². According to the administrative division they were located in the Sokołów Podlaski County, the eastern part of the Mazovian Voivodeship, with luvisols and rusty soil mostly. Each experimental area was divided into three transects, with the length varying from 18 to 80 m, stretching from the edge of the pond to the edge of the field. Around each pond in every transect three concentric moisture sections were marked: I – wet, with water stagnating in early spring after snow has melted or after heavy rainfall; II – periodically wet, with water stagnating only in early spring after snow has melted; III – dry, without any water stagnating there (Franczak and Franczak, 2015). The number of plant and soil samples taken from the transects varied from 85 to 100 in each experimental area. Additionally, bottom sediment samples were taken from each pond. 5-7 plants of each species were collected from each transect.

Plant material (from above-ground parts, without roots) was ground to particles of 0.25 mm in diameter, and 1 g of it was weighed and put into a porcelain crucible. Then the crucible was placed in a muffle furnace for 15 h to oxidise organic matter at the temperature of 450 °C. Next 10 ml of hydrochloric acid solution (1:1) was added and the mixture was put into a sand bath to evaporate, to decompose carbonates and to remove silica. After adding 5 ml of 10% hydrochloric acid solution, the contents of the crucible were filtered through a hard filter into a 100 ml volumetric flask and water was added up to the mark. Cobalt and arsenic concentration was determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). An internal quality control procedure was used to verify the accuracy of the methods. It was assumed that the average recovery for each spike concentration should be between 85 and 115% of true value. Two measurements were taken for each series of samples with the recovery being within the 85÷115% range. The limit of detection for arsenic and cobalt was 0.01 mg·kg⁻¹. Soil pH, the value of which ranged from 5.45 to 6.50, was measured with the potentiometric method.

All of the data were statistically analysed and differences between means were assessed using analysis of variance (The Statistica programme, Version 10.0 StatSoft, was applied). Tukey's test was used to determine LSD_{0.05} for means that were significantly different (StatSoft, Inc. 2011). The intensity of cobalt and arsenic accumulation by the herbs was determined with the accumulation coefficient (AC).

$$AC = c_p / c_s$$

where c_p is the metal content in the plant and c_s is the metal content in the soil (Wesołowski and Radecka, 2003). The results were interpreted as follows:

AC < 0.01 - no accumulation;

AC < 0.1 - slight accumulation;

AC - 1 - medium accumulation;

AC > 1 - high accumulation.

Additionally, the correlation coefficient between the content of each metal in the soil and the same content in the plants was calculated.

Results and discussion

Cobalt concentration in the biomass of herbs used in the experiment significantly varied, depending on the plant species and on the moisture section, and ranged from

0.011 to 0.931 mg·kg⁻¹ DM (*Table 1*). There was no significant variation in this concentration between moisture sections in the area with arable land. Out of all experimental areas and all moisture sections the highest average concentration of this metal was in *Mentha arvensis* (0.600 mg·kg⁻¹), and the lowest, over 16 times lower, in *Achillea millefolium* L. (0.037 mg·kg⁻¹). The average cobalt concentration in herbs sampled from different moisture sections was the highest in the periodically wet sections (0.261 mg·kg⁻¹), while the lowest was in the dry sections (0.189 mg·kg⁻¹).

