



# Distribution of micro- (Fe, Zn, Cu, and Mn) and risk (Al, As, Cr, Ni, Pb, and Cd) elements in the organs of *Rumex alpinus* L. in the Alps and Krkonoše Mountains

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## Abstract

**Background and aims** *Rumex alpinus* is a native plant in the mountains of Europe whose distribution has partly been affected by its utilization as a vegetable and medicinal herb. The distribution of micro and risk elements in its organs is not well-known. The study examined the safety of consuming *R. alpinus* from the Krkonoše Mountains, the Czech Republic, and the Alps (Austria and Italy).

**Methods** We determined the total and plant-available content of Fe, Zn, Cu, Mn, Al, As, Cr, Ni, Pb, and Cd in the soil and the total content in organs of *R. alpinus*.

**Results** The uptake and distribution of elements by plants were characterized by bioaccumulation (BF) and translocation (TF) factors. The level of elements

accumulation by *R. alpinus* is considerably different, depending on local geological substrates and environmental conditions. *Rumex alpinus* has considerable tolerance to Zn, Cu, As, Cr, Ni, with an easy accumulation strategy. High Al and Cd content in below-ground biomass (rhizome) indicate a defensive mechanism for them. Although the aboveground biomass (emerging, senescent, mature leaves, petiole) has some degree of accumulation of risk elements, the results showed that *R. alpinus* is an excluder.

**Conclusion** *Rumex alpinus* does not accumulate risk elements in organs (leaf and petiole) that are consumed based on the permissible limit according to World Health Organization (2001) and can therefore be used without concern. Caution must, therefore, be taken when consuming these plant parts in heavily contaminated soils.

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**Keywords** *Rumex alpinus* weed · Above/ belowground biomass · Bioaccumulation/ Translocation factors · Excluder · Micro/risk element

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## Introduction

The Monk's rhubarb (Alpine dock), *R. alpinus* L. (*R. alpinus*), is a perennial plant inhabiting nutrient-rich areas, stream banks, spring areas, pastures, and meadows. It is one of the historic food plants for preparing the dish farchon, made of steamed *Chenopodium bonus-henricus*, *Urtica dioica*, and *R. alpinus* in the Alps region (Maude and Moe 2005). In Albania, this species is the most quoted and used wild food plant, used as a vegetable mainly cooked with dairy products and rice or as filling for several homemade pies, e.g., savory pie (Pieroni and Quave 2014).

In alpine localities, some organs of this species have been used for various purposes, e.g., leaves as a surrogate for sauerkraut or spinach, stems peeled and applied instead of rhubarb, eaten fresh or put into cakes, biscuits, and puddings (Dickson and Dickson 2000; Štastná et al. 2010). The leaves, seeds, rhizomes, and roots of *R. alpinus* are most often used for the treatment of several health disorders, e.g., diarrhea, dysentery, constipation, stomach disorders, kidney disorders, eczema, jaundice, fever, and cancer (Hartwell 1970; Rácz et al. 1992; Jang et al. 2012).

In traditional Austrian medicine, the leaves and roots of *R. alpinus* have been used internally for the treatment of viral infections (Bogl et al. 2013). *R. alpinus* has also emerged as being suitable for the treatment of inflammation and different bacterial infections (Vasas et al. 2015). Given the benefits of *R. alpinus* concerning health-related issues, it is appropriate to perform a detailed analysis of the bioaccumulation of elements in organs of this species. The nutritional status of plants is best reflected by the content of elements in the leaves (Marschner et al. 1996). However, the detailed distribution of trace and risk elements in the belowground and aboveground organs of *R. alpinus* is so far not studied.

Anthropogenic activities, e.g., mining metals and metallurgy, remain the most vital sources of trace/risk elements (such as As, Cu, Zn, Cd, and Pb) in soils. Notwithstanding, differing content of micro/risk elements can occur from the natural lithogenic background of different environments (Kabata-Pendias and Pendias 2001; Sun et al. 2019; Pavlović et al. 2021). However, under natural conditions, only a minimal fraction of trace/risk elements are present in available forms to plants (Kabata-Pendias and Pendias 2001). In some natural soils developed

from metal-rich parent materials, up to 60% of heavy metals can occur in available forms (Karczevska et al. 1998). The mobility and availability of elements depend on soil biogeochemical properties, e.g., pH, redox condition, dissolved organic carbon (DOC), clay content, and metal oxides (Sungur et al. 2020).

Soil contamination with trace elements represents a risk for crop production, food quality, and human health because of their high toxicity and the ability of plants to bioaccumulate them. However, plants have differing strategies for accumulating risk elements in different organs. For instance, Pb accumulates primarily in root cells because of the blockage by Casparian strips (Dogan et al. 2018). Thus, a higher fraction of accumulated Pb remains in roots, and only less amount gets transported to the aerial plant parts (Kiran and Prasad 2017). Supported by negative charges that exist on root cell walls can also trap Pb ions (Tariq 2018). Meanwhile, Pb uptake by roots and its transportation to above-ground parts can increase with increasing concentration of Pb (Kohli et al. 2018).

Notable, *R. alpinus* grows mainly in mountainous areas, especially in protected areas with minimal anthropogenic activities. Therefore, it may seem that the contents of risk elements would be relatively low there. Nevertheless, in the Krkonoše Mountains, Czech Republic, *R. alpinus* has been widely distributed in locations with historic mining of Cu or As ores with a reported high possibility of soil contamination by micro- and risk elements (Lokvenc 2007; Tásler 2012). Moreover, *R. alpinus* usually grows in nutrient-rich soils in the surrounding areas of human settlements with a tendency for contamination by risk elements.

We decided to analyze the content of trace and risk elements in different organs of *R. alpinus* in differing localities to determine whether the consumption of *R. alpinus* organs represents a health risk for humans. To study plant-soil interactions, we calculated the bioaccumulation factor (Baker 1981; BF) and the plant-to-soil content ratio for selected elements (Klink et al. 2014; Vondráčková et al. 2014).

In this study, we determined the content of elements in soils and plant organs of *R. alpinus* in the Krkonoše Mountains and the Alps, asking the following research questions: (1) to what extent can accumulate micro- and risk elements and (2) which

organs of *R. alpinus* are accumulators of micro- and risk elements?

## Materials and methods

### Study area

This study was conducted in typical stands of *R. alpinus* in the Krkonoše Mountains (Czech Republic) and the Alps (Austria and Italy) (Fig. 1). We selected the localities according to their wide distribution of the *R. alpinus* plants (see Table 1 for a detailed description of each study locality).

The four study localities in the Krkonoše Mountains (Libuše hut- LB; Vítkovice v Krkonoších- VT; Pec pod Sněžkou- PC; and Horní Mísečky- HM; Fig. S1) are all characterized by podzol soils located on phyllite and granite geological substrates (Němeček and Kozák 2005). In Ramsau am Dachstein (DCH) and Zillertal (ZL), Austria, the soils are Luvic and Calcaric Cambisols located on limestone and granite-gneiss bedrock, respectively (Jones et al. 2005). Additionally, in Madesimo (MD), Italy, the soil is a Vertic Cambisol underlain by sandstone bedrock (Jones et al. 2005).

### Soil sampling and preparation

To cover the variability of soil samples in the sampled locations of *R. alpinus* stands, we adopted a specific sampling design. We sampled the upper 10 cm soil layers with a soil probe (Purchhauer type, core diameter: 30 mm). Moreover, we randomly collected ten sub-samples that were mixed into one representative sample per locality (LB, VT, PC, HM, DCH, and MD). The soil samples were air-dried and subsequently oven-dried at 70 °C for 48 h. The samples were ground in a porcelain mortar and homogenized by sieving through a 2-mm sieve after the removal of roots and other debris. To minimize variability in chemical composition, a representative sample was mixed and divided into three replicates (6 localities, with 3 replicates for each). In total, we collected 18 samples for further chemical analysis. We collected mixed samples to produce short-range variations, making reliable results and generalizations.

### Plant organ sampling and preparation

*R. alpinus* is a perennial plant with a horizontal rhizome (Klimeš 1992). Each year, three to five large leaves grow with petioles ranging from 70 to 80 cm and laminae

**Fig. 1** Locations of studied sites in the Krkonoše (Giant) Mountains, Czech Republic, Ramsau am Dachstein and Zillertal in Austria, and Madesimo, Italy, where *Rumex alpinus* samples were collected



**Table 1** Description of studied localities in the Krkonoše Mountains, Czech Republic, and in the Alps of Austria and Italy

Locality	Description	Geographical location	Altitude [m a.s.l.]	Mean annual precipitation [mm]	Mean annual temperature [°C]	Soil type	Geological Substrate
Libuše hut- (LB), CZ	Bank of Úpa river	50°41'19"N 15°46'43"E	700	850	6.5	Podzol	Phyllite
Vítkovice v Krkonoších (VT), CZ	Ditch close to the road	50°41'56"N, 15°31'41"E	650	900	5.5	Podzol	Phyllite
Pec pod Sněžkou (PC), CZ	Ruderal area under the hotel	50°41'46"N, 15°44'8"E	815	850	5.5	Podzol	Phyllite
Horní Mísečky (HM), CZ	Eutrofied grass-land	50°44'2"N, 15°34'5"E	1050	1000	4.5	Podzol	Phyllite
Ramsau am Dachstein (DCH), A	Cattle pasture	47°27'1"N, 13°37'1"E	1650	1100	3.8	CalcaricCambisols	Limestone
Zillertal (ZL), A	Cattley/horse pasture	47°14'21"N 12°7'39"E	1650	933	3.9	Luvic Cambisol	Granite gneiss
Madesimo (MD), I	Cattle pasture	46°26'13"N, 9°21'27"E	1600	2000	2	Vertic Cambisols	Sandstone

CZ Czech Republic, A Austria, and I Italy

up to 50 cm long and 20 cm wide, creating a dense canopy 3 - 8 m<sup>2</sup> wide and 30 - 200 cm high. Young (emerging) leaves of *R. alpinus* most often are formed throughout the vegetation season. The inflorescence growing on the top of the stem produces 1500 - 5000 fruits and remains on the plant until winter (Šťastná et al. 2010). Organs of *R. alpinus* were collected in a monodominant stand that covered 100 m<sup>2</sup> at all localities (Fig. 2). Samples of above- (emerging, mature, and senescent leaves; stem; and petiole) and belowground (rhizomes) organs were collected from all localities (Fig. 2).

We randomly collected ten emerging- semi-developed leaf blades (E), ten fully developed mature leaf blades (M), ten senescent yellow, red, or brown semi-dry leaf blades (S), ten petioles from mature leaves (Pe), ten stems without flowers and seeds (St) and three rhizomes (R) developed in the last two years (Fig. 2). The plants were at least 20 m apart, and the examined organs were collected from one plant. The collected samples also were kept in paper bags and transported to the laboratory. All plant organs were cleaned of soil and other residues in distilled H<sub>2</sub>O and dried for 48 h at 70 °C. Additionally, all ten samples from each organ for one locality were mixed to form a representative sample. The organs were ground and homogenized in an IKA A11 basic analytical

mill (IKA®-Werke GmbH & Co., KG, Germany). Each representative organ sample from each locality again was divided into three sub-samples (replicates) for further chemical analysis. Samples from the Krkonoše Mountains were collected twice; in July (summer- S) and October 2018 (autumn- A). Samples from the Alps were collected only in July 2018 (summer), approximately at the same time as in the Krkonoše Mountains.

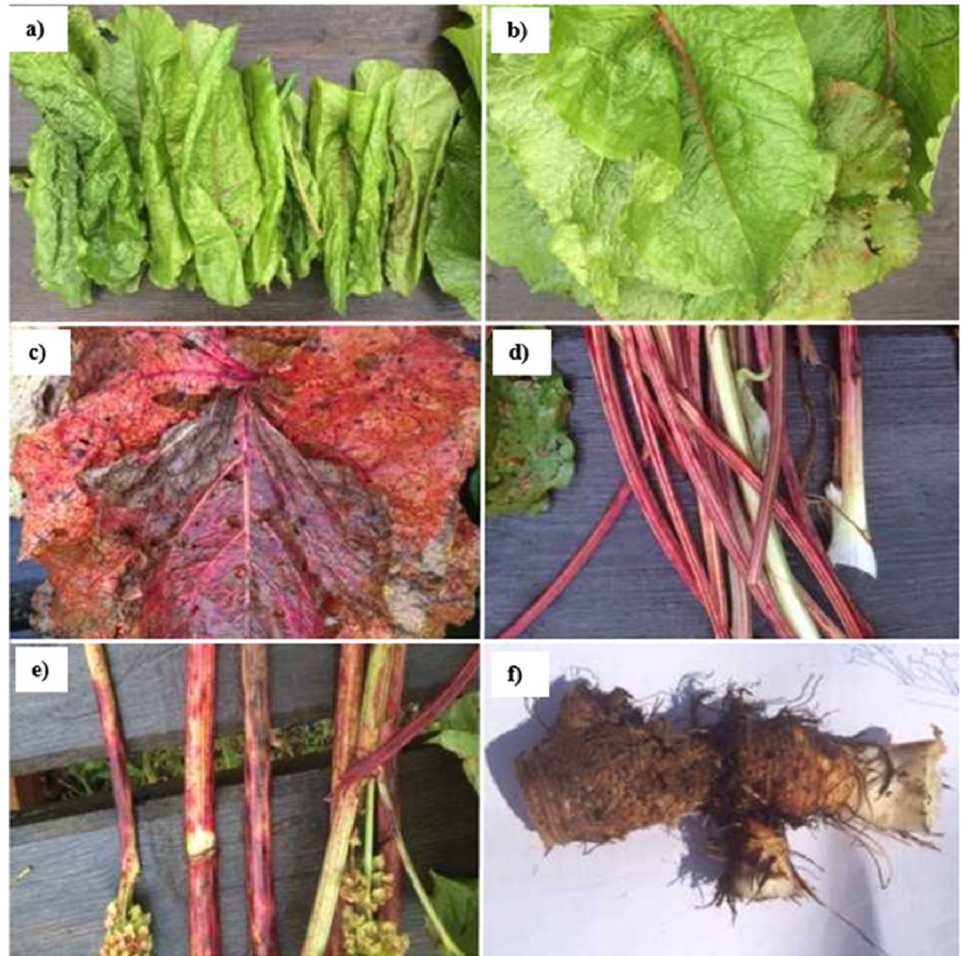
#### Chemical analyses of soils and plant organ samples

The total contents of Fe, Zn, Cu, Mn, Al, As, Cr, Ni, Pb, and Cd in the soils and plant organs were extracted using the USEPA 3052 extraction procedure (International Organization for Standardization, USEPA 1996) with an extraction mixture of 65% HNO<sub>3</sub>, 36% HCl, and 38% HF. Usage: A mass of 0.25 g of homogenized *R. alpinus* individual organs was mineralized in a mixture of 9 mL HNO<sub>3</sub>, 3 mL HCl and 1 mL HF and heated in a sealed 60 mL VWR® PTFE Jar on a hot plate at 150 °C for 24 h.

