The elemental composition of *Hypericum perforatum* plants sampled in environmentally different habitats by the example of West Siberia

Tatyana I. Siromlya1, Yulia V. Zagurskaya2* & Irina I. Bayandina3

**ABSTRACT**

Medicinal plants can amass chemical elements in their tissues due to their ability to tolerate potentially toxic ions in the environment. The *Hypericum perforatum* L. plants were cultivated in three regions of West Siberia (Kemerovo, Novosibirsk, Kamchak Village (Altai Republic)). Elemental analysis was conducted by atomic emission spectrometry. Chemical elements extractable with 10% HCl were assayed by atomic absorption spectrometry. The concentrations of Co, Ni, Cu and Zn have been relatively stable. The coefficients of biogeochemical mobility of Cd, Cu and Zn had high rates, which confirm the possibility of accumulation of these elements in *H. perforatum*. The concentration of chemical elements in extracts (10% HCl) was increased as well as their total contents, the degree of extraction was highest for K, Ni, Fe, Cu, Mn, Mg (90% or more), the minimum for Na (20–40%) and significantly changed in environmentally different habitats.

**Keywords:** St. John’s wort, environmental factor, elemental composition, accumulator Cd, West Siberia

**Резюме**

Сиromля Т. И., Загурская Ю. В., Баяндина И. И. Элементный состав растений *Hypericum perforatum*, отобранных в экологически различных местообитаниях на примере Западной Сибири. Растения могут накапливать химические элементы в различных концентрациях в зависимости от экологических факторов. Растения *Hypericum perforatum* выращивали из генетически однородного материала в трех регионах Западной Сибири (г. Кемерово, г. Новосибирск, с. Камлак Республики Алтай). Элементный химический состав растений определяли с помощью атомно-эмиссионной спектрометрии, анализ экстрактов (10% HCl) проводили методом атомно-абсорбционной спектрометрии. Относительно стабильными оказались концентрации Co, Ni, Cu и Zn. Коэффициенты биогеохимической подвижности Cd, Cu и Zn оказались высокими, что подтверждает возможность накопления этих элементов в *H. perforatum*. Найболее высокая степень извлечения в 10% HCl отмечена для K, Ni, Fe, Cu, Mn, Mg (90% или более), низкая для Na (20–40%) и отличалась в различных экотопах.

**Ключевые слова:** зверобой, экологический фактор, элементный состав, аккумулятор Cd, Западная Сибирь

The influence of industrial pollution on human health is an environmental challenge. People undergo not only the direct action of pollutants present in the air and water but also their indirect action, by consuming potential toxicants with food of plant or animal origin (Singh et al. 2010). Therefore, controllable field experiments on the effect of chemical element (CE) migration, partly driven by anthropogenic factors, are of special importance (Perel’man & Kasimov 1999, Kabata-Perdias 2010).

Saint John’s wort (*Hypericum perforatum* L.) is among the best studied herbs, as it is a valuable and efficient medicinal plant used by herbalists since times immemorial. It is present in drug codices of many countries. However, data on its elemental composition are inconsistent (Radanovic et al. 2002, Gomez et al. 2004, 2005, Ayan et al. 2006, Gasser et al. 2009, Hussain et al. 2009, Helma et al. 2011, Bezlava et al. 2012, Tokaloğlu 2012, Gogoasa et al. 2013, Đurović et al. 2013, Lovkova & Buzuk 2013, Bu et al. 2013, Radulescu et al. 2013, Mihaljev et al. 2014, Senila et al. 2014, Pavlova et al. 2015, Filipiak-Szok et al. 2015, Owen et al. 2016, Veljković et al. 2015, Badea 2016, Glavač et al. 2017, Fischer et al. 2017, Derkach & Khomenko 2018, Nikolova et al. 2018), and little is known about CE accumulation in cultivated plants, particularly, in Siberia. One of the features of *H. perforatum* attracting scientists’ attention is its ability to concentrate cadmium (Lovkova & Buzuk 2013, Jurca et al. 2011). Cadmium affects plant growth and development due to its toxic effects (Hussain et al. 2015, Rimane et al. 2016, Farooq et al. 2019, Qin et al. 2020) even at low concentrations (White & Brown 2010, Dias et al. 2013). It has also been reported to concentrate zinc and copper (Lovkova & Buzuk 2013, Picard 1965). These elements are often accumulated in poorly soluble or insoluble forms, much less toxic (Konieczynski et al. 2011, Pytlakowska et al. 2012, Zhou et al. 2014, Zeljčakov et al. 2008); however, the safety of the pharmacological plant material is assessed from the bulk contents of elements, set forth for all plant species, according to Sanitary Regulations and Standards 2.3.2.1078-01 (Russia).
Most of the studies on elemental compositions in West Siberia are dedicated to the health safety of medicinal plants in regions with high industrial load or with industrial or transportational pollution. In rare cases, excess of threshold limit values of Pb, Cu, and Zn and of permissible exposure limits of Ni and Fe (Siromlya 2011) is noted. Of climatically similar regions of southern West Siberia, Gorny Altai (Altai Republic) is recognized environmentally friendly, whereas industrial cities and metropolises are troubled, in particular, because of the overall anthropogenic water and air pollution.