Table 1. Cobalt concentrations (mg·kg⁻¹) in the biomass of some herbs

| Species | A | | | | B | | | | C | | | | Mean | | | |
|--------------------------------|-------|-------|-------|-------------|-------------|-------|-------|-------|-------------|-------|-------|-------|-------------|-------|-------|-------|
| | I | II | III | Mean | I | II | III | Mean | I | II | III | Mean | I | II | III | Mean |
| <i>Potentilla anserina</i> L. | 0.123 | 0.102 | 0.140 | 0.122 | 0.136 | 0.123 | 0.145 | 0.135 | 0.133 | 0.112 | 0.103 | 0.116 | 0.131 | 0.112 | 0.129 | 0.124 |
| <i>Mentha arvensis</i> | 0.623 | 0.741 | 0.563 | 0.642 | 0.745 | 0.931 | 0.684 | 0.787 | 0.541 | 0.342 | 0.232 | 0.372 | 0.636 | 0.671 | 0.493 | 0.600 |
| <i>Achillea millefolium</i> L. | 0.036 | 0.058 | 0.039 | 0.044 | 0.023 | 0.054 | 0.063 | 0.047 | 0.023 | 0.026 | 0.011 | 0.020 | 0.027 | 0.046 | 0.038 | 0.037 |
| <i>Comarum palustre</i> L. | 0.236 | 0.203 | 0.298 | 0.246 | 0.148 | 0.201 | 0.198 | 0.182 | 0.303 | 0.318 | 0.287 | 0.303 | 0.229 | 0.241 | 0.261 | 0.244 |
| <i>Lysimachia vulgaris</i> L. | 0.056 | 0.032 | 0.030 | 0.039 | 0.099 | 0.820 | 0.094 | 0.338 | 0.060 | 0.036 | 0.050 | 0.049 | 0.072 | 0.296 | 0.058 | 0.142 |
| <i>Lycopus europaeus</i> L. | 0.120 | 0.165 | 0.199 | 0.161 | 0.124 | 0.137 | 0.148 | 0.136 | 0.203 | 0.208 | 0.105 | 0.131 | 0.149 | 0.200 | 0.152 | 0.167 |
| Mean | 0.199 | 0.217 | 0.212 | 0.209 | 0.213 | 0.378 | 0.222 | 0.271 | 0.211 | 0.174 | 0.131 | 0.172 | 0.207 | 0.261 | 0.189 | 0.219 |
| LSD _{0.05} for: | | | | | | | | | | | | | | | | |
| S-species | | | | S = 0.041 | S = 0.013 | | | | S = 0.054 | | | | S = 0.034 | | | |
| M-moisture section | | | | M = n.s. | M = 0.007 | | | | M = 0.031 | | | | M = 0.019 | | | |
| S/M; M/S interaction | | | | S/M = 0.071 | S/M = 0.018 | | | | S/M = 0.075 | | | | S/M = 0.047 | | | |
| | | | | M/S = 0.057 | M/S = 0.022 | | | | M/S = 0.093 | | | | M/S = 0.058 | | | |

A - area with the cultivated field; B - area with permanent grasslands; C - area with bushes; I - wet section; II - periodically wet section; III - dry section; n.s. - not significant difference

Among different experimental areas, the highest average concentration of cobalt was in herbs growing around the pond with permanent grasslands (0.271 mg·kg⁻¹), while the lowest was in herbs from the area with bushes (0.172 mg·kg⁻¹). Bakkaus et al. (2005) say that dicotyledonous plants can contain much more cobalt than monocotyledonous plants. Like iron, cobalt in vascular plants is transported to tissues in an active and passive way (Palit et al., 1994). Because of the similarity of the uptake process there might be an antagonistic relationship between cobalt and other metals, in particular iron and manganese. According to Kabata-Pendias and Pendias (1999) cobalt concentration in plants should range from 0.08 to 0.1 mg·kg⁻¹, while the toxic concentration of this metal varies between 30 and 40 mg·kg⁻¹ DM. In the present experiment the average cobalt concentration in the plants was 0.219 mg·kg⁻¹, which is lower than the limit. Symanowicz et al. (2014) found a similar cobalt concentration in *Galega orientalis* Lam., with mineral fertilisers applied.

Arsenic concentration in the analysed herbs varied considerably depending on the plant species and the moisture section (*Table 2*). Concentration of this metal ranged from 0.301 to 1.36 mg·kg⁻¹ DM. The highest concentration of arsenic was found in *Lysimachia vulgaris* L. (0.848 mg·kg⁻¹) and in *Comarum palustre* L. (0.757 mg·kg⁻¹), with the lowest in *Achillea millefolium* L. (0.448 mg·kg⁻¹). The average concentration of this metal in all the herbs was 0.645 mg·kg⁻¹. The natural barrier for the uptake of

arsenic by the above-ground parts of plants is the root system. Das et al. (2004) say that the concentration of arsenic in plant roots is ten times higher than in the above-ground parts. According to the Ministry of Health Regulation (2003) arsenic concentration in medicinal herbs and dry culinary herbs should not be higher than 0.5 mg·kg⁻¹ DM. In the experiment described here, out of six analysed herbs, only *Achillea millefolium* L. had arsenic concentration below the limit. In all the other herbs the concentration was much higher than the limit set by the Regulation of 2003. Thus, in *Lysimachia vulgaris* L. the concentration was 70% higher, and in *Comarum palustre* L. it was 50% higher.

