Oecologia Montana 2017,
26, 35-46

Accumulation of selected element deposition in the organs of *Fallopia japonica* during ontogeny

P. BÖHMOVÁ[1] and R. ŠOLTÉS[1,2]

[1]Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic;
[2]Podtatranská 19, Poprad, SK-059 60 Slovak Republic;
e-mails: petka.bohmova@gmail.com; rudolf.soltes@gmail.com

Abstract. The aim of the study was to track the movement of selected elements in the plant organs of *Fallopia japonica* during ontogeny in a site near the village of Lisková in Liptov Basin, Slovakia. The statistical graphics system STATISTICA, Release 7 was used for correlation analysis, and for ordination analysis the CANOCO 4.5 for Windows package was used. Elements determined included sulfur, chlorine, potassium, calcium, titanium, chromium, manganese, iron, copper, zinc, rubidium, strontium, molybdenum, tin, antimony, barium, and lead. We studied the movement of the elements in the rhizome, stems, leaves and flowers. The tissue samples were analyzed by X-ray spectrometry. Five significant trends in element movement during vegetation season have been recognised: a fluent downward trend in concentrations (S, K, Zn, Cr, Rb, Mn); a downward trend in concentrations falling below the detection limit in September, at the time of creation of flowers (Fe, Cu); a downward trend in concentrations and increase in concentrations in September, at the time of flowers creation (Ba, Pb, Cl); upward trend in concentration (Ca, Sr); and no systemic movement (Mo, Sb). Correlation between stem parts including leaves and rhizome or bryophyte is lowest during early spring and increases during the year. The concentration of the elements in the flowers correlates highly with the apical part of the stem. The results suggest that the rhizome and the environment affect the chemistry of *Fallopia japonica* only slightly. The results of the ordination analysis indicate that *Fallopia japonica* is only marginally dependent on the uptake of the element from the rhizome.

Key words: *Fallopia japonica*, XRF spectrometry, element accumulation, element mobility, Slovakia

Introduction

The investigated site is located in northern Slovakia, near the village of Lisková (District Ružomberok). The site is highly polluted, the most significant sources of immissions are OFZ Istebné and 150-200 kilometers NW remote Ostrava region, Kraków region and Silesia region.

The largest producer of sulphur dioxide nearby is Mondi SCP Ružomberok.

The more frequently used bio-indicators are mosses, as they have high cation exchange capacity, lacking cuticle and simple thalli organisation (Tyler 1990). However, vascular plants have been used as bio-indicators quite often as well. Nevertheless, the path of accumulation of heavy metals in vascular plants is different. Heavy metal ions accumulate first in the cortex of the roots, from where a small proportion passes through the endodermis and is subsequently distributed into the different plant organs via the xylem and phloem (Salemaa *et al.* 2004).

Hijano *et al.* (2005) used the coniferous tree *Pinus pinea*, broadleaf tree *Quercus ilex* and various bush species (*Pyracantha coccinea, Nerium oleander*) for SO_2 monitoring in the surrounding of Madrid city. Malallah *et al.* (1996) used *Vicia faba*. Krizek *et al.* (2003) searched for the use of vascular plants as a bio-indicator at the molecular level. Lee *et al.* (1991) looking for monitoring of arsenic, used the aquatic perennial vascular plant *Hydrilla verticillata* Casp.

In Slovakia, Mičieta and Murín (1998) tested the suitability of selected vascular plant species (*Pinus sylvestris, Pinus nigra* and *Pinus mugo*) for use as bio-indicators of environmental pollution. Korčeková *et al.* (2015) focused on the usage of the needles of *Pinus sylvestris* as a bio-indicator of air pollution surrounding the town of Ružomberok. Šoltésová (in Šoltés *et al.* 1992) used other species of vascular plants as bio-indicators of emission burdens in the High Tatras. The author identified the most polluted areas. Šoltés *et al.* (2014) used the vascular plant *Calluna vulgaris* to compare levels of accumulated elements in 2011 with the situation in 1987-1988. When evaluating contaminated sites, Mráz and Šoltés (2015) chose *Fallopia japonica* to use for reseach as a bio-indicator of heavy metal deposition in the study area of Ružomberok.

Fallopia japonica (Hout.) R. Decr. (Japanese knotweed) is an invasive rhizomatous perennial geophyte native to eastern Asia, and was introduced into Europe in the 19th century. It has become the most destructive and aggressive of all alien weeds. *Fallopia japonica* is able to grow in diverse soil types, with various pH ranges and nutrient content. The first data about the occurrence of *F. japonica* in Slovakia came from the 1920s and 1930s (Eliáš 2004). In the first half of the 20th century in the Nitra catchment area the alien plant species *Fallopia japonica* was introduced (Fehér 2007). In the Tatra Region, *F. japonica* was detected for the first time in 1967 (Eliáš 2002). The phenology of *Fallopia japonica* is rather well known. The plant sprouts in late April, and the main growing period occurs in May and June (Alberternst and Böhmer 2011). The blooming season is from September to October (Horn 1997). Because it blooms at a time when other plants are not flowering, the flowers are attractive to swarms of bees (Mráz and Šoltés 2015). The plants grow very quickly and reach a height up to 3 m. The underground part of plant is characterized by a huge rhizome system, which is able to spread over an area 15-20 m away and penetrate the soil 2 m below ground (Pauková 2013). Undoubtedly, the plant has recently been the most dangerous neophyte. In many places in Slovakia this species found suitable habitats and created viable populations with 100% coverage, where no other species is able to compete. Sołtysiak and Brej (2014) examined urban conditions of the spread of *Fallopia japonica*. The investigation has shown that in urban conditions knotweeds are able to spread on soil with various pH ranges and nutrient content. One of the threats which supports plant invasion and establishs them in new ecosystems is allelopathy. Allelopathy inhibitions seed germination and the inhibitory effect of extract from Japanese Knotweed rhizomes was proven (Sołtysiak and Brej 2012). Secondary compounds isolated from *F. japonica* include the anti-cancer phytoalexin resveratrol. Several authors have dedicated their work to exploitation of species as a bio-indicator. Heavy metals were accumulated significantly more in roots and rhizomes (Berchová-Bímová *et al.* 2014). In the case of young plants cultivated from seeds, the concentration of iron is much higher in comparison to well grown plants *F. japonica* showed a tendency to translocation of cadmium from roots to overhead parts of the plant. The translocation index of cadmium for leaves of *F. japonica* amounts to over 1.0. The same result was observed for lead (Dwiecki and Koziol 2005). *F. japonica* concentrates toxic metals in roots and restricts metal transfer into the aboveground parts, or gathers heavy metals in aboveground parts, particularly in leaves (metal accumulators) (Sołtysiak *et al.* 2011). Soil under *F. japonica* generally had higher exchangeable nutrient concentrations. Invaded stands had 3.2- to 5.4-fold larger nutrient stocks in abovegroud biomass compared to the resident vegetation (Dassonville *et al.* 2007).