The goal of the present work is to elucidate the general and regional features of CE accumulation by H. perforatum under different environmental conditions by the example of southern West Siberia.

**MATERIAL AND METHODS**

Plants of *H. perforatum* cv. Zolotodolinskii were grown via seeds from genetically uniform material in three regions of southern West Siberia on experimental fields of (1) the Kuzbass Botanical Garden, Institute of Human Ecology, Kemerovo; (2) the Michurinian Garden, Novosibirsk State Agrarian University, Novosibirsk; and (3) the Gorny Altai Botanical Garden, Kamlak Village, Altai Republic.

Aerial parts were cut from ten *H. perforatum* plants on each experimental plot in 2011 and 2012. Sampling dates coincided with the start of mass-scale flowering in the locality and year. A total of 60 plants were examined. Samples were dried and processed according to the State Pharmacopoeia of the USSR, Edition 11. Analyses were done in triplicate, and commercial *H. perforatum* samples (n=4) from the Nonchernozem belt of European Russia were used for reference.

Elemental analysis of dry-calcined plants was conducted by atomic emission spectrometry. The concentrations of As and Hg, determined according to GOST R 51766-2001 (The state standard of Russia, maintained by Euro-Asian Council for Standardization, Metrology and Certification, http://eascby/en/) and GOST R 53183-2008, were below the detection limit. Chemical elements extractable with 10% HCl were assayed by atomic absorption spectrometry on a Kvant device, often designated also BAI or Cb. We also calculated the index of plant biochemical activity (BCA), proposed by Aivazyan (1974) and determined as the ratio between the bulk Ax value and the number of tested CEs n, calculated separately for cationogenic and anionogenic elements.

The degree of consumption of soluble CE forms present in soil was characterized by the index of biogeochemical mobility Bx, proposed by Kasimov: the ratio between CE content in plant dry matter and the content of soluble CE forms extractable from soils by weak solvents (Perel'man & Kasimov 1999).

Statistical evaluation of experimental results was carried out with STATISTICA 6.1 software. The correspondence of the distributions of CEs under study to the normal distribution was assessed by the Shapiro-Wilk test. The hypothesis of equal variances in normally distributed samples was checked by Cochran's test. As non-Gaussian distributions were found in most CEs under study in at least one of the localities, they were described by median values (Me) and quartiles Q1 and Q3 as measures of dispersion. The table also presents variation coefficients V. Some figures also present experimental maximum (max) and minimum (min) values. The statistical significances of differences in normally distributed samples with uniform variances were determined by one-way parametrical analysis of variance. At nonequal variances, as well as at non-Gaussian distributions, the Crusal-Wallis nonparametrical analysis of variance was applied. In all statistical methods, the threshold level of significance was taken to be p = 0.05.

**RESULTS**

Elemental compositions in plants vary broader than in soils owing to different physiological roles of CEs and selective uptake. The variability of concentrations of some CEs in aerial parts of *H. perforatum* within a single plot may exceed 60% in spite of the genetic and physiological uniformity of the plant material examined. With this regard, statistically significant differences in bulk contents of elements are observed only for potassium (p = 0.042), phosphorus (p = 0.046), and nickel (p = 0.045) (Table 1).

Our data indicate that by no means all CEs tested are fully extracted by HCl. The highest degrees (up to 90% or even more) of extraction were reached for K, Ni, Fe, Cu, Mn, and Mg, whereas the lowest (20-40%) were for Na. These values varied considerably from one sampling locality to another (Fig. 1)

The biological absorption and plant biogeochemical mobility indices show considerable variability (Fig. 2). The BAC values for cationogenic CEs calculated for *H. perforatum* are slightly higher than for anionogenic: KBAC is within 5.5–6.8, and ABAC, 3.3–5.0. The highest values of these indices were found in the Kuzbass Botanical Garden.