Table 2. Arsenic concentrations (mg·kg⁻¹) in the biomass of some herbs

| Species | A | | | | B | | | | C | | | | Mean | | | |
|--------------------------------|-------|-------|-------|-------------|-------------|-------|-------|-------|-------------|-------|-------|-------|-------------|-------|-------|-------|
| | I | II | III | Mean | I | II | III | Mean | I | II | III | Mean | I | II | III | Mean |
| <i>Potentilla anserina</i> L. | 0.871 | 0.645 | 0.620 | 0.712 | 0.541 | 0.954 | 0.530 | 0.675 | 0.501 | 0.451 | 0.403 | 0.452 | 0.638 | 0.683 | 0.518 | 0.613 |
| <i>Mentha arvensis</i> | 0.641 | 0.784 | 0.502 | 0.642 | 0.870 | 0.987 | 0.623 | 0.827 | 0.417 | 0.514 | 0.498 | 0.476 | 0.643 | 0.773 | 0.541 | 0.652 |
| <i>Achillea millefolium</i> L. | 0.501 | 0.412 | 0.436 | 0.450 | 0.512 | 0.578 | 0.509 | 0.533 | 0.411 | 0.368 | 0.301 | 0.360 | 0.475 | 0.453 | 0.415 | 0.448 |
| <i>Comarum palustre</i> L. | 0.899 | 0.785 | 0.623 | 0.769 | 0.880 | 1.020 | 0.877 | 0.926 | 0.544 | 0.688 | 0.501 | 0.578 | 0.774 | 0.831 | 0.667 | 0.757 |
| <i>Lysimachia vulgaris</i> L. | 0.991 | 0.836 | 0.521 | 0.783 | 0.841 | 1.360 | 0.801 | 1.00 | 0.754 | 0.801 | 0.723 | 0.759 | 0.862 | 0.999 | 0.682 | 0.848 |
| <i>Lycopus europaeus</i> L. | 0.421 | 0.499 | 0.413 | 0.444 | 0.647 | 0.921 | 0.578 | 0.715 | 0.412 | 0.566 | 0.509 | 0.496 | 0.493 | 0.662 | 0.500 | 0.552 |
| Mean | 0.721 | 0.661 | 0.519 | 0.633 | 0.716 | 0.970 | 0.653 | 0.779 | 0.507 | 0.565 | 0.489 | 0.520 | 0.648 | 0.734 | 0.554 | 0.645 |
| LSD _{0.05} for: | | | | | | | | | | | | | | | | |
| S-species | | | | S = 0.010 | S = 0.025 | | | | S = 0.023 | | | | S = 0.035 | | | |
| M-moisture section | | | | M = 0.006 | M = 0.014 | | | | M = 0.013 | | | | M = 0.020 | | | |
| S/M; M/S interaction | | | | S/M = 0.014 | S/M = 0.043 | | | | S/M = 0.033 | | | | S/M = 0.060 | | | |
| | | | | M/S = 0.017 | M/S = 0.035 | | | | M/S = 0.041 | | | | M/S = 0.049 | | | |

A - area with the cultivated field; B - area with permanent grasslands; C - area with bushes; I - wet section; II - periodically wet section; III - dry section

When it comes to moisture sections the highest average concentration of arsenic was in herbs from periodically wet sections (0.734 mg·kg⁻¹), it was lower in wet sections and the lowest in dry sections (0.554 mg·kg⁻¹). This diversity might have been caused by leaching of plant protection products into groundwater. The average arsenic concentration was also different in different experimental areas. The most arsenic was in plants growing around the pond with permanent grasslands and the lowest in the area with bushes, on average 0.779 mg·kg⁻¹ and 0.520 mg·kg⁻¹, respectively.

Cobalt and arsenic concentration in bottom sediment of the three different ponds varied (Table 3). The highest concentration of cobalt (3.12 mg·kg⁻¹) and arsenic (4.38 mg·kg⁻¹) was found in the bottom sediment sampled from the pond surrounded by arable land. In the sediment from the pond with bushes the concentration was much lower, with 2.03 mg Co·kg⁻¹ and 2.87 mg As·kg⁻¹. Many publications (Dhar et al., 1997; Kondo et al., 1999; Karim, 2000; Anawar et al., 2003), report that arsenic can get into groundwater and accumulate in bottom sediment. Reduction processes, taking place in permanently flooded areas, can be the main source of contamination of groundwater by arsenic in south eastern Asia, in countries like Bangladesh, India and Vietnam (Burton et al., 2008).