According to several authors, *Fallopia japonica* concentrates elements in roots and translocates elements from roots to overhead parts (Dwiecki and Koziol 2005, Soltysiak *et al.* 2011). Bryophytes are completely independent on the substrate, and the majority of the nutrients are received from precipitation or from dry deposition (Ratcliffe 1975, Burton 1990). Comparison of correlation elements in plant organs with concentrations of these elements in the rhizome of *Fallopia japonica* and in the tissues of bryophytes collected in the immediate vicinity of the sampling site, should give an answer to the question of to what extent the above-ground plant organs (leaves, stems, flowers) accumulate elements from the environment and to what extent they are influenced by the concentrations of these elements in the rhizome.

When setting targets, we assumed that *F. japonica* showed a tendency to translocation of elements from roots to overhead parts of the plant (Dwiecki and Koziol 2005). Sołtysiak *et al.* (2011) argue that *F. japonica* concentrates toxic metals in roots and restricts metal transfer into the aboveground parts. The aim of the study was:

- To determine the movement of the monitored elements inside the plant during ontogeny.
- To verify to what extent in the process of ontogeny the concentrations of the elements in the above-ground parts of the plant are affected by the concentrations of these elements in the rhizome and how much is affected by the accumulation from the air, or if any accumulation from the air exists.

Material and Methods

The sampling site is located in the Liptov Basin, near Lisková village, with an altitude of 492 m a.s.l., (19° 20,400' E; 49° 05,234' N). The geographical coordinates are recorded in the system WGS 84. Ten samples were collected and the results are averaged. All samples were collected in 2016. The following parts of *Fallopia japonica* were sampled: rhizomes, stems, leaves, and flowers. In the spring season, when the plants grow rapidly, 4.6 cm per day (Horn 1997), sampling was done with greater frequency. Sampling terms were as follows: April 4, April 13, April 19, May 15, July 05, August 20, September 10, October 20.

The plant material was grinded by the ball mill into a fine powder. The tissue samples were analyzed by X-ray spectrometry, using the hand-held XRF spectrometer DELTA CLASSIC (USA). The following elements were found: S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Rb, Sr, Mo, Sn, Sb, Ba, Pb (Stephens and Calder 2004).

The statistical graphics system STATISTICA, Release 7 was used for the correlation analysis (Stephens and Calder 2004). For statistical analysis we used ordinal frequency data without transformation. For ordination analysis, the CANOCO 4.5 for Windows package was used (Ter Braak and Šmilauer 2002).

Results

Rhizome and bryophytes sampling results

A huge root system serves as a reservoir of nutrients. Therefore, to assess the origin of nutrients in the vegetative parts of plants, it is important to know the content of these substances in the rhizome. Bryophytes are completely independent on the substrate, concentrations of the investigated elements in the moss tissues provide information on environmental contamination. The ratio of the elements in the examined sample then allows us to assess the source of these elements (rhizome, environment). For sampling, pleurocarpous moss *Brachythecium salebrosum* was used. Ten samples were taken (rhizome, bryophytes), and the averaged results of the analysis are presented in the table (Table 1).

Stem, leaves and flower sampling results

A series of measurements of the concentration of elements was launched on April 4 and again on

	S	Cl	K	Ca	Ti	Cr	Mn	Fe	Cu	Zn	Rb	Sr	Mo	Sn	Sb	Ba	Pb
Rhizome	473	1,244	9,544	21,288	91	13.5	42.2	1,325	1.1	39.5	11.6	42.6	4.4	4.8	5.4	30.9	7.5
Bryophyte	577	862	11,114	22,690	340	132	151	6,647	13.7	99.4	21	30.2	8.1	9.6	11.4	87.6	15

Table 1. Averaged results of the rhizome (ten samples averaged) and bryophyte (*Brachythecium salebrosum*, five samples averaged) analysis (ppm). Date of sampling: Rhizome - April 4, 2016; Bryophyte – October 20, 2016.

April 13, April 19, May 15, July 05, Sept. 10 and Oct. 20. The sprouts on April 4 were less than a week old - the height up to 10 cm - thus the samples were not divided to the basal and apical parts. At the sampling on April 13, the plant height had reached approximatly 25-40 cm, so the samples were divided into basal and apical parts. At the sampling on April 19, May 15, July 05, Sept. 10 and Oct. 20 the plant height was sufficient for taking basal, middle and apical parts. 10 samples were taken of each. Average results are given in Table 2 (2a, 2b, 2c).

As of April 13, the leaves were sufficiently developed, and were also sampled (Table 3). In late August, the plant produced flower buds. This process occured quickly, and attentive monitoring enabled sampling immediately after the bud was created (August 20). 10 samples of flower buds were taken. Open flowers were sampled on September 10. The results of chemical analysis of the flowers are in Table 4.

Discussion

To analyze to what extent the concentration of accumulated elements in plant organs is affected by their content stored in the rhizome (collected in April 4) and to what extent by air borne pollutants, the correlation analysis was used. Bryophytes are completely dependent on the substrate. The terrestrial species Brachythecium salebrosum was used as a bio-indicator, collected directly from the site in October 20. In the spring season, when the plants grow rapidly, 4.6 cm per day (Horn 1997), sampling was conducted once per week. During the main growing season, the sampling interval was extended to approximately once every five or six weeks.

Mobility of elements in observed parts of the plant during the growing season

Sampling started on April 4 and ended by October 20. On April 4, the plants were too low (<10 cm), so whole (undivided) plants were taken. April 13, plant height allowed only division at the basal and apical parts. April 19, the plants were tall enough for complete sampling; dividing the basal, middle and apical parts.