**DISCUSSION**

Two major factors affect CE uptake and metabolism in plants: genetic and environmental (Perel'man & Kasimov 1999). The fact that we investigated genetically uniform material allowed us to determine properly the dependence of CE content and availability on just environmental factors, in particular, concentrations of various forms of elements in soils and the putative influence of industrial pollution. The potentially toxic elements dominated by lithogenic input (Ni and Co) were found predominantly in soil residual fractions, while elements with stronger anthropogenic contributions
Table 1. Statistical indices of the bulk contents of chemical elements (CE) in *H. perforatum* plants, ppm in absolutely dry matter. * – pharmacological material; ** – clark in terrestrial vegetation (Romankevich 1988); bdl – below detection limit; – – no data.

| CE  | Me (min–max) | Kemerovo | Novosibirsk | Altai | ** Pharm. Owen et al. (2016) | M | ** Clark |
|-----|--------------|----------|-------------|-------|------------------------------|---|----------|
| B   | 37.5–69.1    | 44       | 46.1        | 51.1  | 20                           | 24| 11.4     |
| Ba  | 27.2–32.1    | 31       | 27.5        | 34.4  | 23                           | 14| 11.8     |
| Be  | 0.0025–0.031 | 18       | 0.027       | 0.026 | 18                           | 27| 0.020    |
| Ca  | 4899–5295    | 28       | 7137        | 8350  | 45                           | 44| 0.097    |
| Cd  | 1.21–1.48    | 36       | 1.34        | 1.47  | 54                           | 34| 0.77     |
| Co  | 0.26–0.32    | 22       | 0.20        | 0.35  | 68                           | 14| 0.07     |
| Cr  | 1.58–2.59    | 51       | 0.86        | 0.63  | 54                           | 34| 0.43     |
| Cu  | 10.1–11.3    | 59       | 9.5         | 7.0   | 61                           | 45| 5.45     |
| Ga  | 0.14–0.15    | 11       | 0.10–0.09   | 0.16  | 32                           | 39| 0.19     |
| Ga  | 0.12–0.21    | 9        | 0.12–0.21   | 9     | 39                           | 0.05|
| Fe  | 213–330      | 62       | 137         | 117–165| 66                          | 271| 156      |
| K   | 6024–5726    | 17       | 8195        | 4665–11843| 45 | 10850| 208662| 110000|
| Mg  | 0.35–0.73    | 25       | 0.57        | 0.47–0.67| 28 | 0.53| 0.26     | 0.8   |
| Mn  | 97.3–105.8   | 35       | 99.9        | 51.6–143.8| 49 | 105.1| 150.18 | 3200  |
| Mo  | 0.44–0.49    | 1        | 0.29        | 0.25–0.32| 16 | 0.50| 0.31     | 0.6   |
| Na  | 95–273       | 63       | 106         | 96–333 | 57 | 135| 89       | 1200  |
| Ni  | 2.90–3.40    | 37       | 2.54        | 2.28–2.88| 57 | 471| 0.71     | 2.0   |
| P   | 4036–5069    | 20       | 3070        | 2970–3170| 5 | 4316| 1.88     | 2000  |
| Pb  | 0.33–0.41    | 15       | 0.32        | 0.21–0.24| 40 | 0.32| 0.35     | 2.5   |
| Si  | 1602–1680    | 6        | 1058        | 958–2160| 45 | 1871| 2668     | 3000  |
| Sn  | 0.12–0.12    | 13       | 0.17        | 0.16–0.18| 8  | 0.18| 0.20     | 0.25  |
| Sr  | 33.6–51.8    | 56       | 76.7        | 51.1–86.7| 33 | 93.1| 17.3     | 32    |
| Ti  | 32.3–59.3    | 18       | 18.4        | 15.4–31.1| 42 | 33.5| 12.9     | 40    |
| V   | 0.87–1.92    | 16       | 0.37        | 0.32–0.89| 56 | 0.54| 0.33     | 1.5   |
| Y   | 0.15–0.17    | 12       | 0.17        | 0.15–0.24| 28 | 0.23| 0.27     | 0.8   |
| Yb  | 0.028–0.030  | 10       | 0.021       | 0.020–0.028| 30 | 0.029| 0.017   | 0.0015|
| Zn  | 60.5–65.5    | 37       | 54.2        | 36.4–59.3| 27 | 614| 21.5     | 50    |
| Zr  | 1.51–1.64    | 14       | 1.40        | 1.20–1.82| 30 | 1.77| 1.29     | 7.5   |

Figure 1. Relative contents of CEs extractable with 10% HCl: (1) Kemerovo, (2) Novosibirsk, (3) Altai, (4) pharmacological material.