Table 3. Cobalt and arsenic concentration in bottom sediment (mg kg^{-1} DM)

| Metal | A | B | C | Mean |
|-------|------|------|------|------|
| Co | 3.12 | 2.63 | 2.03 | 2.59 |
| As | 4.38 | 3.54 | 2.87 | 3.60 |

A - area with the cultivated field; B - area with permanent grasslands; C - area with bushes

The amount of cobalt and arsenic in the soil was also related to the moisture section and to the experimental area (Table 4). On average the highest concentration of both metals was in the soil of the depression with arable land. Out of all three moisture sections the highest concentration of cobalt and arsenic was in the wet section (1.74 mg kg^{-1} and 2.06 mg kg^{-1} , respectively). The amount of those metals in the soil was much lower than their amount in non-contaminated soil reported by Kabata-Pendias and Pendias (1999). The release of arsenic into the soil is a complicated process, chemical and physicochemical, but first of all it involves a biochemical reduction of the components, both solid and liquid parts (Anawar et al., 2003; Solaiman et al., 2009; Postma et al., 2010). One of the main factors affecting this process is humus content in the soil.

Table 4. Cobalt and arsenic concentration in soil (mg kg^{-1} DM)

| Area | Co | | | | As | | | |
|--------------------------|--------------|------|--------------|------|--------------|------|--------------|------|
| | I | II | III | Mean | I | II | III | Mean |
| A | 1.94 | 2.29 | 1.03 | 1.75 | 2.94 | 3.05 | 2.40 | 2.80 |
| B | 1.75 | 1.23 | 1.48 | 1.49 | 1.85 | 1.69 | 0.989 | 1.51 |
| C | 1.52 | 1.36 | 0.984 | 1.29 | 1.39 | 1.26 | 1.32 | 1.32 |
| Mean | 1.74 | 1.63 | 1.17 | 1.51 | 2.06 | 2.00 | 1.57 | 1.88 |
| LSD _{0.05} for: | | | | | | | | |
| M-moisture section | M = 0.288 | | | | M = 0.166 | | | |
| Ar-area | Ar = 0.288 | | | | Ar = 0.166 | | | |
| M/B; B/M-interaction | M/Ar = 0.499 | | Ar/M = 0.499 | | M/Ar = 0.288 | | Ar/M = 0.288 | |

A - area with the cultivated field; B - area with permanent grasslands; C - area with bushes; I - wet section; II - periodically wet section; III - dry section

There was a wide range of accumulation coefficient values of the soil-plant system (Figs. 2 and 3). The accumulation coefficient for arsenic was higher than for cobalt but for both heavy metals it was lower than one, on average 0.111 for cobalt and 0.353 for arsenic. When the value of accumulation coefficient is higher than one, then the accumulation of the metal in plants is high (Kloke et al., 1984). According to the above publication both cobalt and arsenic have the lowest accumulation coefficient of all heavy metals, ranging from 0.01 to 0.1. The values obtained in this experiment are within the same range. The accumulation coefficient was closely related to the herb species, with the highest value for cobalt in *Mentha arvensis*, and arsenic in *Lysimachia vulgaris* L.. *Achillea millefolium* L. had the lowest value of the accumulation coefficient both for cobalt and arsenic.