During the investigation period, we recorded a downward trend in concentrations of the following elements: sulfur, potassium, zinc, chromium, rubidium, manganese (Fig. 1).

Sulfur assimilation is understood as the reduction of received sulphates and synthesis of amino acids and proteins. The special importance of sulfur rests in the chemical nature of CoA and its ability to transfer the acetyl group to the citric acid in Krebs

Table 2. Averaged results of stem analysis (ppm), ten samples averaged.

	April 4	April 13	April 19	May 15	July 05	Sept 10	Oct. 20
S	2,097	1,994	1,157	846	405	445	255
Cl	2,964	5,074	2,592	2,542	1,762	3,518	2,793
K	61,507	74,316	45,193	43,112	40,078	41,761	22,431
Ca	5,180	8,678	4,994	6,294	6,906	11,430	10,335
Ti	5.9	0	0	0	0	0	0
Cr	8.7	31.2	6,2	8	9.9	7.7	5.2
Mn	27.5	51	17.7	15.7	18.3	22.8	14.2
Fe	240.8	283.2	39.5	40.6	31.9	0	0
Cu	8.1	9.8	1.5	2.1	0	0	0
Zn	120.0	82.7	45.5	37.6	21.8	17.2	7.2
Rb	22.2	20.5	14.9	11.4	11.3	11.1	8
Sr	2.2	7.8	8	14.1	20	26.6	27.4
Mo	4.1	5.5	3.5	3.5	4.3	4.3	3.4
Sn	0.0	7.7	9	6.1	8.1	9.9	7.3
Sb	9.5	11.2	8.5	10.2	11.3	11.4	8.6
Ba	1.9	26.4	3.9	0	2.7	11.4	1.2
Pb	7.0	10.4	3.8	3.7	6.2	7	4.7

Table 2a. Basal stem parts results (ppm).

	April 4	April 19	May 15	July 05	Sept. 10	Oct. 20
S	2,097	1,706	1,297	440	430	314
Cl	2,964	4,105	2,741	1,799	3,025	3,020
K	61,507	66,391	54,187	38,382	37,980	22,478
Ca	5,180	6,422	8,502	6,622	9,884	13,281
Ti	5.9	0	2.1	0	0	0
Cr	8.7	6.8	14.9	14.4	9.6	9.7
Mn	27.5	22.9	26.3	22.7	26.1	20.2
Fe	240.8	74	167.5	27.5	0	3.3
Cu	8.1	6.9	4.4	0	0	0.5
Zn	120.0	85.4	74.7	30.1	16.6	9.1
Rb	22.2	22.3	18.7	11.9	12.2	10.7
Sr	2.2	6.7	9.1	16.7	21.3	31.4
Mo	4.1	4.6	4.6	4.5	4.8	4.6
Sn	0	8.3	9.3	4.6	9.4	7.9
Sb	9.5	8.9	10.9	9.3	9.3	11.9
Ba	1.9	2.5	5.8	6.4	15.6	3.9
Pb	7.0	6.7	6.3	6.9	8.3	6.3

Table 2b. Middle stem parts results (ppm).

	April 4	April 13	April 19	May 15	July 05	Sept. 10	Oct. 20
S	2.097	3,211	2,192	1,695	641	395	388
Cl	2.964	7,236	4,456	3,838	1,697	2,129	2,355
K	61.507	97,425	68,391	57,927	28,375	22,508	16,009
Ca	5.180	11,795	7,603	7,041	7,136	7,630	21,247
Ti	5.9	0	0	0	0	0	0
Cr	8.7	38.3	14.8	12.5	21.2	12.2	10.9
Mn	27.5	75.6	40.3	30.8	27.0	27.6	22.1
Fe	240.8	384.4	204.3	186.5	81.4	0	52.6
Cu	8.1	13.8	11.2	11.6	0	0	3.2
Zn	120.0	141.2	121.2	110	34.7	11.7	10.9
Rb	22.2	25.4	26.5	25.9	12.9	11.6	9.9
Sr	2.2	4.0	2.5	3.5	16.1	15.4	45.9
Mo	4.1	6.2	5.8	5	5.8	4.8	4.4
Sn	0	0	4.5	7.8	7.7	6.9	11.3
Sb	9.5	5.8	10.4	11.8	10.6	8.8	10.5
Ba	1.9	39.3	12.4	3.9	15.9	20.0	4.2
Pb	7.0	11.5	9.6	7.5	9.3	8.9	5.5

Table 2c. Apical stem parts results (ppm).

cycle for energy production, or ATP creation. ATP is the energy source for the synthesis of enzymes, proteins, and biomass. The concentration of sulfur in the stem is highest at the beginning of the growing season, in April, then rapidly decreases and stabilizes in July. Sulfur content in the leaves follows a similar pattern (Table 2, 3; Fig. 1a), which is associated with the most intense biomass production in the spring. The highest concentrations of sulfur are in the apical parts of the shoots (Fig. 1a), that is, the maximum biological activity takes place in the youngest parts of the plant. Rahmonov *et al.* (2014) found the maximal accumulation of sulfur in *Fallopia japonica* (2,571 ppm), which is approximately equal to our identified maximum (Table 2c, 3). High intensity of biomass production is a prerequisite for a high competitive potential of the plant.

Potassium increases the hydrophilicity of protoplasm colloids and thus the ability to bind water, thereby creating the conditions for the course of enzymatic processes within cells. This contributes to the survival of plants exposed to various biotic and

	April 13	April 19	May 15	July 05	Sept. 10	Oct. 20
S	2,567	1,916	1,553	995	984	689
Cl	4,054	2,861	3,115	3,475	3,987	4078
K	50,807	38,526	40,885	22,508	19,792	12,823
Ca	13,184	9,900	9,956	18,516	43,002	53,098
Ti	0	0	0	0	0	26.8
Cr	27.5	9.8	10.5	12.7	9.4	13.5
Mn	72.0	40.1	39.5	32.5	48.6	49.7
Fe	534.4	319.8	305	233	265	525
Cu	18.7	9.8	8.5	0	0.7	0
Zn	152.2	126	118	40.2	30.3	37.6
Rb	23.0	22.8	21.7	13.1	12.7	10.5
Sr	4.9	3.0	2.7	18.6	46.8	65.8
Mo	6.5	6.0	5.4	5.9	5.1	4.9
Sn	7.0	6.0	4.5	3.8	4.3	6.3
Sb	13.3	9.0	8.2	9.5	10.8	10.6
Ba	32.9	7.9	0	8.0	24.1	17.1
Pb	12.3	8.6	5.7	7.4	8.3	6.8

Table 3. Averaged results of leaves analysis (ppm), ten samples averaged.