After 24 h, 1 ml of 30% of H<sub>2</sub>O<sub>2</sub> was added to each sample and evaporated on a hot plate at 50 °C for 24 h. The evaporated samples were then diluted to 20 mL by 2% HNO<sub>3</sub> for two hours and filtrated. Total content was determined by inductively coupled



**Fig. 2** Studied organs of *Rumex alpinus*: (a) emerging- E (b) mature- M, and (c) senescent leaf blades- S, (d) petioles from mature leaves- Pe, (e) stems from flowering plants- St, and (f) two-year-old rhizome- R



plasma–optical emission spectrometry (ICP–OES; 720 Series, Agilent Technologies Inc., USA). The plant organs were represented by three samples, which were measured separately. In determining the total content of elements in the soil samples, we used the same approach (USEPA 1996) as in the case of the plant organs.

The plant-available fractions of Fe, Zn, Cu, Mn, Al, As, Cr, Ni, Pb, and Cd in the soil were analyzed by Mehlich-III reagent (Mehlich 1984) followed by ICP–OES (Varian VistaPro, Mulgrave, Australia). The extractant composition was as follows: 0.2 M  $\text{CH}_3\text{COOH}$ +0.25 M  $\text{NH}_4\text{NO}_3$ +0.013 M  $\text{HNO}_3$ +0.015 M  $\text{NH}_4\text{F}$ +0.001 M EDTA; usage: 25 cm<sup>3</sup> reagent per 2.5 cm<sup>3</sup> soil. The plant-available content of the elements in soil samples was analyzed in an accredited national laboratory, Eko-Lab Žamberk ([www.ekolab.zamberk.cz](http://www.ekolab.zamberk.cz)). We determined the soil pH ( $\text{H}_2\text{O}$ ) in two replicates for all samples at

a ratio (soil–water) of 1:2 using a Voltcraft PH-100 ATC pH meter (pH 212) manufactured by I & CS spol. sr.o. (Czech Republic).

#### Statistical analyses

Data on pH, elemental contents in the organs of *R. alpinus*, and soil samples were tested by the Kolmogorov–Smirnov test of normality and met assumptions for the use of parametric tests. There was relative homogeneity of variance among the obtained data. Factorial ANOVA was used to determine the significant difference among the content of elements in different organs of *R. alpinus* from all localities. One-way ANOVA was used to determine the significant difference in the content of elements in the organs and soil from the overall localities. In all cases, post hoc comparison using the Tukey HSD test was applied to identify significant

differences between the content of elements in different organs and soils. All statistical analyses were performed using the STATISTICA 13.3 program ([www.statsoft.com](http://www.statsoft.com)).

#### Estimation of bioaccumulation (BF) and translocation factors (TF)

The BF was calculated by the following equation,

$$\begin{aligned} i. BF &= leaf \div soil \\ ii. BF &= petiole \div soil \\ iii. BF &= rhizome \div soil \end{aligned} \quad (1)$$

The BF of the leaf was estimated for the total element content in emerging and mature leaves by the bioavailable metal contents in the soil, without senescent (degenerative part) leaves. We combined the resulting values of emerging and mature leaves because they did not differ significantly, and the pattern (leaves, petiole, and belowground organ) in Klink et al. (2014) and Vondráčková et al. (2014) was followed.

The TF was calculated by the following equation,

$$TF = leaf \div rhizome \quad (2)$$

We used the mean value of the total content of elements in the mature leaf and rhizome according to Klink et al. (2014) and Vondráčková et al. (2014). One-way ANOVA was used to determine the

significant difference between BF and TF of the studied elements in all localities.

## Results

### pH and content of elements in soils

We recorded a significant effect of locality on pH [H<sub>2</sub>O] (Table 2). The pH of the soil samples from all analyzed localities ranged from 5.2–6.1. Except for a slightly acidic reaction in DCH, soils in all other localities were moderately acidic, resulting in increased availability of elements to plants.

The statistical descriptions of the total and plant-available contents of the studied elements are shown in Tables 2 and 3, respectively. There was a significant effect of locality on the total contents of the micro-(Fe, Zn, Cu, and Mn) and risk (Al, As, Cr, Ni, Pb, and Cd) elements (Table 2). The total content of Fe ranged from 13.6 to 27.5 g kg<sup>-1</sup> in DCH and HM, respectively. The total content of Zn ranged from 48 in DCH to 182 mg kg<sup>-1</sup> in PC. The content of total Cu ranged from 4.7 in DCH to 39.8 mg kg<sup>-1</sup> in LB. The total Mn ranged from 178 in MD to 693 mg kg<sup>-1</sup> in VT.

Moreover, the total Al content ranged from 11.1 in LB to 27.1 g kg<sup>-1</sup> in VT. The content of total As ranged from 3.9 in DCH to 70.9 in HM. The total Cr content ranged from 20.6 in MD to 53.2 mg kg<sup>-1</sup> in

**Table 2** Total content (mean ± SE) of elements in upper 10 cm soil layers from the studied localities. The *p* value for each element was obtained by one-way ANOVA. Using Tukey

(HSD) post hoc test, the content of individual elements with the same letter for each locality was not significantly different

Locality	LB	VT	PC	HM	DCH	MD	<i>F</i> -value	<i>P</i> value
pH (H <sub>2</sub> O)	5.2 ± 0.17 <sup>d</sup>	5.7 ± 0.04 <sup>bcd</sup>	5.9 ± 0.11 <sup>cd</sup>	5.6 ± 0.13 <sup>abc</sup>	6.1 ± 0.33 <sup>a</sup>	5.3 ± 0.67 <sup>ab</sup>	22.1	0.001
Fe (g kg <sup>-1</sup> )	21.1 ± 0.6 <sup>ab</sup>	24.2 ± 2.1 <sup>a</sup>	23.8 ± 1.4 <sup>a</sup>	27.5 ± 4.0 <sup>a</sup>	13.6 ± 0.1 <sup>b</sup>	18.2 ± 2.3 <sup>ab</sup>	3.0	0.05
Zn (mg kg <sup>-1</sup> )	179 ± 12.6 <sup>a</sup>	165 ± 37.3 <sup>a</sup>	182 ± 36.8 <sup>a</sup>	124 ± 19.4 <sup>ab</sup>	48 ± 0.6 <sup>b</sup>	58 ± 0.5 <sup>b</sup>	3.3	0.05
Cu (mg kg <sup>-1</sup> )	39.8 ± 1.4 <sup>a</sup>	28.3 ± 3.3 <sup>ab</sup>	23.3 ± 1.5 <sup>ab</sup>	31.9 ± 6.2 <sup>ab</sup>	4.7 ± 0.1 <sup>c</sup>	19.8 ± 5.2 <sup>bc</sup>	6.6	0.002
Mn (mg kg <sup>-1</sup> )	655 ± 6.2 <sup>a</sup>	693 ± 101 <sup>a</sup>	603 ± 29 <sup>ab</sup>	581 ± 216 <sup>ab</sup>	519 ± 19 <sup>ab</sup>	178 ± 79 <sup>b</sup>	1.6	0.24
Al (g kg <sup>-1</sup> )	11.1 ± 2.1 <sup>b</sup>	27.1 ± 5.2 <sup>a</sup>	14.6 ± 2.0 <sup>b</sup>	14.4 ± 2.4 <sup>b</sup>	13.1 ± 0.5 <sup>b</sup>	18.1 ± 1.3 <sup>ab</sup>	3.4	0.33
As (mg kg <sup>-1</sup> )	<b>54.5</b> ± 5.9 <sup>ab</sup>	22.5 ± 2.3 <sup>c</sup>	16.3 ± 2.6 <sup>cd</sup>	<b>70.9</b> ± 6.2 <sup>a</sup>	3.9 ± 0.4 <sup>d</sup>	<b>41.9</b> ± 1.1 <sup>b</sup>	29.9	0.001
Cr (mg kg <sup>-1</sup> )	36.1 ± 1.5 <sup>abc</sup>	46.1 ± 3.5 <sup>a</sup>	43 ± 1.6 <sup>ab</sup>	53.2 ± 6 <sup>a</sup>	22.6 ± 0.4 <sup>bc</sup>	20.6 ± 9.5 <sup>c</sup>	5.4	0.01
Ni (mg kg <sup>-1</sup> )	20.7 ± 19.1 <sup>ab</sup>	26.6 ± 24.1 <sup>a</sup>	22.2 ± 19.3 <sup>ab</sup>	28 ± 5.8 <sup>a</sup>	11.6 ± 10.8 <sup>b</sup>	11.7 ± 3.2 <sup>b</sup>	3.3	0.05
Pb (mg kg <sup>-1</sup> )	44.5 ± 3.5 <sup>ab</sup>	42.1 ± 8.2 <sup>abc</sup>	39.9 ± 5.3 <sup>abc</sup>	70.8 ± 12.8 <sup>a</sup>	13.2 ± 0.2 <sup>c</sup>	21.5 ± 3.6 <sup>bc</sup>	4.9	0.01
Cd (mg kg <sup>-1</sup> )	1.32 ± 0.17 <sup>a</sup>	1.18 ± 0.22 <sup>a</sup>	0.89 ± 0.14 <sup>ab</sup>	1.35 ± 0.05 <sup>a</sup>	0.43 ± 0.01 <sup>b</sup>	0.46 ± 0.12 <sup>b</sup>	5.3	0.01

**Abbreviations:** LB Libuše hut, VT Vítkovice v Krkonoších, PC Pec pod Sněžkou, HM Horní Míšečky, DCH Ramsau am Dachstein, MD Madesimo. The values above the permissible limit for agricultural soils are in bold, according to Decree of the Ministry of the Environment No.153/2016 Coll.: As 40; Cd 1.5; Cu 200; Ni 150; Pb 300; Zn 400

**Table 3** Mean ( $\pm$  SE) plant-available content of elements in the upper 10 cm soil layers of the studied localities

Locality		LB	Vítkovice	PC	HM	DCH	Madesimo	<i>p</i> value
Fe	(mg kg <sup>-1</sup> )	404 $\pm$ 9.5 <sup>d</sup>	527 $\pm$ 5.1 <sup>c</sup>	260 $\pm$ 6.9 <sup>c</sup>	657 $\pm$ 21.2 <sup>a</sup>	204 $\pm$ 13 <sup>f</sup>	554 $\pm$ 14.3 <sup>b</sup>	< 0.001
Zn	(mg kg <sup>-1</sup> )	39.5 $\pm$ 4.7 <sup>b</sup>	10.6 $\pm$ 0.3 <sup>d</sup>	62.1 $\pm$ 0.7 <sup>a</sup>	25.2 $\pm$ 0.2 <sup>c</sup>	5.1 $\pm$ 0.1 <sup>c</sup>	6.2 $\pm$ 0.1 <sup>e</sup>	< 0.001
Cu	(mg kg <sup>-1</sup> )	15.4 $\pm$ 0.5 <sup>a</sup>	4.26 $\pm$ 0.2 <sup>b</sup>	5.9 $\pm$ 0.8 <sup>b</sup>	0.67 $\pm$ 0.01 <sup>c</sup>	0.86 $\pm$ 0.01 <sup>c</sup>	7.9 $\pm$ 0.6 <sup>bd</sup>	< 0.001
Mn	(mg kg <sup>-1</sup> )	162 $\pm$ 3.1 <sup>b</sup>	99 $\pm$ 1.8 <sup>d</sup>	189 $\pm$ 3.8 <sup>a</sup>	59 $\pm$ 0.8 <sup>e</sup>	131 $\pm$ 1.1 <sup>c</sup>	43 $\pm$ 2.4 <sup>f</sup>	< 0.001
Al	(mg kg <sup>-1</sup> )	931 $\pm$ 24 <sup>d</sup>	1474 $\pm$ 72 <sup>b</sup>	648 $\pm$ 16.2 <sup>f</sup>	1720 $\pm$ 77 <sup>a</sup>	697 $\pm$ 16.5 <sup>e</sup>	1256 $\pm$ 68 <sup>c</sup>	< 0.001
As	(mg kg <sup>-1</sup> )	3.64 $\pm$ 0.6 <sup>a</sup>	0.36 $\pm$ 0.01 <sup>b</sup>	1.11 $\pm$ 0.2 <sup>c</sup>	0.76 $\pm$ 0.1 <sup>bc</sup>	0.35 $\pm$ 0.04 <sup>b</sup>	0.91 $\pm$ 0.01 <sup>c</sup>	< 0.001
Cr	(mg kg <sup>-1</sup> )	0.12 $\pm$ 0.01 <sup>a</sup>	0.20 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.01 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	0.20 $\pm$ 0.01 <sup>a</sup>	0.31 $\pm$ 0.01 <sup>a</sup>	0.081
Ni	(mg kg <sup>-1</sup> )	1.85 $\pm$ 0.1 <sup>a</sup>	1.35 $\pm$ 0.03 <sup>ac</sup>	1.03 $\pm$ 0.01 <sup>ac</sup>	0.77 $\pm$ 0.02 <sup>bc</sup>	0.34 $\pm$ 0.01 <sup>b</sup>	0.87 $\pm$ 0.03 <sup>c</sup>	< 0.001
Pb	(mg kg <sup>-1</sup> )	19.6 $\pm$ 1.7 <sup>a</sup>	7.4 $\pm$ 0.4 <sup>b</sup>	20.8 $\pm$ 2.1 <sup>a</sup>	2.2 $\pm$ 0.2 <sup>c</sup>	4.4 $\pm$ 0.7 <sup>d</sup>	5.1 $\pm$ 0.8 <sup>d</sup>	< 0.001
Cd	(mg kg <sup>-1</sup> )	0.51 $\pm$ 0.02 <sup>a</sup>	0.27 $\pm$ 0.01 <sup>a</sup>	0.27 $\pm$ 0.01 <sup>a</sup>	0.23 $\pm$ 0.0 <sup>a</sup>	0.14 $\pm$ 0.01 <sup>ab</sup>	0.06 $\pm$ 0.01 <sup>b</sup>	0.042

*Abbreviations:* LB Libuše hut, VT Vítkovice v Krkonoších, PC Pec pod Sněžkou, HM Horní Mísečky, DCH Ramsau am Dachstein, MD Madesimo

HM. The Ni content ranged from 11.65 in DCH to 28 mg kg<sup>-1</sup> in HM. The total Pb content ranged from 13.2 in DCH to 70.8 mg kg<sup>-1</sup> in HM. The total Cd content ranged from 0.43 in DCH to 1.35 mg kg<sup>-1</sup> in HM.