Figure 2. The biological absorption (black) and biochemical mobility (open) indices of CEs in *H. perforatum* plants grown in southern West Siberia (Me, Min-Max) (Cd, Zn, Pb and Cu) showed much higher portion in the more mobile and bioavailable fractions obtained from sequential chemical extraction (Said et al. 2019). The most relevant toxic elements from a human health point are Cd, Hg, Pb and As (Silva et al. 2005).

The USSR Soil Classification and Diagnostics (1977) classifies soils in our experimental fields as gray forest (Novosibirsk and Kemerovo) and meadow-chernozem (Gorny Altai). Their physicochemical and agrochemical properties, as well as the bulk contents and concentrations of soluble forms of CEs have been reported in (Siromlya et al. 2017). Soils in the region are generally characterized by broad diversity, which is caused by their complex genesis, diversity of cover deposits, moistening regimes, etc. This diversity is reflected in soil elemental composition (Syso 2007).

Our results are supported by Dobrovol’skii (2003), who found that concentrations of dispersed elements broadly vary in related species even within a region. This fact indicates that the averaged data, commonly reported in such studies, do not reflect the natural nonuniformity of CE contents, which may be quite significant. In addition, non-Gaussian distribution is observed in many cases, making the comparison of arithmetic means incorrect. Median values are more appropriate.
Analysis of experimental and literature data brings us to the following inferences as to the elemental composition of aerial parts of *H. perforatum*.

1. The contents of Co, Ni, Cu, and Zn are relatively stable, because these elements rank among the most vital for plants. Probably, their sufficient quantities are accumulated in plants even under different ambient factors.

2. The contents of Cd, Cr, Pb, Na, Ca, B, and V may vary by an order of magnitude even within a single locality, because *H. perforatum*, like all higher plants, displays a certain degree of selectivity in CE uptake from the soil solution, although the selectivity is not absolute.

3. The contents of Fe and Mn may vary by hundreds of times, because, owing to their variable oxidation states, their forms in soils directly depend on soil acidity and redox potential, which affects their accessibility for plants.

A specific regional feature of the element composition in *H. perforatum* is high concentrations of Ba and Sr. They are several times as high as in other regions of the world. An elevated barium content (74 ppm) was detected by Yi et al. (2004). However, the experimental plots where our plants were grown contained about 600 ppm Ba and 200 ppm Sr, which corresponds to the average level in soils of the globe (Kabata-Pendias 2010) and southern West Siberia (Syso 2007). It is reasonable to assume that it is the manifestation of natural climatic settings and soil geochemistry, on which CE mobility depends substantially. This assumption is supported by the fact that similar high Ba and Sr concentrations were found in our earlier studies of other plant species in southern West Siberia (Siromlya 2011).

In comparison to the clarkes in land vegetation (Romankevich 1988), our samples contained less Be, Ca, Co, Mn, Na, Pb, V, and Zr and more B, Cd, and Yb. It should be noted that the clarkes of Cd and Yb are very low: such low values are not reported in the literature. Probably, it is more consistent to compare our data with regional levels, although the mean background values in meadow habitats of West Siberian forest-steppe have been calculated only for 11 CEs (ppm in absolutely dry matter): Fe (108), Mn (33), Zn (24), B (13), Cu (5.6), Mo (2.0), Ni (0.8), Cr (0.6), Pb (0.6), Co (0.2), and Cd (0.2) (Syso et al., 2014). Only the concentration of Co in our samples is close to the contents in meadow herbs. Concentrations of Pb and Mo are lower, and of other CEs, higher (two- or threefold, or even more), particularly, Cd. This observation seems natural, because *H. perforatum* is known to concentrate Cd. The bulk content of this element in soils of experimental plots was about 0.5 ppm, which is within its maximum permissible concentration according to Sanitary–Hygienic Standard 2.1.7.2511-09; therefore, under the experimental conditions *H. perforatum* can be considered a selective concentrator, having high Cd concentrations regardless of Cd content in the environment (Uritmsheva 2015). The result of this specific feature of the species, noted in other works as well (Lovkova & Buzuk 2013, Jurca et al. 2011), is that the ecological assessment of *H. perforatum* specimens grown in the localities under study shows their inconsistency with the demands imposed by Sanitary Regulations and Standards 2.3.2.1078-01 (for herb-based dietary supplements) on Cd content, although in some samples Cd contents did not exceed the permissible limit, 1 ppm (see table). The commercial *H. perforatum* material analyzed for reference contains less Cd on the average, about 0.8 ppm, which meets the Standards; less B, Ba, Co, Cr, La, Mn, Ni, P, Sr, and Zn; but more K and Pb than our samples.