Sampling term	August 20	September 10
S	1,445	1,011
Cl	5,247	5,707
K	47,719	32,315
Ca	9,598	11,775
Ti	8.2	2.4
Cr	29.4	21.7
Mn	57.6	43.2
Fe	445	401
Cu	21.9	7.9
Zn	85.8	37.3
Rb	20.2	15.3
Sr	11.9	19.3
Mo	6.4	6.4
Sn	8.6	9.2
Sb	10.4	10.4
Ba	30.8	28.5
Pb	11.6	10.4

Table 4. Averaged results of flowers analysis (ppm), ten samples averaged.

abiotic stresses (Wang *et al.* 2013). When potassium is present, the plants use iron more efficiently (Rubin 1966). Plants accumulate large quantities of this element, which constitutes > 2% of plant dry weight In order to optimize their performance as nutrient uptake organs, plant roots develop mechanisms of acclimation to the current K^+ status in the rhizosphere (Ashley *et al.* 2005). In these plants, potassium serves an essential role as an osmoticum and charge carrier (Rudulier *et al.* 1984). The highest concentrations of potassium existing in stems and leaves are recorded in the spring, which corresponds to the time of highest enzyme activity. Following this, concentration of potassium gradually decreases. The highest concentrations of potassium were found in the apical part of the stem (Fig. 1b).

It has long been known that zinc is essential to life as an integral part of a number of enzymes. Increasing evidence also suggests that zinc is important in the stability of macromolecules, particularly the components of various biological membranes (Chvapil 1973). Zinc-deficient leaves are highly light-sensitive, rapidly becoming chlorotic and necrotic when exposed to high light intensity (Cakmak 2000). Zinx is a component of carbonic anhydrase, an enzyme that catalyzes reaction of dehydration or hydration of carbonic acid (Rubin 1966). The highest concentrations of zinc in stems and leaves was recorded during the spring months (April – May, Fig. 1c). Enzymatic activity gradually decreases, and zinc concentration decreases as well. Higher zinc content was recorded in the apical stem parts (Fig. 1c), which are youngest and therefore enzymatically most active.

Chromium has received relatively little attention from plant scientists, and there is still a need to get a complete picture of Cr–plant interaction (Shanker *et al.* 2009). Plants with high concentrations of chromium exhibited severe chlorosis, necrosis, other growth abnormalities and anatomical disorders (Samantaray *et al.* 1998). Toxic effects of chromium on plant growth and development depend primarily on its valence state. Cr^{6+} is highly toxic, and causes severe damage to cell membranes which includes degradation of photosynthetic pigments causing deterioration in growth (Hayat *et al.* 2012). In general, the concentration of chromium in the stems and leaves of *Fallopia japonica* decreases