Except for Cr, there was a significant effect of locality on the content of plant-available elements (Table 3). The available fraction of Fe ranged from 204 to 657 mg kg<sup>-1</sup> in DCH and HM, respectively. The plant-available content of Zn ranged from 5.1 in DCH to 62 mg kg<sup>-1</sup> in PC. The plant-available Cu ranged from 0.7 in HM to 7.9 mg kg<sup>-1</sup> in MD. The available Mn content ranged from 43 in MD to 189 mg kg<sup>-1</sup> in PC. The plant-available content of Al ranged from 648 to 1720 mg kg<sup>-1</sup> in PC and HM, respectively. The available As content ranged from 0.35 in DCH to 3.64 mg kg<sup>-1</sup> in LB. The plant-available Cr ranged from 0.12 in LB and HM to 0.31 mg kg<sup>-1</sup> in MD. The content of available Ni ranged from 0.34 in DCH to 1.85 mg kg<sup>-1</sup> in LB. The plant-available content of Pb ranged from 2.15 in HM to 21 mg kg<sup>-1</sup> in PC. Finally, the available content of Cd ranged from 0.06 in MD to 0.51 mg kg<sup>-1</sup> in LB. There was no significant correlation between the total and plant-available elements in the soils ( $r = 0.04$  to  $0.76$ ;  $p > 0.7$ ) and a negative relationship in the case of Cr ( $r = -0.7$ ;  $p = 0.11$ ) (see Table S1).

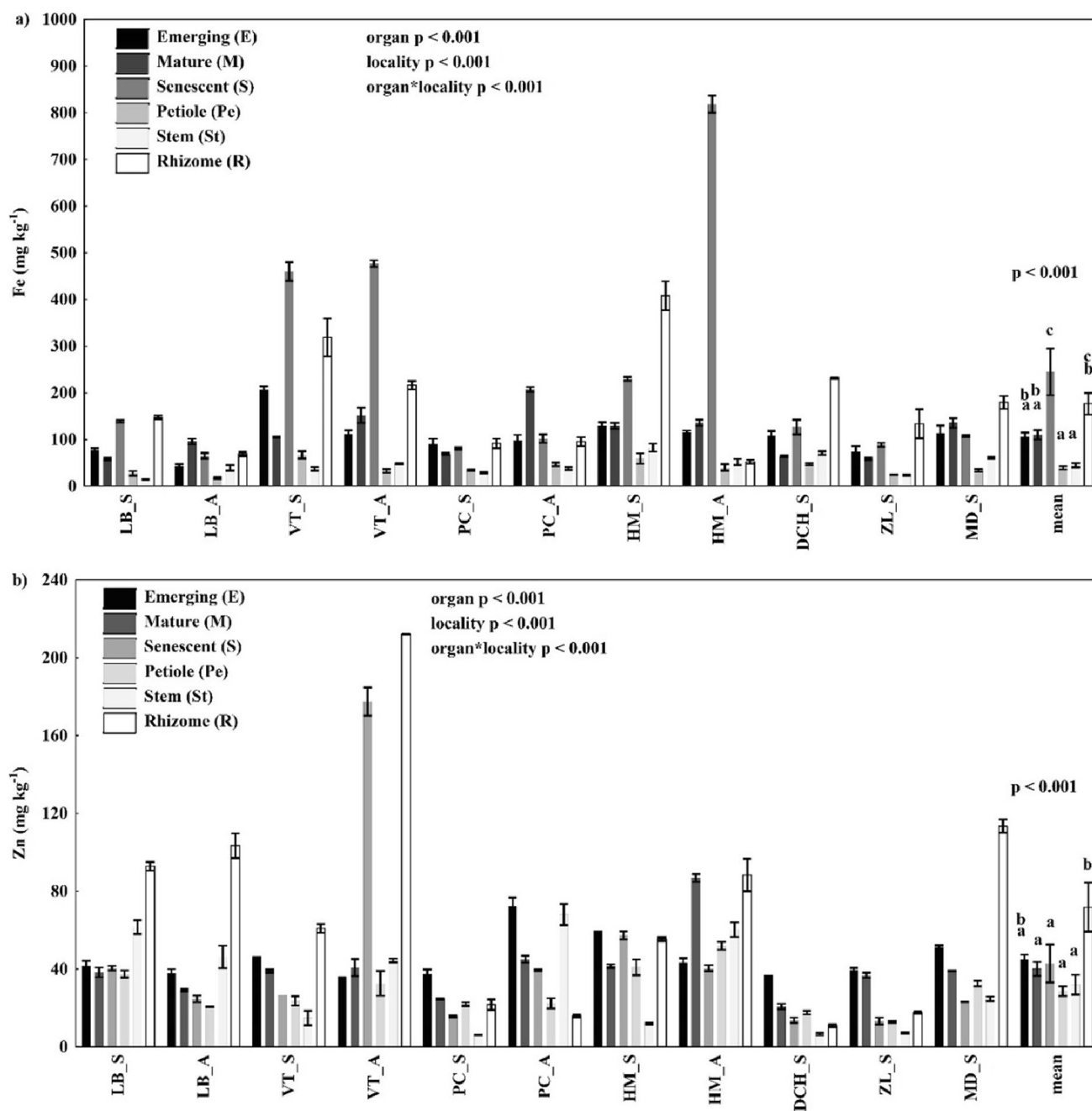
#### Content of total elements in plant organs

The contents of the elements in all analysed organs for all localities are given in Figs. 3, 4, 5 and 6. The

overall means of the studied elements are presented in Table 4. There was a significant effect of organ, locality, and organ/locality interaction on the content of all analyzed elements. In the Krkonoše Mountains, there was a significant effect of organs and seasons (summer-S and autumn-A) on the content of Fe, Cu, Mn, Al, As, and Cr, and vice versa in the case of Zn, Ni, Pb, and Cd (Figs. 7 and 8). The content of Fe ranged from 15 in the stem from LB\_S to 818 mg kg<sup>-1</sup> in senescent leaves at HM\_A. The mean Fe content in the organs for all localities was ranked Pe < St < E < M < R < S (Fig. 3a). The content of Zn ranged from 6 in stems from PC\_S to 212 mg kg<sup>-1</sup> in rhizomes from VT\_A. The mean Zn content in the organs for all localities and collection seasons was ranked Pe < St < S < M < E < R (Fig. 3b). The Cu content ranged from 0.7 in stems from PC\_A to 13.1 mg kg<sup>-1</sup> in emerging leaves from VT\_S. The mean Cu content in the organs for all localities and collection seasons was ranked St < Pe < S < R < M < E (Fig. 4a). The Mn content ranged from 4.5 in the stem from ZL to 322 mg kg<sup>-1</sup> in senescent leaves in MD. The mean Mn content in the organs for all localities and collection seasons was ranked St < R < Pe < E < M < S (Fig. 4b).

The content of Al ranged from 15 in emerging leaves to 1590 mg kg<sup>-1</sup> in the petiole from LB\_A and VT\_S. The mean Al content in the organs for all localities and collection seasons was ranked E < St < M < R < S < Pe (Fig. 5a). The content of As ranged from 0.009 in emerging leaves in ZL to 5 mg kg<sup>-1</sup> in senescent leaves in PC\_A. The mean As





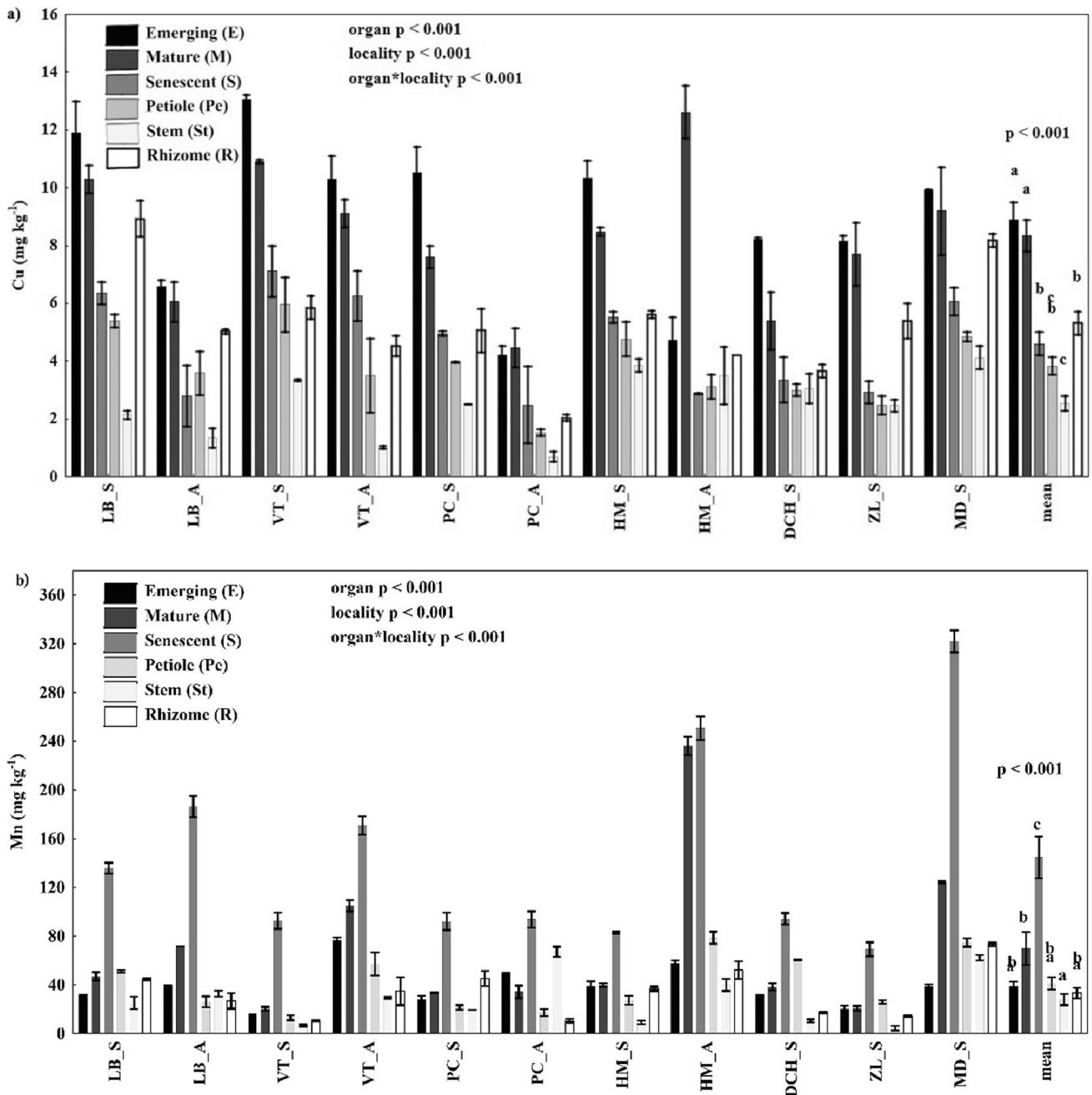
**Fig. 3** Effect of locality on the content (mean  $\pm$  SE) of (a) Fe and (b) Zn in different organs of *R. alpinus*. The  $p$  value was obtained by factorial ANOVA. The content of elements in individual organs' overall localities was evaluated by One-way ANOVA. Using the Tukey post hoc test, mean contents in sites with the same letter were not significantly different. Abbreviations of localities: LB\_S (Libuše hut\_Summer), LB\_A (Libuše

hut\_Autumn), VT\_S (Vítkovice v Krkonoších\_Summer), VT\_A (Vítkovice v Krkonoších\_Autumn), PC\_S (Pec pod Sněžkou\_Summer), PC\_A (Pec pod Sněžkou\_Autumn), HM\_S (Horní Mísečky\_Summer), HM\_A (Horní Mísečky\_Autumn), DCH\_S (Ramsau am Dachstein\_Summer), ZL\_S (Zillertal\_Summer), and MD\_S (Madesimo\_Summer)

content in the organs for all localities and collection seasons was ranked  $St < M < E < R < S < Pe$  (Fig. 5b). The level of Cr ranged from 0.06 in mature leaves in DCH to 6.6 mg kg<sup>-1</sup> in rhizome in ZL, and the mean

content in the organs for all localities and collection seasons was ranked  $Pe < E < M < S < St < R$  (Fig. 5c). The Ni content ranged from 0.01 in the stems of PC\_S to 6.6 mg kg<sup>-1</sup> in the rhizomes of VT\_A. The mean