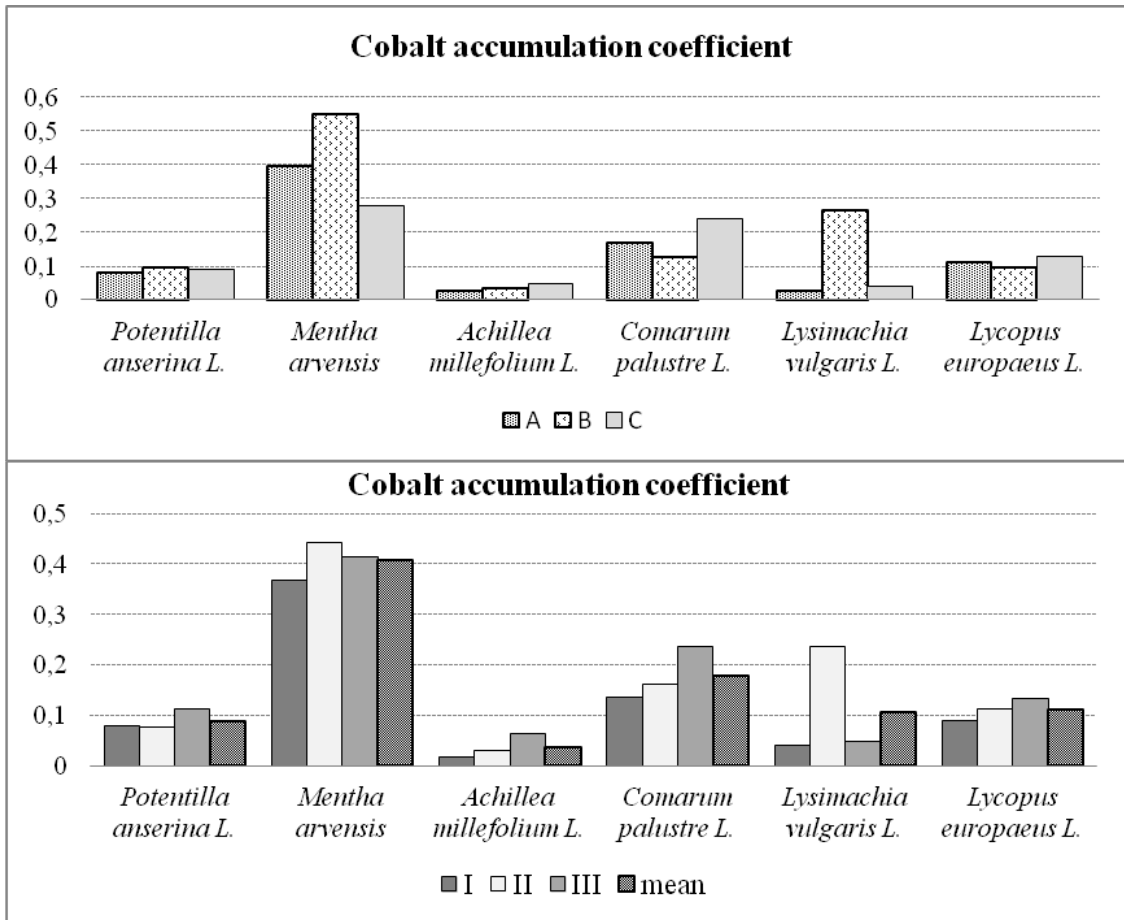
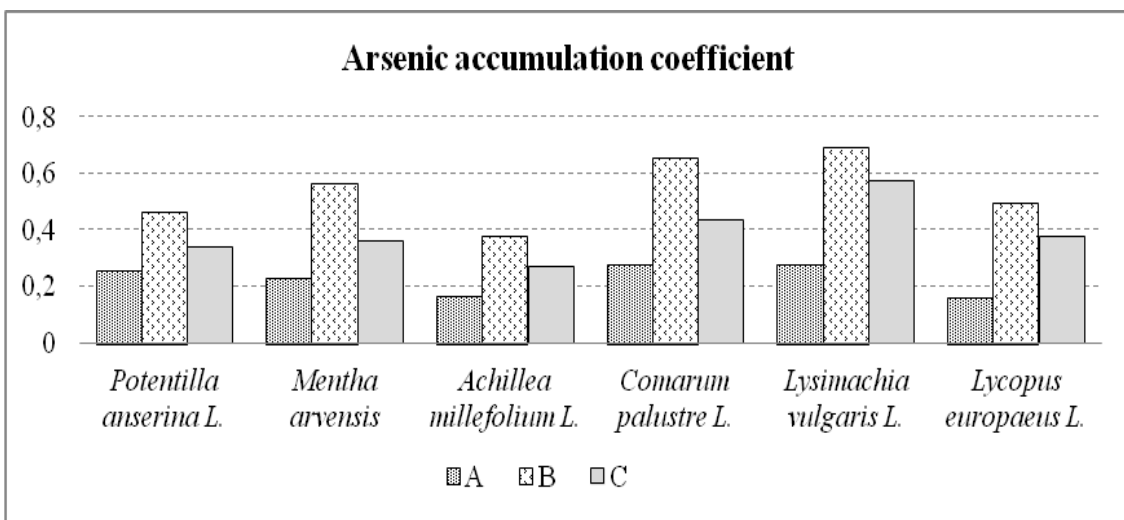