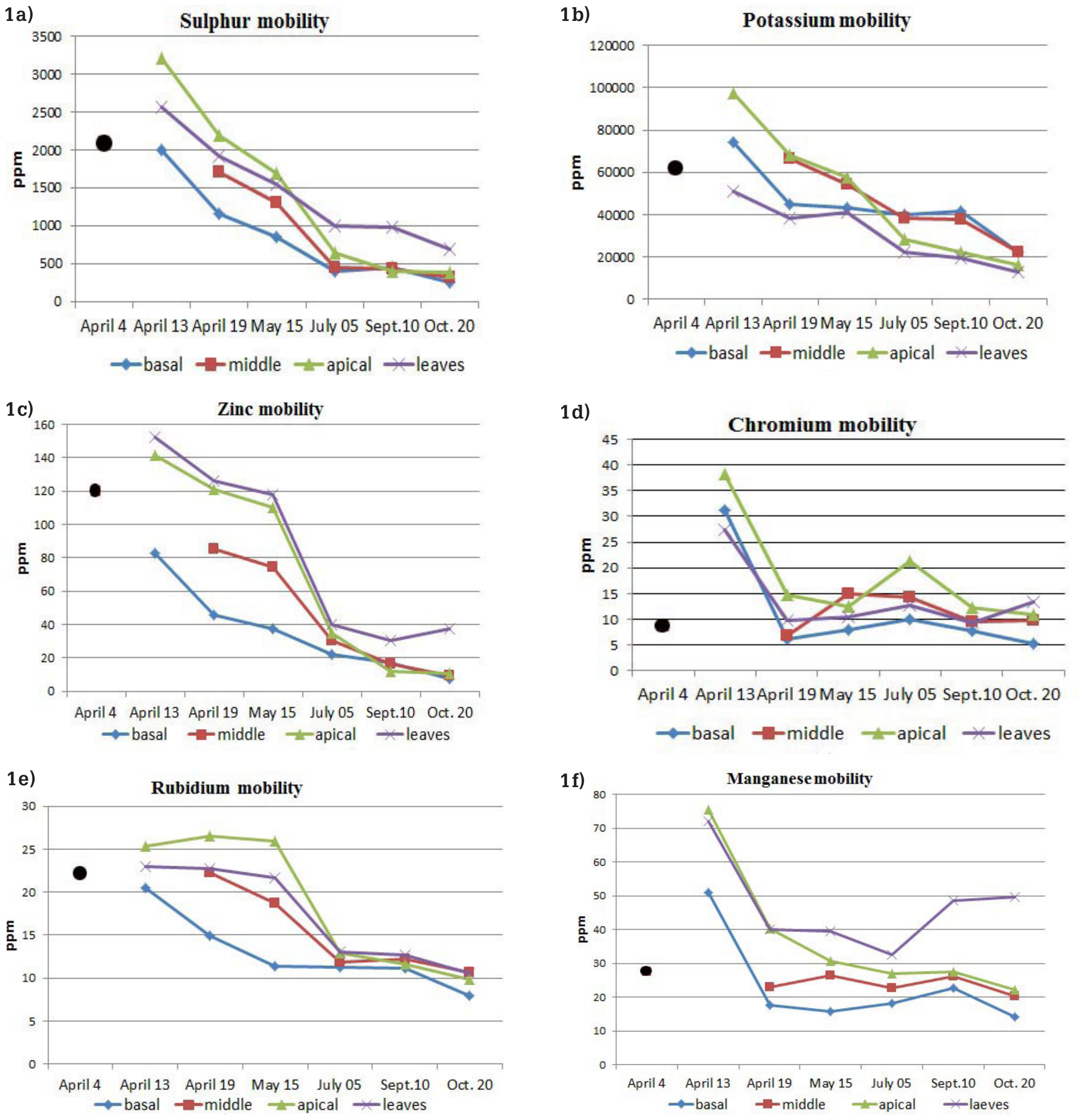

Fig. 1. Sulphur, potassium, zinc, chromium, rubidium and manganese mobility in the stem parts and leaves. Full circles – initial concentration, the sample undivided.

through the year (Fig. 1d), which can be attributed to declining biochemical activity. The highest concentrations of chromium were recorded in the apical part of the stem (Fig. 1d).

Interaction between Rb, Na and K was observed tp affect the blade size and sucrose content (El-Sheikh and Ulrich1970). Rubidium increased the growth of plants significantly when supplied in small doses. High rubidium concentrations are toxic (El-Sheikh *et al.* 1967). If potassium is intruduced, uptake of rubidium slowed (Cline and Hungate 1960). Throughout the year, rubidium has a tendency to decrease in concentration in the stem as well as in the leaves (Fig. 1). The highest concentrations of rubidium were recorded in the apical parts of stem (Fig. 1e).

Manganese is an essential element for plants, intervening in several metabolic processes, including photosynthesis, and as an enzyme antioxidant-cofactor. If there are low levels of manganese, much of the iron is left in reduced form, becoming toxic. However an excess ofmanganese is also toxic for plants. When manganese is present in the cells, mechanisms that can tolerate this toxicity are also observed (Millaleo *et al.* 2010). Manganese is also involved in the process of assimilation of nitrogen (Rubin 1966). The highest concentrations of manganese in stems and leaves are recorded in the spring, which is associated with the highest enzyme activity. Then, over the course of the year, concentration of manganese gradually decreases. The highest concentrations of manganese are recorded in the apical, i.e. the youngest parts of the stem, which assumes the highest enzyme activity (Fig. 1f). These elements are seen as stars in the ordination diagram below (Fig. 6).

In September, iron and copper in the stems fell below the concentration detection limit. This decrease may be explained by transporting elements into forming flowers where there is a high enzymatic activity (Fig. 2a, 2b).

Iron is an essential micronutrient for plants, because it plays a critical role in metabolic processes such as DNA synthesis, respiration, and photosynthesis. Iron is significant in various physiological and bio-

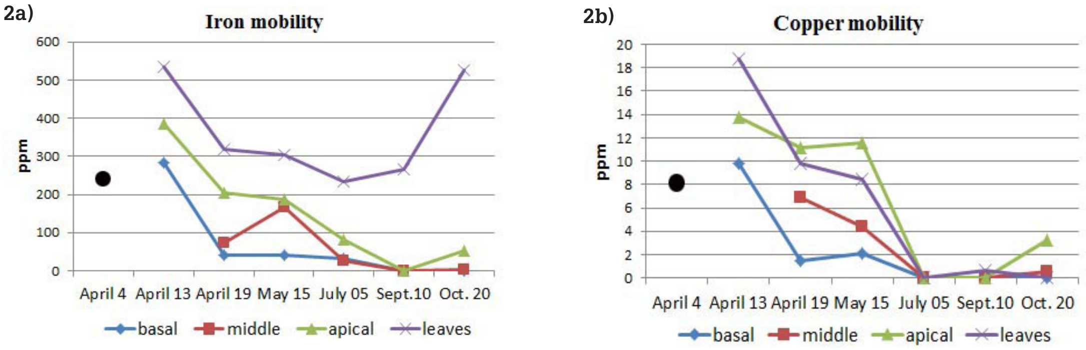

Fig. 2. Iron and copper mobility in the stem parts and leaves. Full circles – initial concentration, the sample undivided.

chemical pathways in plants. It serves as a component of many vital enzymes like cytochromes. Iron is involved in the synthesis of chlorophyll (Rout and Sahoo 2015). However, iron is toxic when it accumulates to high levels. Plants must therefore respond to iron stress in terms of both iron deficiency and iron overload (Connolly and Guerinot 2002). In accordance with the most intensive photosynthesis and respiration in the spring time, the concentration of iron in tissues is high. In September, the iron concentration in stem decreased below the detection limit (Fig. 2a). This is related to the formation of flowers where iron is transported, and where there is a high intensity of biochemical processes. In the flowers, we recorded more than 400 ppm of iron (Table 4). The highest concentration of iron was observed in the apical, i.e. youngest parts of stem (Fig. 2a).

The mose important physiological activity of copper is its participation in the plant tissue oxidation system. Copper acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses and cell wall metabolism (Yruela 2005). Copper ions act as cofactors in many enzymes such as cytochrome C oxidase, amino oxidase, plastocyanin and polyphenol oxidase. These enzymes are involved in the dark reactions of photosynthesis (Rubin 1966). At the cellular level, copper also plays an essential role in oxidative phosphorylation and iron mobilization. Copper can also be potentially toxic, and problems arise when excess copper is present in cells (Yruela 2005). Excess copper inhibits plant growth and impairs important cellular processes. Sufficienct of copper assists with efficient use of the microelements - Zn and Mn (Rubin 1966). The concentration of copper in spring is high, and correlates to the hightened enzymatic activity (Fig. 2b). In July and September, when flowers are created, the copper concentration in the stem decreased below the detection limit. As in the case of iron, we recorded a slight increase in the copper concentration during October. The highest concentrations of copper was observed in the apical parts of the stem (Fig. 2b). Iron and copper are seen in ordination diagram (Fig. 6) as right-triangles.

Barium, lead and chlorine have exhibited a downward trend during the investigated period. However, in September, these elements recorded a substantial increase in concentrations. It is hypothesized that this increase is related to the flowering of the plant. Inhibition of photosynthesis is a common feature of barium and lead (Fig. 3).