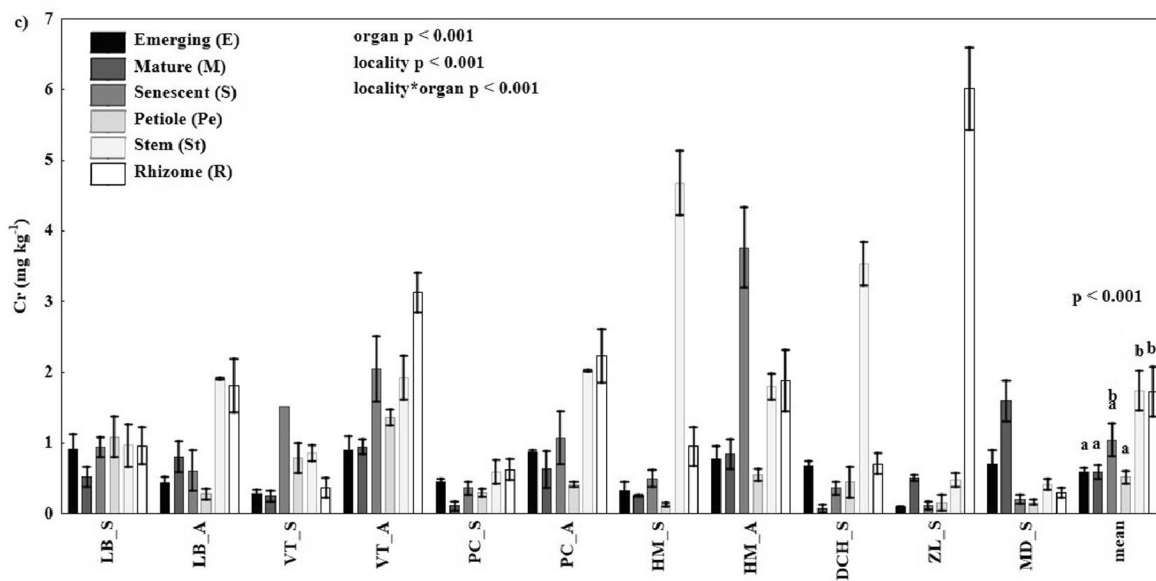
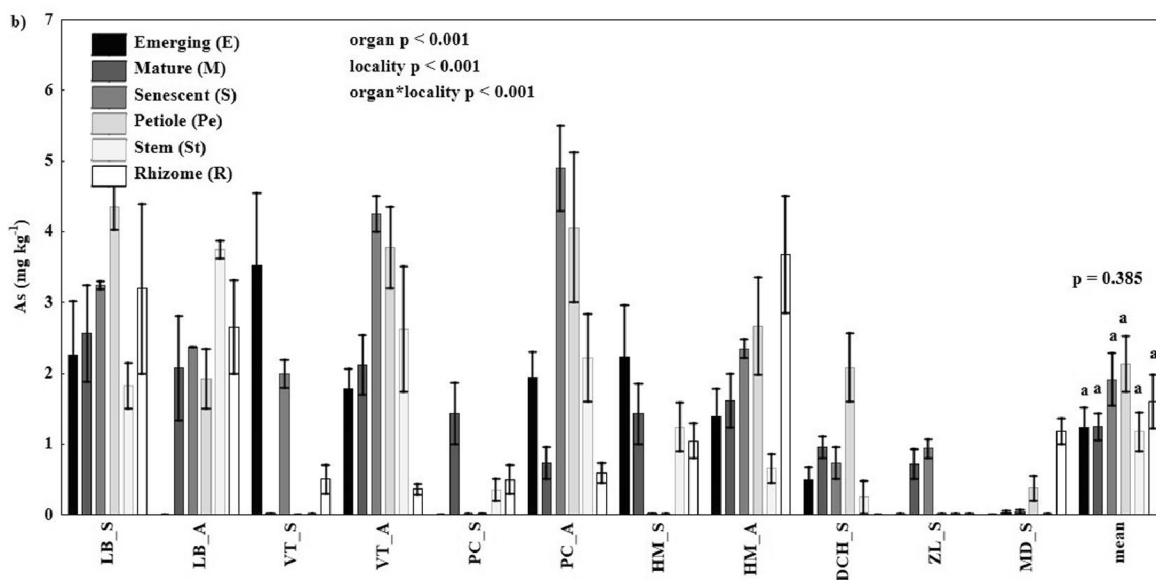
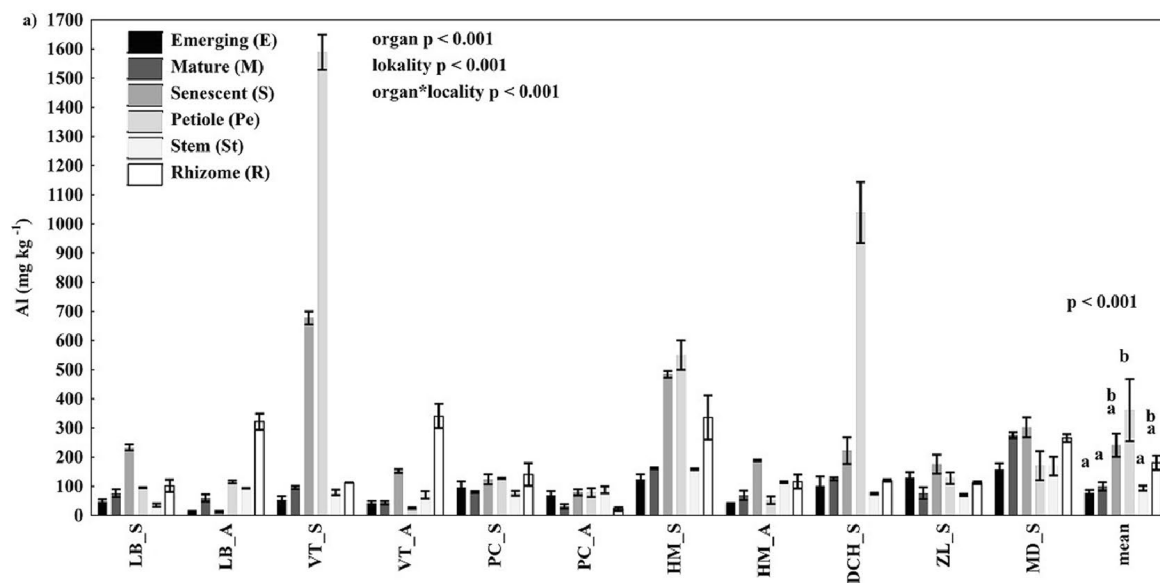


**Fig. 4** Effect of locality on the content (mean  $\pm$  SE) of (a) Cu and (b) Mn in different organs of *R. alpinus*. The  $p$  value was obtained by factorial ANOVA. The concentration of elements in individual organs' overall localities was evaluated by One-way ANOVA. - Using the Tukey post hoc test, mean contents in sites with the same letter were not significantly different. Abbreviations of localities: LB\_S (Libuše hut\_Summer),

LB\_A (Libuše hut\_Autumn), VT\_S (Vítkovice v Krkonoších\_Summer), VT\_A (Vítkovice v Krkonoších\_Autumn), PC\_S (Pec pod Sněžkou\_Summer), PC\_A (Pec pod Sněžkou\_Autumn), HM\_S (Horní Mísečky\_Summer), HM\_A (Horní Mísečky\_Autumn), DCH\_S (Ramsau am Dachstein\_Summer), ZL\_S (Zillertal\_Summer), and MD\_S (Madesimo\_Summer)

Ni content in the organs for all localities and collection seasons was ranked  $Pe < St < M < Se < E < R$  (Fig. 6a). The Pb content ranged from 0.001 in the petiole and rhizome in LB\_S to 8.2 mg kg<sup>-1</sup> in

senescent leaves in HM\_A. The mean Pb content in the organs for all localities and collection seasons was ranked  $R < M < Pe < St < S < E$  (Fig. 6b). The content of Cd in organs across the Alps localities was mostly



**Fig. 5** Effect of locality on the content (mean  $\pm$  SE) of (a) Al, (b) As, and (c) Cr in different organs of *R. alpinus*. The *p* value was obtained by factorial ANOVA. The content of elements in individual organs' overall localities was evaluated by One-way ANOVA. Using the Tukey post hoc test, mean contents in sites with the same letter were not significantly different. Abbreviations of localities: LB\_S (Libuše hut\_Summer), LB\_A (Libuše hut\_Autumn), VT\_S (Vítkovice v Krkonoších\_Summer), VT\_A (Vítkovice v Krkonoších\_Autumn), PC\_S (Pec pod Sněžkou\_Summer), PC\_A (Pec pod Sněžkou\_Autumn), HM\_S (Horní Mísečky\_Summer), HM\_A (Horní Mísečky\_Autumn), DCH\_S (Ramsau am Dachstein\_Summer), ZL\_S (Zillertal\_Summer), MD\_S (Madesimo\_Summer)

below detection except for the rhizome, which was 0.8 to 1 mg kg<sup>-1</sup> in Zl and MD, respectively. In the Krkonoše Mountains, the Cd content ranged from 0.1 in emerging leaves in VT\_S to 3.9 mg kg<sup>-1</sup> in rhizomes from VT\_A. The mean Cd content in the organs for all localities and collection seasons was ranked R < M < Pe < St < Se < E (Fig. 6c).

Bioaccumulation factor (BF) leaf  $\div$  soil, petiole  $\div$  soil, rhizome  $\div$  soil

The results of the BF for leaf/soil, petiole/soil, and rhizome/soil of micro- and risk elements are shown in Table 5. There was a significant effect on locality for all elements. The mean bioaccumulation factor for Zn, Cu, As, Cr, and Ni was >1 indicating that the *R. alpinus* can accumulate these elements, with different accumulation trends in each locality. The results of the mean BF for petiole/soil for Zn, Cu, As, Cr, Ni, and Cd were BF > 1. Similar results were measured for the mean BF for rhizome/soil, where Zn, Cu, As, Cr, Ni, and Cd were BF > 1.

TF (leaf  $\div$  rhizome)

The mean TF of Al, Cr, and Fe was <1 (Table 6). Only in PC\_A was the TF of Al above 1, and in MD, Cr was above 1. TF of Cd was <1 in all localities. The TF for Fe was above 1 in autumn in localities LB, PC, and HM. The TF of Cu, Mn, Ni, and Pb was mainly above 1.