Figure 2. Cobalt accumulation coefficient in plants growing in different experimental areas and different moisture sections. (A - area with the cultivated field; B - area with permanent grasslands; C - area with bushes; I - wet section; II - periodically wet section; III - dry section; S - species; Ar - area)



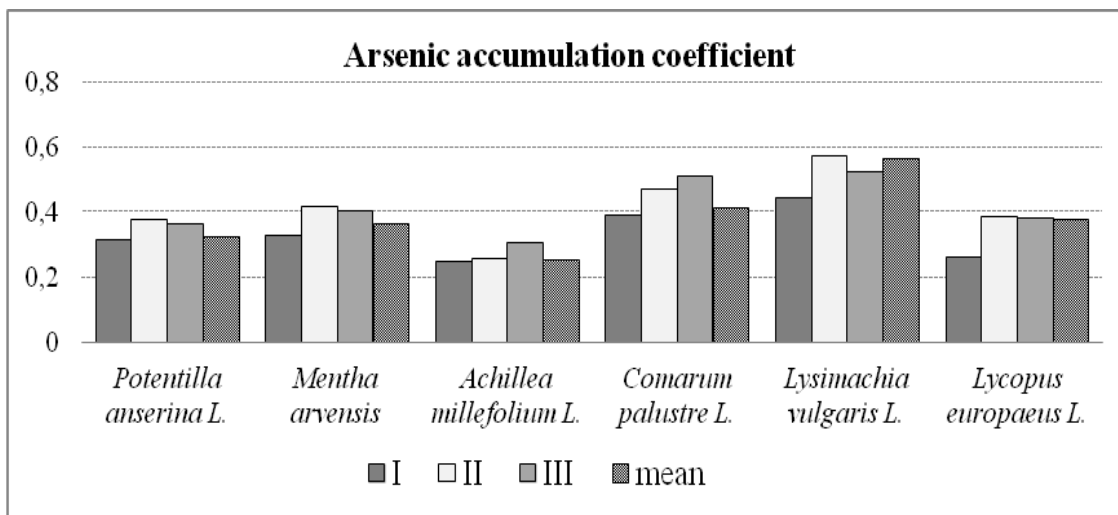


Figure 3. Arsenic accumulation coefficient in plants growing in different experimental areas and different moisture sections. (A - area with the cultivated field; B - area with permanent grasslands; C - area with bushes; I - wet section; II - periodically wet section; III - dry section; S - species; M - moisture section)

In plants growing in periodically wet and dry sections accumulation coefficient was much higher than in plants growing in wet sections where the concentration of both metals was the highest. There was also a relationship between cobalt and arsenic accumulation and the experimental area. The accumulation coefficient was much higher in plants from the area with permanent grassland than in the area with arable land. Accumulation of soil heavy metals by plants depends on many factors, first of all on pH but also on soil moisture and organic matter content. According to Kabata-Pendias and Pendias (1999) plants can alter chemical mobility and, at the same time, availability of metals in the rhizosphere. Additionally, the correlation coefficient between cobalt and arsenic content in the soil and in the plants was calculated but there was no significant relationship between those values (Table 5).

Table 5. Correlation coefficient between cobalt and arsenic concentration in the soil and in the biomass of herbs

| Species | Co | As |
|--------------------------------|--------|--------|
| <i>Potentilla anserina</i> L. | 0.149 | 0.533 |
| <i>Mentha arvensis</i> | 0.385 | 0.264 |
| <i>Achillea millefolium</i> L. | 0.094 | 0.150 |
| <i>Comarum palustre</i> L. | -0.596 | 0.227 |
| <i>Lysimachia vulgaris</i> L. | -0.217 | 0.042 |
| <i>Lycopus europaeus</i> L. | -0.215 | -0.295 |

$p \leq 0.05$; critical value for $r = 0.632$; $N = 9$

Conclusions

There were significant differences between cobalt and arsenic content in the biomass of different species. Out of all moisture sections and experimental areas the highest

concentration of cobalt was in *Mentha arvensis*, for arsenic it was the highest in *Lysimachia vulgaris* L. and *Comarum palustre* L., and the lowest in *Achillea millefolium* L.

The highest content of cobalt and arsenic was found in bottom sediment and in the soil of the area with arable land, which might have been a result of plant protection products application and intensive fertilization of cereals.

In the soil-plant system the accumulation coefficient was higher for arsenic than for cobalt. For both of the metals the coefficient was lower than one, which means that the accumulation was low.

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