A severe nutritional deficiency disease was observed when chlorine supply was limited - growth was correlated with chlorine supply in the diet (Broyer *et al.* 1954). Chloride increases the level of sucrose in storage roots and increases production of dry matter as well. Succulence in many plants is stimulated by salinity (Jennings 1976). During the

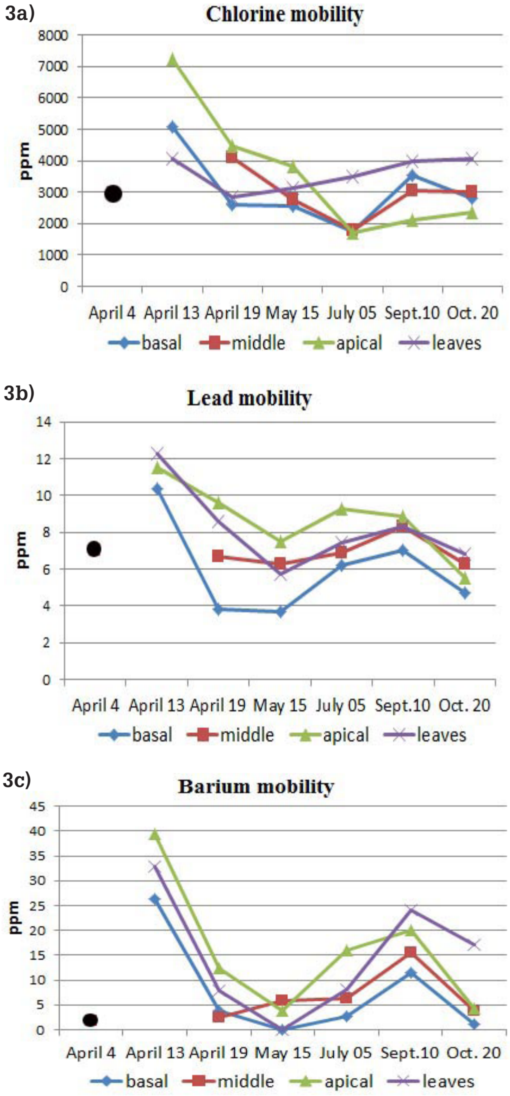

Fig. 3. Chlorine, lead and barium mobility in stem parts and leaves. Full circles - Initial concentration, the sample undivided.

year, we observed a decrease in the concentration of chlorine in the stem tissues but the concentration of chlorine in the leaves remained static (Fig. 3a).

Lead is easily absorbed and accumulated in different plant parts. Uptake of lead in plants is regulated by pH, particle size, and cation exchange capacity of the soil as well as by root exudation and other physico-chemical parameters. Excess lead causes a number of toxicity symptoms in plants, including stunted growth, chlorosis and blackening of root systems. Lead inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability. After entering the cell, lead inhibits activities of many enzymes, upsets mineral nutrition and water balance and affects membrane structure and its permeability (Sharma and Dubey 2005). The spring decrease in lead concentrations in stems and leaves is followed by a peak in September. The highest average concentrations of lead are seen in the apical part of stems (Fig. 3b).

Barium inhibits photosynthetic activity and plant growth (Suwa *et al.* 2008). The element is fixed in the tissues where it was received and is transported only slightly (Rubin 1966). Barium concentrations in stems and leaves during the year is atypical. After the spring decrease in concentrations, it shows a peak in September. Nevertheless, the highest average concentrations of barium are reported in the apical part of stems (Fig. 3c).

Unlike the other elements, calcium and strontium have exhibited an upward trend in concentration during the investigation period (Fig. 4). Calcium and strontium are alkaline earth metals. These elements are fixed in the plant's reception point and move only slightly. Tin is also included with this group as the apical part of stem exhibits related symptoms including an increasing trend in concentration. These elements create a distinct group of crosses in the ordination diagram (Fig. 6).

Strontium was not observed to be directly involved in metabolic processes. However, Tamponnet *et al.* (2008) found that there is a linear relationship between the absorbing power of calcium and strontium. Strontium is fixed in the tissues where it was received and is transported only slightly (Rubin 1966). While the concentration of most elements decline during the year, concentration of strontium rose in all parts of the stem and in the leaves. The highest concentrations of strontium are found in the basal parts of the shoots (Fig. 4a).

Calcium is an important part of protoplasmic structure, and a component of the cell wall. Calcium is fixed in the tissues where it was received and is transported only slightly. The element affects the development of the root system (Rubin 1966). Calcium serves as a versatile messenger in many adaptation and developmental processes in plants (Bastič and Kudla 2012). It is necessary to control Ca^{2+} for cell survival, as regulation of cellular calcium is an essential cell function (Bush 1995). The Ca^{2+} concentration in tissues increases throughout the year. This applies to the stems and leaves, and a possible explanation is calcium transport to the cell walls. The highest concentrations of calcium were found in the basal part of the stem (Fig. 4b).

Müller *et al.* (2015) found an accumulation of tin in the roots. Similar results were obtained by Ashraf *et al.* (2011), finding that roots showed the highest tin concentration followed by leaves, shoots and flowers. We can't confirm this finding. The annual course of the observed tin concentration in stems is mostly static (Fig. 4c), except in the apical section where we have seen an upward trend in concentration, (distribution similar to strontium and calcium). Therefor, detrended correspondence analysis grouped tin with strontium and calcium (Fig. 6).

In the case of molybdenum and antimony we have not recorded any systemic movement during the investigated period (Fig. 5). In the ordination diagram, these elements stand close together as diamonds (Fig. 6).