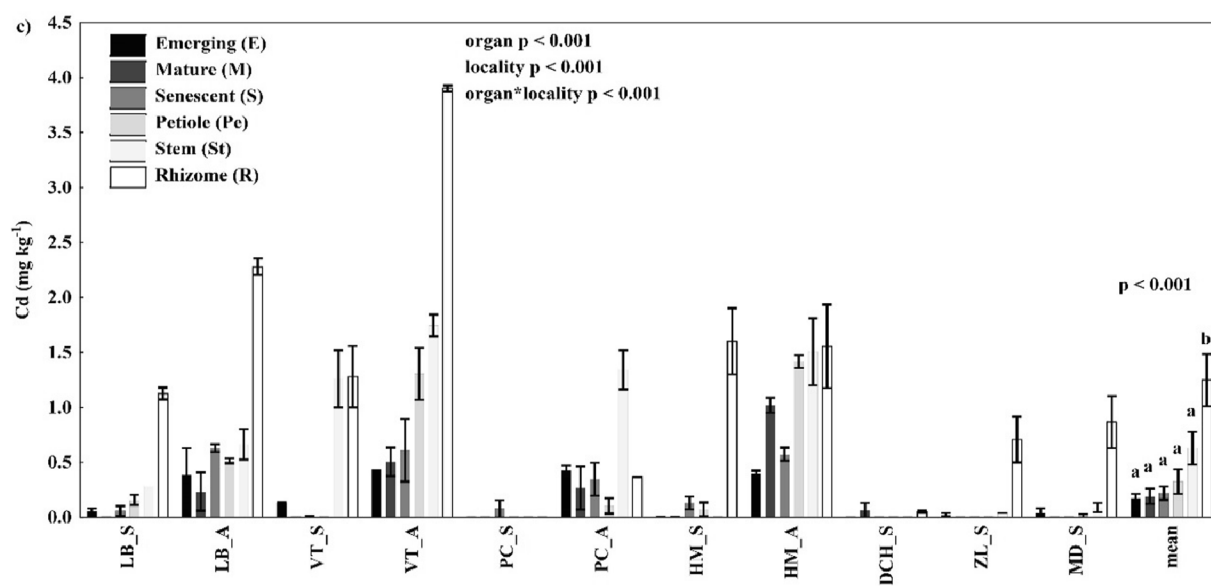
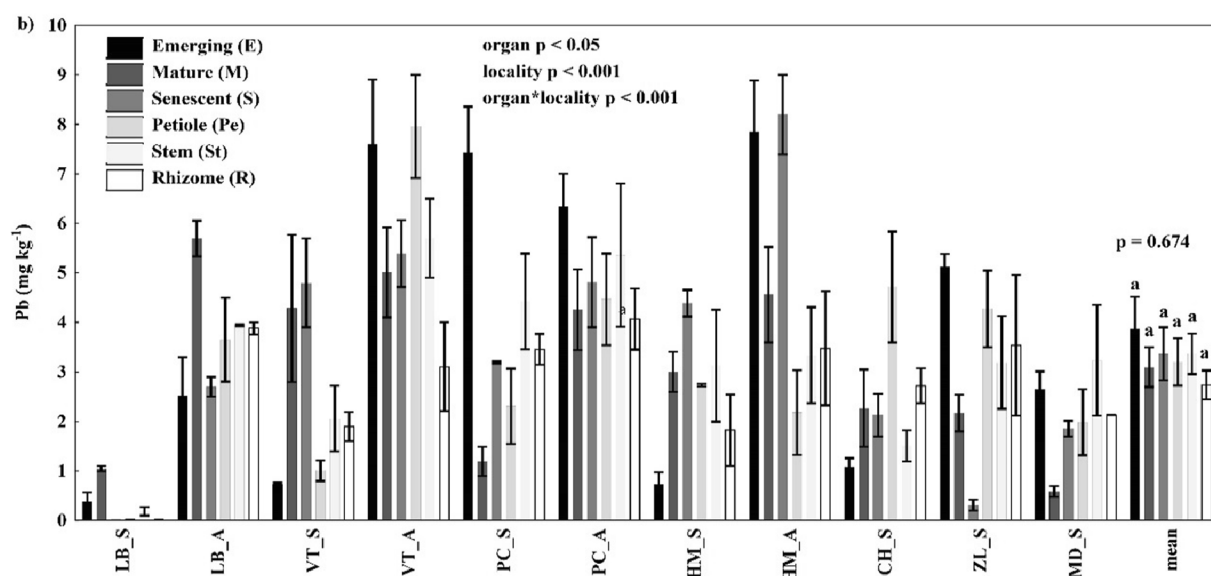
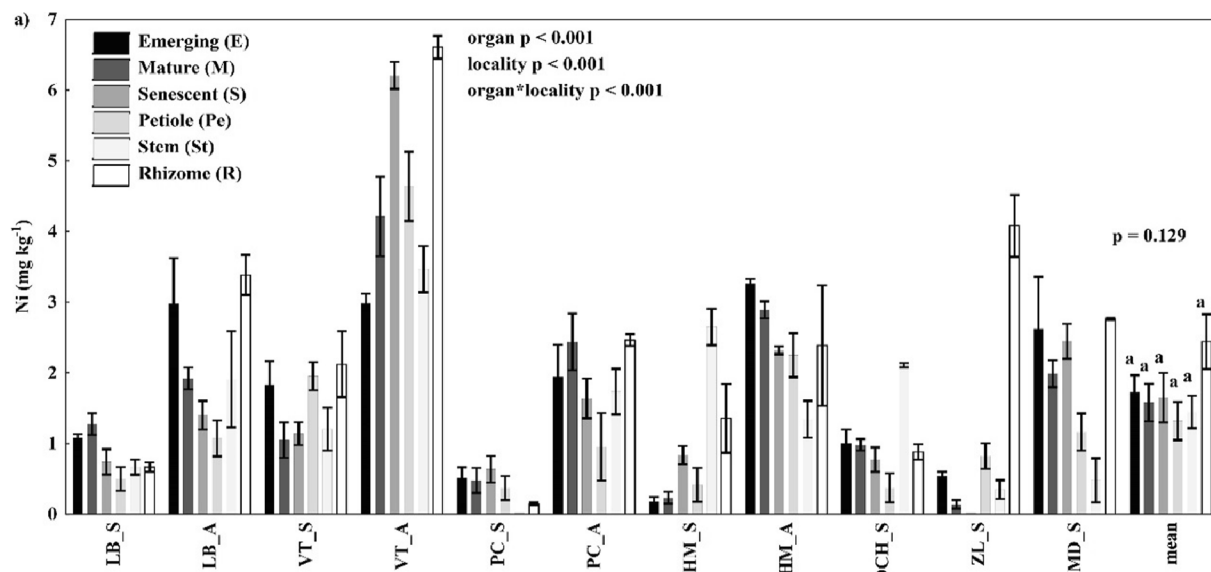
## Discussion

The main message of this study is that the edibility of *R. alpinus* can be questionable, considering

the accumulation and distribution of risk elements in different organs of this species. The levels of elements accumulation by *R. alpinus* are noticeably site-specific, accounted for by the differences in geological substrate, environmental condition, and the kind of anthropogenic activity. The release of trace/risk elements relates to lithogenic and anthropogenic sources, resulting in subsequent accumulation in different parts of *R. alpinus*. Moreover, the accumulation of risk elements such as As, Cr, Ni, Pb, and Cd was affected by seasons (ČHMÚ, 2018). In addition to acidic soil, dissolution by precipitation (H<sub>2</sub>O) during the autumn contributed to the release of elements (Truog 1947), reflected in higher contents in the organs during autumn compared with summer in localities of the Krkonoše Mountains.

## Chemical characterization of soil

The reduced soil acidity in all localities was due to the high contents of Ca and Mg resulting predominantly from the geological substrates. The acidity of the soils can contribute to the release of elements for plant uptake. The contents of total Zn and Cu were below the permissible limits (400 for Zn and 200 mg kg<sup>-1</sup> for Cu) for agricultural soils (Decree of the Ministry of the Environment No. 153/2016 Coll.). In recent years the average value of Zn in agricultural soils in the Czech Republic has ranged from 105 to 120 mg kg<sup>-1</sup> (Poláková et al. 2016). All localities, therefore, exceeded this average value in the Krkonoše Mountains. The high total content of Zn in the soils from the Krkonoše Mountains relates to the historic mining and smelting of Zn (Kafka 2003). According to Adriano (2001), the average content of Cu in agricultural soils ranges from 1 to 50 mg kg<sup>-1</sup> and corresponds with the investigated localities. At the Krkonoše and DCH localities, the total Mn content in the soil exceeded more than twice the average value according to Kabata-Pendias and Pendias (2001), which is 270 mg kg<sup>-1</sup> in podzol. The higher Mn is probably due to the acidic conditions of the soils, which can cause some extent of dissolution of total Mn content for subsequent absorption by the plants in different localities. Plant uptake of Mn is a function of the Mn oxidation state in the soil. At neutral or higher pH, Mn<sup>3+</sup> and Mn<sup>4+</sup> predominate, and insoluble Mn oxides will form (Rengel 2000; Marschner 2012). Thus, Mn shows some translocation





**Fig. 6** Effect of locality on the content (mean  $\pm$  SE) of (a) Ni, (b) Pb, and (c) Cd in different organs of *R. alpinus*. The *p* value was obtained by factorial ANOVA. The content of elements in individual organs' overall localities was evaluated by One-way ANOVA. Using the Tukey post hoc test, mean contents in sites with the same letter were not significantly different. Abbreviations of localities: LB\_S (Libuše hut\_Summer), LB\_A (Libuše hut\_Autumn), VT\_S (Vítkovice v Krkonoších\_Summer), VT\_A (Vítkovice v Krkonoších\_Autumn), PC\_S (Pec pod Sněžkou\_Summer), PC\_A (Pec pod Sněžkou\_Autumn), HM\_S (Horní Mísečky\_Summer), HM\_A (Horní Mísečky\_Autumn), DCH\_S (Ramsau am Dachstein\_Summer), ZL\_S (Zillertal\_Summer), MD\_S (Madesimo\_Summer)

restriction phenomenon, with the divalent form of Mn most efficiently accumulated in plants (Marschner 2012). Microorganisms can affect the solubility of Mn - by either reducing or oxidizing Mn, thereby affecting its availability to plants (Geszvain et al. 2012). Moreover, different plants have differing mechanisms in the uptake of elements resulting from their physiology and metabolic processes. The reduction in the solubility of Mn and its availability can also result from reduced soil moisture partly due to a lack of total precipitation (Porter et al. 2004; Vaněk et al. 2012; Fig. 7d), which was 50% lower than the average in August 2018 (<https://www.chmi.cz>). The total contents of Al, Cr, Ni, Pb, and Cd were within the reported range for most agricultural soils (Kabata-Pendias and Pendias 2001; Decree of the Ministry of the Environment No.153/2016 Coll.). In the localities of the Krkonoše Mountains, the contents of these elements were higher than in the Alps, which relate to past mining activities (Lokvenc 2003, 2007; Tásler 2012).

The Cr content in soils is directly related to parent rocks (geogenic elements), with an average value of 50 mg kg<sup>-1</sup> (Adriano 2001). The average total content in the Czech Republic is approximately 41 mg kg<sup>-1</sup> (Tásler 2012), which indicates that the limit was exceeded in VT, PC, and HM, resulting from anthropogenic activities. The average Ni content in the soil generally is considered to be approximately 20 mg kg<sup>-1</sup> (Adriano 2001), and the results show it again exceeded in the Krkonoše localities. Notably, contaminated agricultural areas in the Czech Republic have Ni values >200 mg kg<sup>-1</sup> (Poláková et al. 2016), which is the limit according to the Decree of the Ministry of the Environment No. 153/2016 Coll. and was not exceeded. The permissible limit of Pb in agricultural soils is 100 mg kg<sup>-1</sup> (Kabata-Pendias and

Pendias 2001); there was no exceedance of Pb levels in the examined localities. For comparison, the Czech limit for soils with a pH  $\leq$  of 6.5 is 300 mg kg<sup>-1</sup> (Decree of the Ministry of the Environment No. 153/2016 Coll.). The As contents were above the Czech legislative (Decree of the Ministry of the Environment CZ No. 153/2016) limit of 40 mg kg<sup>-1</sup> at LB, HM, and MD, indicating a risk to the safety of food or feed, direct danger to human or animal health in contact with this soil, and a negative impact on the production function of agricultural land. In the Krkonoše localities, the soils remain contaminated by As, probably due to arsenopyrite mining in the past (Tásler 2012).

According to the regression model, there was no pattern between the content of the total micro/risk elements and plant-available portions of the same elements (Table S1). Even though there were good correlation coefficients, which indicate possible relations, none recorded a significant relationship. Hence, the content of each fraction of the studied elements is independent.

Distribution of Fe, Zn, Cu, Mn, Al, As, Cr, Ni, Pb, and Cd in the organs

The amount of risk elements extracted depends on the contents in the harvestable parts of plants and other plant biomass. The mean contents of Fe, Zn, Cu, and Mn in all organs of *R. alpinus* were found to be relatively low and within the ranges of geochemical background values given by Kabata-Pendias and Pendias (2001). The variability in the content of microelements in the organs of *R. alpinus* relates to compartmentalization and translocation in the vascular system (Hänsch and Mendel 2009; Vondráčková et al. 2014). Zinc and Fe ions enter the plant xylem through the symplastic pathway (Olsen and Palmgren 2014; She et al. 2018). The content of Zn was highest in the rhizome, consistent with the findings of Bohner (2005). Moreover, the content of Fe was lowest in the stem, which is consistent with studies by Gaweda (2009) and Vondráčková et al. (2014). The highest content of Fe was in senescent leaves, and this finding corresponds with a study by Bohner (2005). The same pattern as Fe was exhibited in the case of Mn compared with a study by Bohner (2005), who reported an elevated content of Mn in mature leaves of *R. alpinus*. We recorded the lowest content of Mn in mature

**Table 4** The mean values of individual organs are described in the graphs of individual elements. Content of total elements (Mean  $\pm$  SE) in organs of *R. alpinus*, and these numbers correspond to columns of mean values in Figs. 3, 4, 5 and 6. The *p* value for each element was obtained by one-way ANOVA. Using Tukey (HSD) post hoc test, the content of the same element with the same letters for organs was not significantly different