Analysis of the content of ash fraction insoluble in 10 % HCl (standardized in official monographs) allows estimation of the content of admixtures of mainly mineral origin, and analysis of the elemental composition of the extracts provides information on the amount of biogenic CEs eliminating the effect of contaminating dust and, possibly, the presence of tightly bound and hardly available CE species (Siromlya 2011). The amounts of CEs extractable with 10 % HCl increase in the same order as their bulk content: from Pb, Co, and Cd to Mg, Ca, and K. Certain variations are observed among the localities, but no clear correlation was detected. For example, the least amount of Pb but the greatest amount of Cu were extracted from *H. perforatum* plants grown in Gorny Altai, although no statistically significant differences in the bulk contents of these CEs in plants from different localities were found. More Pb and K was extracted from commercial *H. perforatum* materials, and their bulk contents in the commercial sample studies were also higher.

Different inferences from studies of CE absorption rates have been made. Dobrovolski (2003) notes that Ax values are surprisingly uniform over zonal vegetation types, although they vary for some CEs. Perekhman & Kasimov (1999) indicate that the Ax value for a CE is not constant. For major elements, the genetic factor is crucial, whereas minor CEs are more affected by the landscape and geochemistry (ecology), particularly, in ore and industrial regions. With regard to the rate of biologic uptake, CEs of biologic accumulation (Ax > 1) and CEs of biologic capture (Ax < 1) are traditionally recognized, and they are subdivided into four groups.

In general, the Ax coefficients calculated for *H. perforatum* meet the classification; however, some CEs (Cr, Mo, Mg, etc.) show considerable variability. The Ax value for Cd differs most profoundly, exceeding 50, although this element is commonly classified with elements of medium accumulation or even medium capture. In contrast, no drastic differences were found for Ax values for Ba or Sr, whose high contents were noted in plants of the region under study in comparison to data from the literature. Barium also belongs to medium accumulation and strong capture elements, and strontium, to the group of intense and medium accumulation (Perelman & Kasimov 1999), though the Ax values calculated by us were two to three times higher than those reported by Dobrovolski (Dobrovolskij 2003).

It has been noted that Bx values, characterizing the availability of CEs and the percentage of utilization of soluble CE species present in the soil considerably exceed the corresponding Ax values for most CEs (Perelman & Kasimov 1999). Nevertheless, in our studies Bx values for Ca, Sr, Cd, and Pb (the most mobile elements in soils (Siromlya et al., 2017)) were lower (Fig. 2). The high Bx values for Cu and Zn may result from the low contents of soluble forms of these elements (according to agrochemical criteria of soil CE supply). Also, it is conceivable that *H. perforatum* acts as a selective concentrator of these elements,
which is supported by data reported in (Lovkova & Buzuk 2013, Picard 1965).

CONCLUSIONS

Our results and data from the literature indicate that the contents of Co, Ni, Cu, and Zn in the aerial part of H. perforatum are the most stable. The amounts of Cd, Cr, Pb, Na, Ca, B, and V vary by an order of magnitude even within a single region, and the amounts of Fe and Mn vary by hundreds of times. The elemental compositions of H. perforatum samples from different areas of southern Western Siberia show practically no statistically significant differences. The common feature of plants in the region is high concentrations of Ba and Sr. High degrees of CE extraction with HCl (over 90 %) are observed for K, Ni, Fe, Cu, Mn, and Mg. The lowest degree is observed for Na: 20–40 %. These values vary considerably with ecological settings. The safety estimate of H. perforatum plants as medicinal herb material with regard to the bulk Cd content does not reflect its potential toxicity, as confirmed by abnormally high biological absorption indices Ax for this element.

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