Molybdenum has an influence on the development of the root system, and improves the nutrition requirements of calcium (Rubin 1966). This element is an essential trace element for several enzymes important to plant metabolism and has an important role in enzymes that catalyse the reduction of nitrogen and nitrates. Molybdenum is essential to

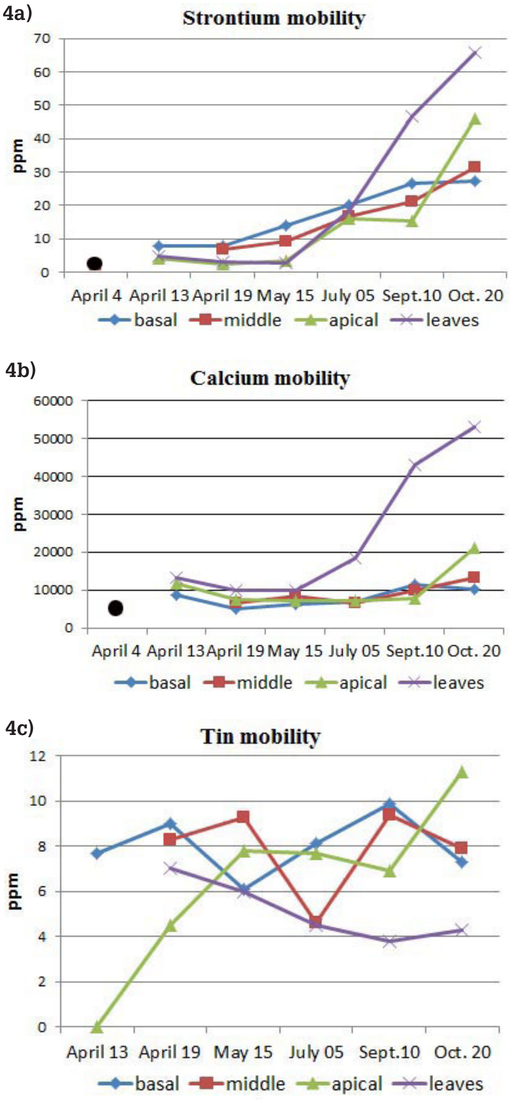

Fig. 4. Strontium, calcium and tin mobility in the stem parts and leaves. Full circles - initial concentration, the sample undivided.

plants, and is necessary for plant production, even though it is only present in plant tissue at a very low concentration (0.5 ppm dry matter) (Turnlund *et al.* 1995). During the year, we have seen a slight decrease in the concentration of molybdenum in the stem tissues and in the leaves (Fig. 5a).

Antimony is present in the environment as a result of natural processes and human activities. Antimony has no known biological function, but the element is generally taken up by terrestrial plants in proportion to the concentration of soluble antimony in soil (Tschan *et al.* 2009). It exists mainly as Sb 3^+ and Sb 5^+ (Filella *et al.* 2002), but trivalent antimony is more toxic (Frézard *et al.* 2013). The concentration of antimony in stems and leaves remained largely unchanged over the course of the year (Fig. 5b).

These elements create a group of up-triangles in the ordination diagram (Fig. 6).

Correlation between processed samples and rhizome or bryophyte

According to some authors (Dwiecki and Koziol 2005, Sołtysiak *et al.* 2011), *Fallopia japonica* translocates elements from roots to overhead parts. Conversely, Bryophytes are completely dependent on the substrate, reflecting environmental contamination (Ratcliffe 1975, Burton 1990). This is why the correlation matrix shows analysis of both rhizome and bryophytes (*Brachythecium salebrosum*). Correlation analysis helps to assess the extent to which the results of analysis of considered parts of the plant correlate with rhizome or air contamination.

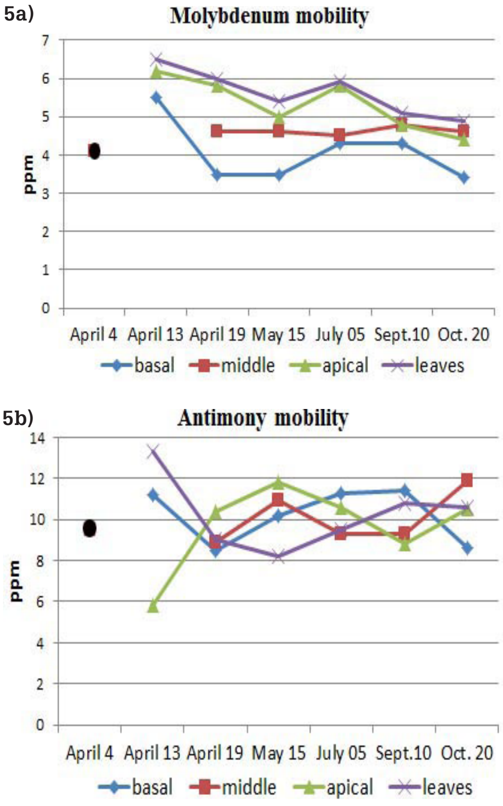

Fig. 5. Molybdenum and antimony mobility in the stem parts and leaves. Full circles – initial concentration, the sample undivided.

Correlation between basal parts of stems and rhizome or bryophyte

In the spring period (April - May), young shoots grow rapidly (4.6 cm per day; Horn 1997). During this period we recorded a significant disproportion in representation of monitored elements between the basal parts of the stem and rhizome or bryophytes respectively, correlation coefficient r<0.5 (Table 5). Later (May - October), the growth is slowed down and via elemental translocation or possible air accumulation the concentrations are balanced and the correlation coefficient (r) reaches values > 0.7.

	Correlation (stem, basal part)	
Variables	**Rhizome**	**Bryophyte**
April 4	0.43	0.44
April 13	0.46	0.47
April 19	0.45	0.46
May 15	0.48	0.49
July 25	*0.51	*0.51
Sept. 10	*0.59	*0.59
Oct. 20	*0.72	*0.71

*indicates significance

Table 5. Correlation analysis, results, stem - basal parts.

	Correlation (stem, middle part)	
Variables	**Rhizome**	**Bryophyte**
April 4	0.43	0.44
April 19	0.44	0.45
May 15	*0.49	*0.50
July 25	*0.51	*0.51
Sept. 10	*0.58	*0.58
Oct. 20	*0.79	*0.77

*indicates significance
Note: On April 13, middle parts were not collected. The samples have been divided only on the basal and apical parts.

Table 6. Correlation analysis, results, stem - middle parts.

Variables	**Correlation (stem, apical part)**	
	Rhizome	**Bryophyte**
April 4	0.43	0.44
April 13	0.46	0.47
April 19	0.45	0.46
May 15	0.46	0.47
July 25	*0.57	*0.57
Sept. 10	*0.64	*0.63
Oct. 20	*0.97	*0.94

*indicates significance

Table 7. Correlation analysis, results, stem - apical parts.