Elements	Emerging leaves	Mature leaves	Senescent leaves	Petiole	Stem	Rhizome	<i>F</i> -value	<i>P</i> value	Normal value *
Fe (mg kg <sup>-1</sup> )	106 $\pm$ 8.8 <sup>bc</sup>	110 $\pm$ 10 <sup>bc</sup>	245 $\pm$ 50 <sup>a</sup>	39.2 $\pm$ 3.3 <sup>c</sup>	45 $\pm$ 4.4 <sup>c</sup>	177 $\pm$ 23 <sup>ab</sup>	11.5	< 0.001	30 – 300 <sup>(3)</sup>
Zn (mg kg <sup>-1</sup> )	45.3 $\pm$ 2.4 <sup>ab</sup>	40.1 $\pm$ 3.6 <sup>b</sup>	42.9 $\pm$ 9.1 <sup>b</sup>	28.6 $\pm$ 2.5 <sup>b</sup>	32 $\pm$ 5.1 <sup>b</sup>	75 $\pm$ 12.5 <sup>a</sup>	4.6	< 0.001	10 – 150 <sup>(2)</sup>
Cu (mg kg <sup>-1</sup> )	8.9 $\pm$ 0.6 <sup>a</sup>	8.3 $\pm$ 0.5 <sup>a</sup>	4.6 $\pm$ 0.4 <sup>b</sup>	3.8 $\pm$ 0.3 <sup>bc</sup>	2.5 $\pm$ 0.3 <sup>c</sup>	5.3 $\pm$ 0.4 <sup>b</sup>	33.8	< 0.001	4 – 15 <sup>(6)</sup>
Mn (mg kg <sup>-1</sup> )	38.8 $\pm$ 3.6 <sup>cd</sup>	17.1 $\pm$ 13.5 <sup>b</sup>	145 $\pm$ 17 <sup>a</sup>	41.3 $\pm$ 5.0 <sup>cd</sup>	27.9 $\pm$ 4.5 <sup>c</sup>	33.3 $\pm$ 4.3 <sup>cd</sup>	21.7	< 0.001	40 – 200 <sup>(4)</sup>
Al (mg kg <sup>-1</sup> )	79 $\pm$ 10 <sup>b</sup>	99.8 $\pm$ 14.5 <sup>b</sup>	214 $\pm$ 40 <sup>ab</sup>	361 $\pm$ 106 <sup>a</sup>	94 $\pm$ 8.7 <sup>b</sup>	181 $\pm$ 24 <sup>ab</sup>	5.3	< 0.001	–
As (mg kg <sup>-1</sup> )	1.2 $\pm$ 0.3 <sup>a</sup>	1.2 $\pm$ 0.2 <sup>a</sup>	1.9 $\pm$ 0.4 <sup>a</sup>	1.8 $\pm$ 0.4 <sup>a</sup>	1.2 $\pm$ 0.3 <sup>a</sup>	1.2 $\pm$ 0.3 <sup>a</sup>	1.6	0.173	1 – 1.7 <sup>(1)</sup>
Cr (mg kg <sup>-1</sup> )	0.6 $\pm$ 0.1 <sup>b</sup>	0.6 $\pm$ 0.1 <sup>b</sup>	1.0 $\pm$ 0.2 <sup>ab</sup>	0.5 $\pm$ 0.1 <sup>b</sup>	1.7 $\pm$ 0.3 <sup>a</sup>	1.7 $\pm$ 0.4 <sup>a</sup>	7.0	< 0.001	0.1 – 0.5 <sup>(1)</sup>
Ni (mg kg <sup>-1</sup> )	1.7 $\pm$ 0.2 <sup>a</sup>	1.6 $\pm$ 0.3 <sup>a</sup>	1.6 $\pm$ 0.4 <sup>a</sup>	1.3 $\pm$ 0.3 <sup>a</sup>	1.4 $\pm$ 0.2 <sup>a</sup>	2.4 $\pm$ 0.4 <sup>a</sup>	1.8	0.128	0.5 – 5 <sup>(5)</sup>
Pb (mg kg <sup>-1</sup> )	3.9 $\pm$ 0.7 <sup>a</sup>	3.1 $\pm$ 0.4 <sup>a</sup>	3.4 $\pm$ 0.5 <sup>a</sup>	3.2 $\pm$ 0.5 <sup>a</sup>	3.3 $\pm$ 0.4 <sup>a</sup>	2.7 $\pm$ 0.3 <sup>a</sup>	0.6	0.674	0.5 – 10 <sup>(2)</sup>
Cd (mg kg <sup>-1</sup> )	0.17 $\pm$ 0.04 <sup>b</sup>	0.19 $\pm$ 0.07 <sup>b</sup>	0.22 $\pm$ 0.06 <sup>b</sup>	0.33 $\pm$ 0.11 <sup>b</sup>	0.63 $\pm$ 0.15 <sup>b</sup>	1.25 $\pm$ 0.24 <sup>a</sup>	10.5	< 0.001	0.05 – 2 <sup>(2)</sup>

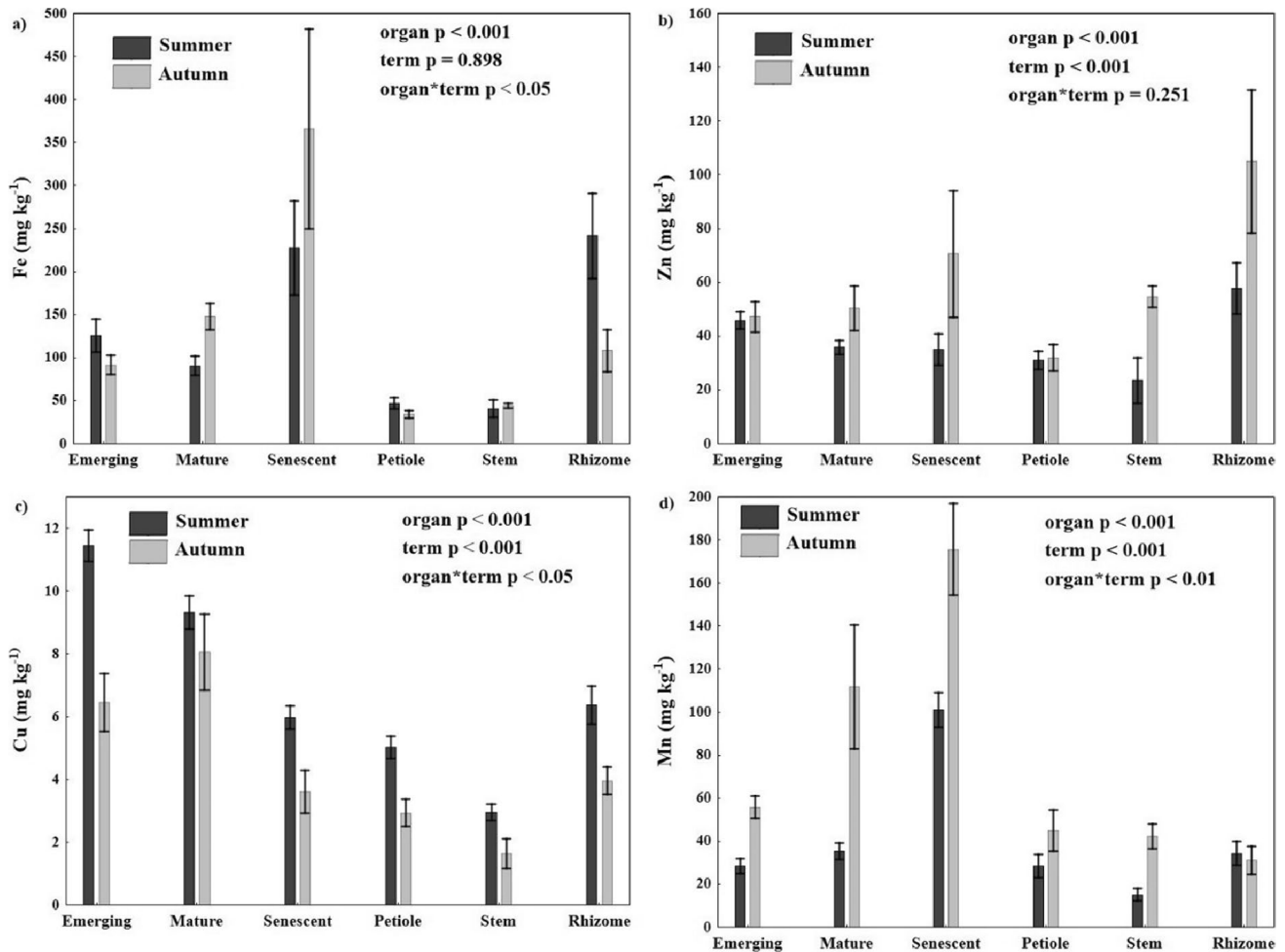
\*—no material; 1 adapted from Kabata-Pendias and Pendias (2001); 2—adapted from Pugh et al. (2002); 3—adapted from Levy et al. (1999); 4—Mahler (2004); 5—Allen (1989); 6—adapted from Güleriyüz et al. (2016)

leaves and the highest in senescent leaves. Gaweda (2009) reported the highest contents of Fe and Mn in *Rumex acetosa* in belowground biomass.

Nonetheless, the Fe and Mn contents in the senescent leaves were higher than those in the other organs, suggesting that the plant was probably supersaturated (Baker 1981). However, the Fe and Mn contents in the leaves (emerging and mature leaves) were within the normal recorded range, according to Levy et al. (1999) and Mahler (2004). A different distribution of Cu was found in emerging and mature leaves, which was not consistent with the distribution of Cu in the belowground organs of *R. acetosa* (Gaweda 2009). The higher content of Cu in the emerging and mature leaves explains why the higher content of Cu localizes in the growing section of the tissues and chloroplasts, respectively (Vaněk et al. 2012). However, the amount of these microelements in *R. alpinus* organs was consistent with the normal values measured by Levy et al. (1999), Kabata-Pendias and Pendias (2001), Pugh et al. (2002), Mahler (2004), Güleriyüz et al. (2016).

Several studies have investigated plants with mechanisms to tolerate high contents of Al (Tolrà et al. 2005; Arunakumara et al. 2013; Vondráčková et al. 2015). The mean total content of Al was lowest in emerging leaves and highest in petioles, followed by rhizomes, due to low transport from belowground organs to leaves: a defensive mechanism against a high content of Al in plants (Poschenrieder et al. 2008). In comparison with an experiment by Vondráčková et al. (2015), concerning *Rumex obtusifolius*, the recorded values in the organs of this study were low, which indicates that *R. alpinus* prevents the intake of Al.

Arsenic occurs naturally in the environment and is present in the soil, groundwater, and plants. The main known side effects associated with long-term intake of inorganic arsenic in humans are skin lesions, cancer, neurotoxicity, cardiovascular disease, and diabetes. Therefore, this element is monitored in plants/feed based on the recommendation of the European Commission (EU) 2015/138). The mean total content of As in individual organs ranged from 1 to 1.8 mg kg<sup>-1</sup>, which slightly exceeds the limit according to Kabata-Pendias and Pendias (2001) but did not exceed the limit according to Commission Regulation (EU) No. 1275/2013, which is 2–10 mg kg<sup>-1</sup>. For comparison, the Environmental Protection Agency (EPA, 2021)

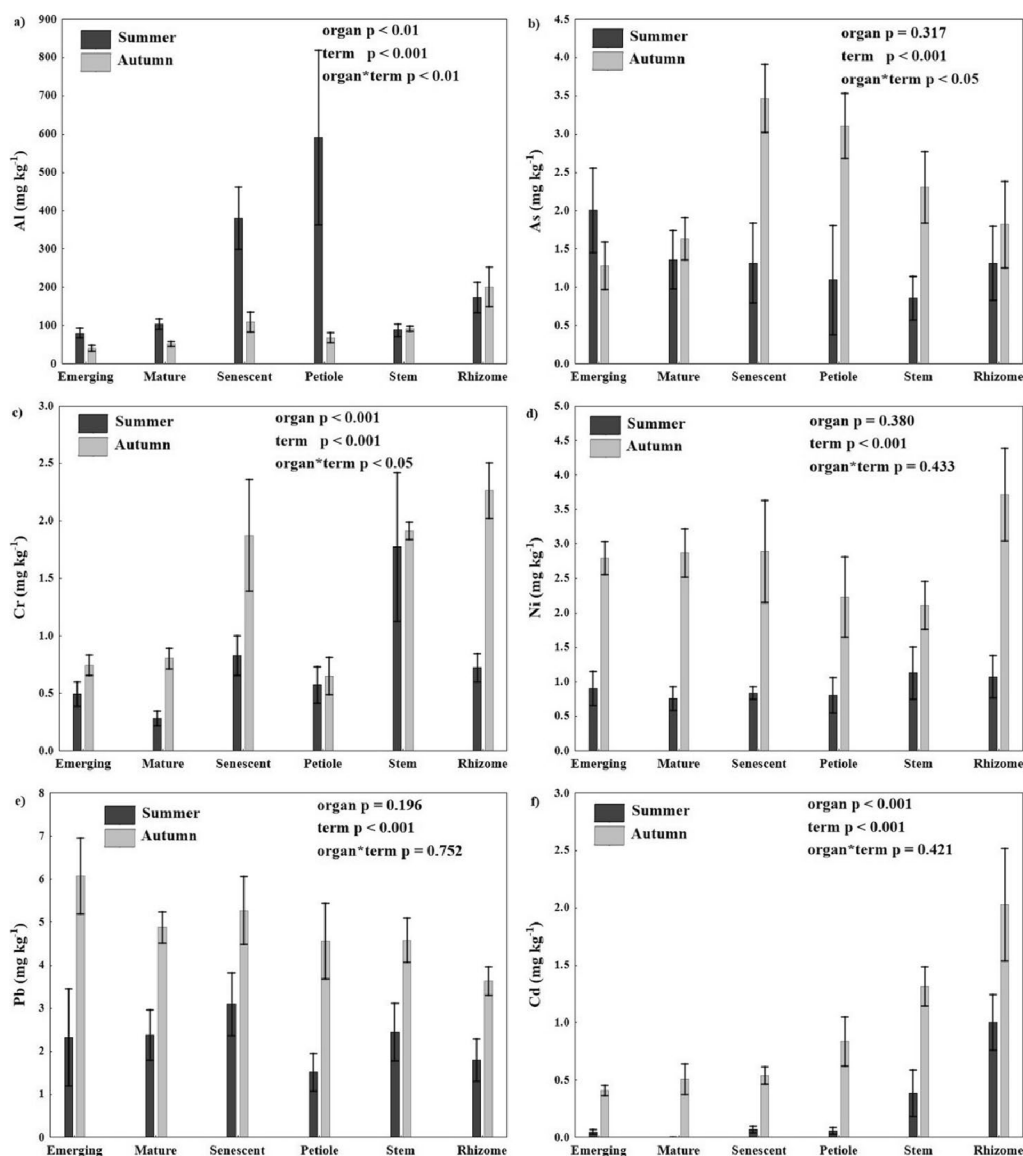


**Fig. 7** Effect of term on the content (mean  $\pm$  SE) of (a) Fe, (b) Zn, (c) Cu, and (d) Mn in different organs of *Rumex alpinus*, collected in the Krkonoše Mountains. The  $p$  value was obtained by factorial ANOVA

allows maximum level limits of arsenic in US drinking water to 10 micrograms per liter ( $\mu\text{g l}^{-1}$ ). Based on current data, the reference maximum daily dose for cancer risk from arsenic is estimated to be between 3.7 and 10.7  $\text{mg kg}^{-1}$ . Although *R. alpinus* grows mainly on slightly arsenic-contaminated soils, it is evident that As accumulates in the plant only in very small amounts. For comparison, according to the World Health Organization (2001), some terrestrial species accumulate concentrations of up to 3000  $\text{mg kg}^{-1}$  at arsenic mine sites. Moreover, the As content in the leaves was lower and higher in petiole and senescent leaves, which indicates a tendency for increased transport of As from protected metabolic organs away from the plant (Kee et al. 2018).