Variables	Correlation, leaves	
	Rhizome	Bryophyte
April 13	0.58	0.58
April 19	0.58	0.58
May 15	0.57	0.57
July 25	0.88	0.86
Sept. 10	1.00	0.96
Oct. 20	0.98	0.94

Note: All data are significant

Table 8. Correlation analysis, results, leaves.

Variables	Correlation, flowers	
	Bryophyte	Stem, apical part July 05
August 20	0.53	1.00
September 10	0.65	0.99

Note: All data are significant

Table 9. Correlation analysis, results, flowers.

Correlation between middle parts of stems and rhizome or bryophyte

Due to the rapid growth in the spring, correlation is low (r<0.5), during the year, when growth is slowed, correlation gradually increases, in October r=0.79 (Table 6).

Correlation between apical parts of stems and rhizome or bryophyte

In the apical parts of the stem, due to the rapid growth in April – May, the correlation is low (r<0.5), later, when growth slows, correlation gradually increases. In October r=0.97 (Rhizome; Table 7).

According to Berchová-Bímová *et al.* (2014), the metal content was significantly higher in the underground parts of plants, and this is confirmed by our measurements also. In the underground parts of the plants we have recorded increased Ca, Fe, Mn, Sr, Ba, and Ti, but significantly less non-metals, sulfur and chlorine (Table 1 compared to Table 2). We investigated the correlation of concentrations of the elements with the concentration of the same elements in the moss tissues *Brachythecium salebrosum* and the rhizome of *Fallopia japonica*. Concentrations of the elements in the moss tissues *Brachythecium salebrosum* and rhizome correlate, and the correlation coefficient is 0.98. Correlation between stem parts (basal, middle, apical part) and rhizome, or bryophytes respectively, is lowest in April 4, influenced by the intense formation of shoots, which results in low concentrations of Ba, Ca, Cr, Sr, Mo, Sn. Conversely, titanium is present only on April 4, then is missing. Correlations of observed concentrations increase during the year in relation to the moss *Brachythecium salebrosum* and to the rhizome (Table 6, 7, 8). Correlation coefficients were highest on October 20 (0.71 to 0.97).

Correlation between leaves and rhizome or bryophyte

The correlation of detected concentration of elements in the leaves with those in the rhizome and the moss tissues is approximately the same, but by the end of

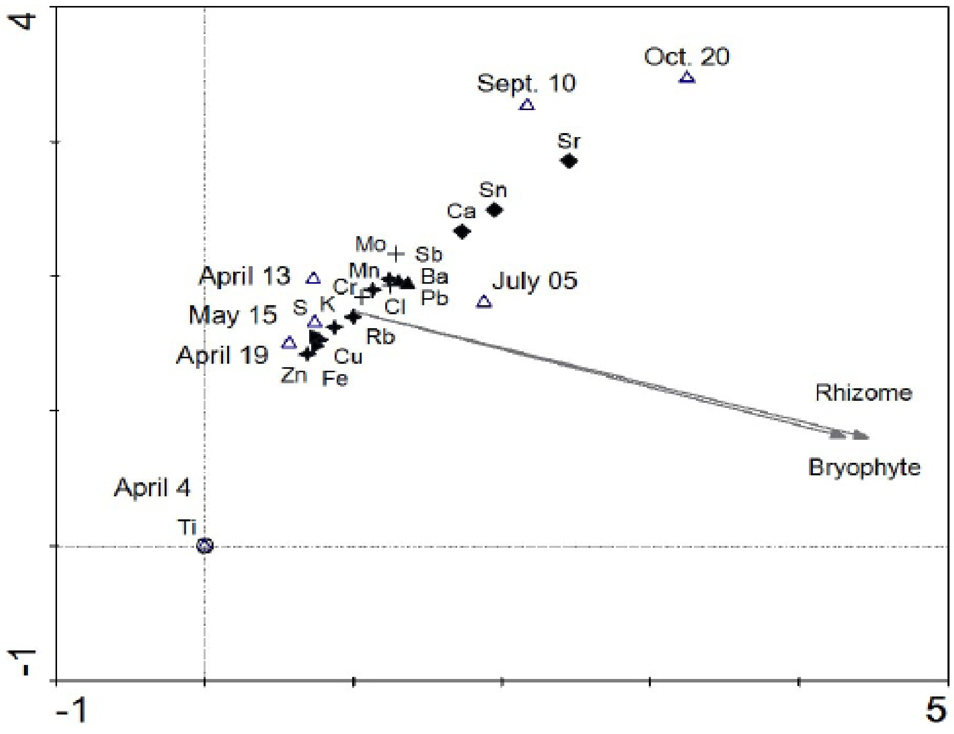

Fig. 6. Detrended correspondence analysis (DCA), ordination diagram of evaluated elements. Diamonds - Sr, Ca, Sn; Crosses – Cl, Pb, Ba; Up-triangles – Mo, Sb; Right-triangles – Fe, Cu; Stars – S, K, Zn, Cr, Rb, Mn.

the growing season the moss becomes slightly more predominant (Table 8). When comparing the correlation parameters of stems and leaves (Tables 5, 6, 7 vs. Table 8) there are strikingly higher values of correlation in the leaves. This can be explained by possible airborn element accumulation by leaves.

Correlation between flowers and the stem or bryophyte

The concentration of the elements in the flower buds and open flowers highly correlate with the apical parts of the stem (Table 9). The correlation with mosses is low, indicating that the flowers do not accumulate elements from the environment.

Ordination of the elements data set

The detrended correspondence analysis (DCA) ordination diagram of 17 variables (elements) doesn't show major differences in any axes (Fig. 6), but supplementary variables show the major differences in the direction of the first axis (Rhizome r = 0.8333, eigenvalue 0.129; Bryophyte r=0.7864, eigenvalue 0.001), species-environment correlations in PC1 0.957, in PC2 0.953. The ordination diagram showed no correlation of environmental data with supplementary variables either. This indicates that in terms of elemental uptake, *Fallopia japonica* is only marginally dependent on the content of elements in the rhizome or on uptake from the environment. The elements are distributed into the different plant organs via the xylem and phloem due to the plant's own regime.

Acknowledgements

The assistence of everyone who helped with valuable advice is also warmly acknowledged. The research was supported by project ITMS (Grant No. 26210120006).

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Received 24 April 2017; accepted 15 May 2017.