The mean total content of Cr in *R. alpinus* organs in this study was lower than that in *R. obtusifolius*,

according to Vondráčková et al. (2014). While their results showed the highest Cr content in the leaves, we obtained the lowest Cr content in emerging and mature leaves and the highest Cr content in stems and rhizomes. However, these results are comparable to those of Gaweda (2009), who studied *R. acetosa* under natural conditions. Notably, the different results relate to the species, anthropogenic activities, and soil chemical properties to varying degrees. In any case, the amount of this element in the examined organs of *R. alpinus* was very low from the point of view of human health. A chronic oral reference dose of 0.003  $\text{mg kg}^{-1}$  of body weight per day for Cr(VI) was established by the US EPA based on organ changes in rat studies. According to the Centre for Food Safety in Hong Kong (2011), a regulatory level of 1 ppm of total chromium in vegetables would not pose adverse effects on



**Fig. 8** Effect of term on the content (mean  $\pm$  SE) of (a) Al, (b) As, (c) Cr (d), Ni, (e) Pb, and (f) Cd in different organs of *R. alpinus*, collected in the Krkonoše Mountains. The  $p$  value was obtained by factorial ANOVA

public health. For comparison, according to the World Health Organization, the highest dose is equivalent to 1210 mg kg<sup>-1</sup> Cr(III) of body weight per day.

Pb is usually taken up by plants from air pollution because of anthropogenic activities (Kabata-Pendias and Pendias 2001). Additionally, Pb intake can pose a serious risk to public health. Lead can reduce intellectual performance in children, and in adults, Pb can lead to cardiovascular disease (Wani et al. 2015). The mean total contents of Pb and Ni in all organs were approximately the same and within the normal range (Pugh et al. 2002). Compared with *R. acetosa*, *Rumex*

*crispus*, and *Rumex* K-1 (Zhuang et al. 2007; Gaweda 2009), where the highest content of Pb was in below-ground organs, we found Pb to be higher in above-ground organs. According to Pawlak et al. (2007), Pb accumulates mainly in roots with an affinity to galacturonic acid. However, for safe consumption of the organs of the studied species, a limit of 3 mg kg<sup>-1</sup> for consumable plants was set by the Commission Regulation (EC) No. 629/ 2008. However, the value was exceeded in the aboveground organs of *R. alpinus*, especially in the emerging leaves. Nevertheless, according to Directive 2002/32/EC of the European



**Table 5** Bioaccumulation factor (Mean  $\pm$  SE) of elements in the studied sites. The *p* value for each element was obtained by one-way ANOVA. Using Tukey (HSD) post hoc test, values with the same letters for each element among the localities are not significantly different. The asterisk indicates \* *p* < 0.01

Locality	Fe*	Zn*	Cu*	Mn*	Al*	As*	Cr*	Ni*	Pb*	Cd*
LB_S_leaves	0.17 $\pm$ 0.003 <sup>a</sup>	1.01 $\pm$ 0.001 <sup>a</sup>	0.7 $\pm$ 0.02 <sup>a</sup>	0.24 $\pm$ 0.01 <sup>abc</sup>	0.07 $\pm$ 0.01 <sup>a</sup>	0.66 $\pm$ 0.01 <sup>a</sup>	6.09 $\pm$ 0.31 <sup>ab</sup>	0.64 $\pm$ 0.03 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.06 $\pm$ 0.02 <sup>ab</sup>
LB_S_petiole	0.07 $\pm$ 0.01 <sup>ab</sup>	0.95 $\pm$ 0.05 <sup>ab</sup>	0.35 $\pm$ 0.01 <sup>a</sup>	0.31 $\pm$ 0.01 <sup>ab</sup>	0.1 $\pm$ 0 <sup>ab</sup>	1.2 $\pm$ 0.1 <sup>a</sup>	9 $\pm$ 2.4 <sup>ab</sup>	0.27 $\pm$ 0.09 <sup>a</sup>	0 $\pm$ 0 <sup>a</sup>	0.31 $\pm$ 0.1 <sup>a</sup>
LB_S_rhizome	0.37 $\pm$ 0.01 <sup>ab</sup>	2.4 $\pm$ 0.1 <sup>ab</sup>	0.58 $\pm$ 0.04 <sup>ac</sup>	0.27 $\pm$ 0.004 <sup>a</sup>	0.11 $\pm$ 0.02 <sup>acd</sup>	0.87 $\pm$ 0.33 <sup>a</sup>	8.1 $\pm$ 2.2 <sup>abcd</sup>	0.36 $\pm$ 0.04 <sup>ac</sup>	0 $\pm$ 0 <sup>a</sup>	2.2 $\pm$ 0.1 <sup>a</sup>
LB_A_leaves	0.17 $\pm$ 0.01 <sup>a</sup>	0.85 $\pm$ 0.02 <sup>ab</sup>	0.4 $\pm$ 0.03 <sup>a</sup>	0.34 $\pm$ 0.001 <sup>b</sup>	0.04 $\pm$ 0.01 <sup>a</sup>	0.29 $\pm$ 0.1 <sup>a</sup>	5.25 $\pm$ 1.2 <sup>abc</sup>	1.3 $\pm$ 0.13 <sup>ab</sup>	0.21 $\pm$ 0.03 <sup>a</sup>	0.62 $\pm$ 0.41 <sup>abc</sup>
LB_A_petiole	0.04 $\pm$ 0.004 <sup>a</sup>	0.52 $\pm$ 0.003 <sup>a</sup>	0.23 $\pm$ 0.05 <sup>a</sup>	0.16 $\pm$ 0.03 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>ab</sup>	0.52 $\pm$ 0.12 <sup>a</sup>	2.3 $\pm$ 0.7 <sup>bc</sup>	0.58 $\pm$ 0.13 <sup>a</sup>	0.19 $\pm$ 0.04 <sup>abc</sup>	1 $\pm$ 0.04 <sup>a</sup>
LB_A_rhizome	0.17 $\pm$ 0.01 <sup>bc</sup>	2.6 $\pm$ 0.2 <sup>ab</sup>	0.33 $\pm$ 0.01 <sup>a</sup>	0.17 $\pm$ 0.04 <sup>a</sup>	0.35 $\pm$ 0.03 <sup>bc</sup>	0.73 $\pm$ 0.18 <sup>a</sup>	15 $\pm$ 3.2 <sup>bcd</sup>	1.8 $\pm$ 0.15 <sup>abc</sup>	0.2 $\pm$ 0.01 <sup>a</sup>	4.5 $\pm$ 0.1 <sup>a</sup>
VT_S_leaves	0.3 $\pm$ 0.01 <sup>bc</sup>	3.9 $\pm$ 0.06 <sup>c</sup>	2.8 $\pm$ 0.03 <sup>a</sup>	0.18 $\pm$ 0.01 <sup>ac</sup>	0.05 $\pm$ 0.003 <sup>a</sup>	4.91 $\pm$ 1.4 <sup>bc</sup>	1.32 $\pm$ 0.04 <sup>c</sup>	1.1 $\pm$ 0.3 <sup>ab</sup>	0.34 $\pm$ 0.1 <sup>a</sup>	0.24 $\pm$ 0.02 <sup>ab</sup>
VT_S_petiole	0.13 $\pm$ 0.01 <sup>bcd</sup>	2.2 $\pm$ 0.2 <sup>bcd</sup>	1.4 $\pm$ 0.22 <sup>a</sup>	0.13 $\pm$ 0.02 <sup>a</sup>	1.1 $\pm$ 0.04 <sup>c</sup>	0.01 $\pm$ 0 <sup>a</sup>	4 $\pm$ 1 <sup>abc</sup>	1.5 $\pm$ 0.15 <sup>ab</sup>	0.14 $\pm$ 0.03 <sup>ab</sup>	0 $\pm$ 0 <sup>a</sup>
VT_S_rhizome	0.6 $\pm$ 0.08 <sup>de</sup>	6 $\pm$ 0.2 <sup>c</sup>	1.4 $\pm$ 0.1 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.08 $\pm$ 0 <sup>acd</sup>	1.4 $\pm$ 0.57 <sup>a</sup>	1.83 $\pm$ 0.7 <sup>a</sup>	1.6 $\pm$ 0.35 <sup>abc</sup>	0.26 $\pm$ 0.04 <sup>a</sup>	4.7 $\pm$ 1 <sup>a</sup>
VT_A_leaves	0.25 $\pm$ 0.02 <sup>abc</sup>	3.6 $\pm$ 0.2 <sup>c</sup>	2.3 $\pm$ 0.15 <sup>a</sup>	0.92 $\pm$ 0.01 <sup>d</sup>	0.03 $\pm$ 0.004 <sup>a</sup>	5.42 $\pm$ 0.97 <sup>c</sup>	4.63 $\pm$ 0.8 <sup>abc</sup>	2.7 $\pm$ 0.16 <sup>c</sup>	0.86 $\pm$ 0.03 <sup>b</sup>	1.7 $\pm$ 0.22 <sup>c</sup>
VT_A_petiole	0.06 $\pm$ 0.01 <sup>a</sup>	3.1 $\pm$ 0.6 <sup>cd</sup>	0.82 $\pm$ 0.3 <sup>a</sup>	0.57 $\pm$ 0.09 <sup>b</sup>	0.02 $\pm$ 0.002 <sup>a</sup>	10 $\pm$ 1.6 <sup>c</sup>	7 $\pm$ 0.6 <sup>ab</sup>	3.5 $\pm$ 0.36 <sup>c</sup>	1.08 $\pm$ 0.14 <sup>cd</sup>	5 $\pm$ 0.9 <sup>b</sup>
VT_A_rhizome	0.41 $\pm$ 0.02 <sup>ad</sup>	20 $\pm$ 0.01 <sup>d</sup>	1.1 $\pm$ 0.1 <sup>bc</sup>	0.35 $\pm$ 0.12 <sup>ab</sup>	0.23 $\pm$ 0.03 <sup>bc</sup>	1.01 $\pm$ 0.21 <sup>a</sup>	15 $\pm$ 1.4 <sup>bcd</sup>	5 $\pm$ 0.12 <sup>d</sup>	0.42 $\pm$ 0.12 <sup>a</sup>	14 $\pm$ 0.1 <sup>b</sup>
PC_S_leaves	0.31 $\pm$ 0.03 <sup>c</sup>	0.5 $\pm$ 0.02 <sup>b</sup>	1.5 $\pm$ 0.11 <sup>a</sup>	0.16 $\pm$ 0.01 <sup>a</sup>	0.14 $\pm$ 0.02 <sup>bc</sup>	0.65 $\pm$ 0.2 <sup>a</sup>	2.24 $\pm$ 0.11 <sup>ac</sup>	0.5 $\pm$ 0.01 <sup>a</sup>	0.21 $\pm$ 0.03 <sup>a</sup>	0.001 $\pm$ 0 <sup>a</sup>
PC_S_petiole	0.13 $\pm$ 0.01 <sup>cd</sup>	0.35 $\pm$ 0.01 <sup>a</sup>	0.68 $\pm$ 0 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>	0.2 $\pm$ 0.002 <sup>ab</sup>	0.2 $\pm$ 0 <sup>a</sup>	2.4 $\pm$ 0.5 <sup>c</sup>	0.36 $\pm$ 0.16 <sup>a</sup>	0.11 $\pm$ 0.04 <sup>ab</sup>	0 $\pm$ 0 <sup>a</sup>
PC_S_rhizome	0.35 $\pm$ 0.04 <sup>ab</sup>	0.35 $\pm$ 0.04 <sup>c</sup>	0.86 $\pm$ 0.13 <sup>abc</sup>	0.24 $\pm$ 0.03 <sup>a</sup>	0.22 $\pm$ 0.06 <sup>abc</sup>	0.45 $\pm$ 0.18 <sup>a</sup>	5 $\pm$ 1.16 <sup>abc</sup>	0.14 $\pm$ 0.02 <sup>a</sup>	0.17 $\pm$ 0.01 <sup>a</sup>	0 $\pm$ 0 <sup>a</sup>
PC_A_leaves	0.59 $\pm$ 0.03 <sup>d</sup>	0.95 $\pm$ 0.05 <sup>a</sup>	0.74 $\pm$ 0.1 <sup>a</sup>	0.22 $\pm$ 0.01 <sup>ac</sup>	0.08 $\pm$ 0.02 <sup>ab</sup>	1.20 $\pm$ 0.3 <sup>a</sup>	6.03 $\pm$ 0.9 <sup>ab</sup>	2.1 $\pm$ 0.4 <sup>bc</sup>	0.25 $\pm$ 0.04 <sup>a</sup>	1.29 $\pm$ 0.3 <sup>bc</sup>
PC_A_petiole	0.18 $\pm$ 0.02 <sup>de</sup>	0.36 $\pm$ 0.04 <sup>a</sup>	0.26 $\pm$ 0.02 <sup>a</sup>	0.09 $\pm$ 0.02 <sup>a</sup>	0.12 $\pm$ 0.02 <sup>ab</sup>	3.7 $\pm$ 0.96 <sup>ab</sup>	3.3 $\pm$ 0.3 <sup>bc</sup>	0.92 $\pm$ 0.46 <sup>a</sup>	0.21 $\pm$ 0.04 <sup>abc</sup>	0.39 $\pm$ 0.3 <sup>a</sup>
PC_A_rhizome	0.37 $\pm$ 0.04 <sup>a</sup>	0.25 $\pm$ 0.01 <sup>c</sup>	0.35 $\pm$ 0.02 <sup>a</sup>	0.06 $\pm$ 0.01 <sup>a</sup>	0.04 $\pm$ 0.01 <sup>d</sup>	0.53 $\pm$ 0.12 <sup>a</sup>	18 $\pm$ 3 <sup>d</sup>	2.4 $\pm$ 0.1 <sup>abc</sup>	0.2 $\pm$ 0.03 <sup>a</sup>	1.4 $\pm$ 0.01 <sup>a</sup>
HM_S_leaves	0.2 $\pm$ 0.001 <sup>abc</sup>	2 $\pm$ 0.02 <sup>d</sup>	14 $\pm$ 0.56 <sup>b</sup>	0.67 $\pm$ 0.03 <sup>e</sup>	0.08 $\pm$ 0.004 <sup>ab</sup>	2.40 $\pm$ 0.2 <sup>abc</sup>	2.53 $\pm$ 0.5 <sup>abc</sup>	0.27 $\pm$ 0.1 <sup>a</sup>	0.87 $\pm$ 0.15 <sup>b</sup>	0.01 $\pm$ 0.003 <sup>a</sup>
HM_S_petiole	0.09 $\pm$ 0.02 <sup>abc</sup>	1.6 $\pm$ 0.2 <sup>ab</sup>	7 $\pm$ 0.9 <sup>b</sup>	0.47 $\pm$ 0.06 <sup>b</sup>	0.32 $\pm$ 0.03 <sup>b</sup>	0.01 $\pm$ 0 <sup>a</sup>	1.13 $\pm$ 0.3 <sup>c</sup>	0.54 $\pm$ 0.31 <sup>a</sup>	1.27 $\pm$ 0.01 <sup>d</sup>	0.32 $\pm$ 0.3 <sup>a</sup>
HM_S_rhizome	0.62 $\pm$ 0.05 <sup>c</sup>	2.2 $\pm$ 0.04 <sup>a</sup>	8 $\pm$ 0.2 <sup>d</sup>	0.62 $\pm$ 0.03 <sup>bc</sup>	0.2 $\pm$ 0.04 <sup>abc</sup>	1.37 $\pm$ 0.32 <sup>a</sup>	8.3 $\pm$ 2.4 <sup>abcd</sup>	1.8 $\pm$ 0.6 <sup>abc</sup>	0.85 $\pm$ 0.33 <sup>ab</sup>	7 $\pm$ 1.3 <sup>ab</sup>
HM_A_leaves	0.19 $\pm$ 0.01 <sup>ab</sup>	2.58 $\pm$ 0.09 <sup>c</sup>	13 $\pm$ 1.26 <sup>b</sup>	2.5 $\pm$ 0.04 <sup>f</sup>	0.03 $\pm$ 0.004 <sup>a</sup>	1.98 $\pm$ 0.5 <sup>ab</sup>	6.99 $\pm$ 1.71 <sup>b</sup>	3.9 $\pm$ 0.04 <sup>d</sup>	2.9 $\pm$ 0.02 <sup>c</sup>	3.01 $\pm$ 0.21 <sup>d</sup>
HM_A_petiole	0.06 $\pm$ 0.01 <sup>a</sup>	2.1 $\pm$ 0.1 <sup>bc</sup>	4.7 $\pm$ 0.6 <sup>c</sup>	1.33 $\pm$ 0.08 <sup>c</sup>	0.03 $\pm$ 0.01 <sup>ab</sup>	3.5 $\pm$ 0.9 <sup>ab</sup>	5 $\pm$ 0.8 <sup>abc</sup>	2.9 $\pm$ 0.4 <sup>bc</sup>	1.01 $\pm$ 0.4 <sup>bcd</sup>	6 $\pm$ 0.3 <sup>b</sup>
HM_A_rhizome	0.08 $\pm$ 0.01 <sup>c</sup>	3.5 $\pm$ 0.33 <sup>b</sup>	6 $\pm$ 0 <sup>f</sup>	0.88 $\pm$ 0.12 <sup>c</sup>	0.07 $\pm$ 0.01 <sup>ad</sup>	4.8 $\pm$ 1.1 <sup>b</sup>	16.4 $\pm$ 3.8 <sup>cd</sup>	3 $\pm$ 1.1 <sup>bd</sup>	1.6 $\pm$ 0.5 <sup>b</sup>	7 $\pm$ 1.6 <sup>ab</sup>
DCH_S_leaves	0.42 $\pm$ 0.03 <sup>e</sup>	5.5 $\pm$ 0.06 <sup>f</sup>	7.9 $\pm$ 0.6 <sup>c</sup>	0.27 $\pm$ 0.01 <sup>bc</sup>	0.16 $\pm$ 0.02 <sup>c</sup>	2.06 $\pm$ 0.5 <sup>abc</sup>	1.88 $\pm$ 0.04 <sup>ac</sup>	2.9 $\pm$ 0.2 <sup>cd</sup>	0.38 $\pm$ 0.11 <sup>a</sup>	0.23 $\pm$ 0.22 <sup>ab</sup>
DCH_S_petiole	0.23 $\pm$ 0.01 <sup>e</sup>	3.4 $\pm$ 0.2 <sup>d</sup>	3.5 $\pm$ 0.24 <sup>c</sup>	0.46 $\pm$ 0.004 <sup>b</sup>	1.5 $\pm$ 0.2 <sup>d</sup>	6 $\pm$ 1.4 <sup>b</sup>	2 $\pm$ 1.1 <sup>bc</sup>	1.1 $\pm$ 0.6 <sup>ab</sup>	1.06 $\pm$ 0.25 <sup>cd</sup>	0 $\pm$ 0 <sup>a</sup>
DCH_S_rhizome	1.13 $\pm$ 0.01 <sup>e</sup>	2.1 $\pm$ 0.2 <sup>a</sup>	4 $\pm$ 0.3 <sup>f</sup>	0.13 $\pm$ 0.005 <sup>a</sup>	0.02 $\pm$ 0 <sup>acd</sup>	0.02 $\pm$ 0 <sup>a</sup>	3.6 $\pm$ 0.7 <sup>ab</sup>	2.6 $\pm$ 0.32 <sup>bcd</sup>	0.61 $\pm$ 0.1 <sup>ab</sup>	0.4 $\pm$ 0.1 <sup>a</sup>
MD_S_leaves	0.23 $\pm$ 0.02 <sup>d</sup>	7.3 $\pm$ 0.06 <sup>g</sup>	1.2 $\pm$ 0.1 <sup>a</sup>	1.9 $\pm$ 0.01 <sup>g</sup>	0.17 $\pm$ 0.01 <sup>c</sup>	0.02 $\pm$ 0.01 <sup>a</sup>	3.74 $\pm$ 0.8 <sup>abc</sup>	2.7 $\pm$ 0.5 <sup>c</sup>	0.32 $\pm$ 0.05 <sup>a</sup>	0.35 $\pm$ 0.34 <sup>ab</sup>
MD_S_petiole	0.06 $\pm$ 0.01 <sup>a</sup>	5.2 $\pm$ 0.2 <sup>e</sup>	0.62 $\pm$ 0.02 <sup>a</sup>	1.7 $\pm$ 0.08 <sup>d</sup>	0.14 $\pm$ 0.04 <sup>d</sup>	0.41 $\pm$ 0.19 <sup>a</sup>	0.5 $\pm$ 0.12 <sup>c</sup>	1.3 $\pm$ 0.3 <sup>ab</sup>	0.39 $\pm$ 0.1 <sup>abcd</sup>	0.25 $\pm$ 0.2 <sup>a</sup>
MD_S_rhizome	0.32 $\pm$ 0.02 <sup>ab</sup>	18 $\pm$ 0.54 <sup>f</sup>	1.04 $\pm$ 0.03 <sup>bc</sup>	1.7 $\pm$ 0.03 <sup>d</sup>	0.2 $\pm$ 0.01 <sup>abc</sup>	1.3 $\pm$ 0.2 <sup>a</sup>	0.95 $\pm$ 0.23 <sup>a</sup>	3 $\pm$ 0.01 <sup>bd</sup>	0.42 $\pm$ 0 <sup>a</sup>	15 $\pm$ 4 <sup>b</sup>
Mean_leaves	0.28 $\pm$ 0.03	2.8 $\pm$ 0.5	4.5 $\pm$ 1.14	0.74 $\pm$ 0.18	0.08 $\pm$ 0.01	1.96 $\pm$ 0.4	4.07 $\pm$ 0.48	1.8 $\pm$ 0.3	0.64 $\pm$ 0.2	0.76 $\pm$ 0.2

Table 5 (continued)

Locality	Fe*	Zn*	Cu*	Mn*	Al*	As*	Cr*	Ni*	Pb*	Cd*
Mean_petiole	0.11 ± 0.01	1.98 ± 0.3	1.96 ± 0.52	0.54 ± 0.12	0.36 ± 0.11	2.6 ± 0.8	3.7 ± 0.6	1.3 ± 0.25	0.55 ± 0.11	1.3 ± 0.5
Mean_rhizome	0.44 ± 0.06	5.7 ± 1.6	2.5 ± 0.6	0.45 ± 0.11	0.17 ± 0.02	1.25 ± 0.3	9.3 ± 1.5	2.2 ± 0.32	0.47 ± 0.11	5.6 ± 1.2

Abbreviations: LB\_S Libuše hut\_Summer, LB\_A Libuše hut\_Autumn, VT\_S Vítkovice v Krkonoších\_Summer, VT\_A Vítkovice v Krkonoších\_Autumn, PC\_S Pec pod Sněžkou\_Summer, PC\_A Pec pod Sněžkou\_Autumn, HM\_S Horní Mísečky\_Summer, HM\_A Horní Mísečky\_Autumn, DCH\_S Ramsau am Dachstein\_Summer, ZL\_S Zillertal\_Summer, MD\_S Madesimo\_Summer

Parliament and the Council on undesirable substances in animal feed, the values were within the regulatory limit.

Another observed risk element in terms of its effects on human health is Cd. Cadmium can accumulate in the human body and cause renal dysfunction, bone damage, reproductive disorders, etc. (Fatima et al. 2019). The content of Cd was lower in above-ground organs and higher in belowground organs. Cd translocation primarily is a function of the retention in rhizome and loading activity in the xylem (Verbruggen et al. 2009; Fig. 6). Retention, however, is mediated through Cd chelating molecules, such as phytochelatins and vacuolar sequestration. According to the recorded mean values, the *R. alpinus* is, therefore, is an accumulator of Cd (Table 5). This provides a basis that the various leaves of the *R. alpinus* are suitable for consumption according to Commission Regulation (EU) No. 1275/ 2013, with strict caution to the kind of locality.

Higher content of Zn in soils can have an antagonistic effect on the absorption of Cd in plants because it competes with Cd for plant surface absorption sites, thereby affecting the absorption of Cd by plant tissues (He et al. 2004). Fei et al. (2018) reported a young leaf protection mechanism with Cd preferentially distributed to senescent leaves to avoid Cd toxic effects emerging in mature leaves (She et al. 2018; Fei et al. 2018). However, the highest content of Cd was in the rhizomes. These values were within the range of typical normal levels in plants (Zhuang et al. 2007; Gaweda 2009). The amount of Cd in the plant organs consumed (especially in the leaves) did not exceed the stated value of 0.5 mg kg<sup>-1</sup> according to Commission Regulation (EU) No. 1275/ 2013. The permitted weekly limit for a 60 kg human is to consume 0.43 mg kg<sup>-1</sup> in food.

Transport and accumulation of elements by *R. alpinus*

From the results of this study, *R. alpinus* has a strategy to exclude Fe, Mn, and Pb, although the content of some of the elements was higher (e.g., Fe) in the soil. *R. alpinus* accumulated Mn only in MD and HM, even with the lowest plant-available content in the soil. The plants probably consider and use this element essential and connected with the ionic species of Mn available in each locality (Marschner 2012). The situation was similar for

**Table 6** Translocation (TF) (Mean  $\pm$  SE) of micro and risk elements of the studied sites. The *p* value for each element was obtained by one-way ANOVA. Using Tukey (HSD) post hoc test, values with the same letters for each element among the localities are not significantly different

Locality	Fe	Zn	Cu	Mn	Al	As	Cr	Ni	Pb	Cd
LB_S	0.46 $\pm$ 0.01 <sup>a</sup>	0.43 $\pm$ 0.01 <sup>ab</sup>	1.2 $\pm$ 0.05 <sup>a</sup>	0.88 $\pm$ 0.05 <sup>a</sup>	0.61 $\pm$ 0.02 <sup>abc</sup>	0.88 $\pm$ 0.34 <sup>a</sup>	0.8 $\pm$ 0.18 <sup>a</sup>	1.8 $\pm$ 0.3 <sup>ab</sup>	0 $\pm$ 0 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>a</sup>
LB_A	1 $\pm$ 0.14 <sup>ab</sup>	0.32 $\pm$ 0.03 <sup>ab</sup>	1.3 $\pm$ 0.11 <sup>a</sup>	2.2 $\pm$ 0.51 <sup>ab</sup>	0.12 $\pm$ 0.03 <sup>b</sup>	0.38 $\pm$ 0.05 <sup>a</sup>	0.38 $\pm$ 0.16 <sup>a</sup>	0.72 $\pm$ 0.01 <sup>ab</sup>	1.1 $\pm$ 0.2 <sup>ab</sup>	0.14 $\pm$ 0.1 <sup>a</sup>
VT_S	0.5 $\pm$ 0.08 <sup>a</sup>	0.7 $\pm$ 0.04 <sup>ab</sup>	2.1 $\pm$ 0.12 <sup>a</sup>	0.68 $\pm$ 0.02 <sup>a</sup>	0.66 $\pm$ 0.03 <sup>ac</sup>	4.7 $\pm$ 2.9 <sup>a</sup>	0.83 $\pm$ 0.29 <sup>a</sup>	0.72 $\pm$ 0.2 <sup>ab</sup>	1.3 $\pm$ 0.2 <sup>ab</sup>	0.05 $\pm$ 0.01 <sup>a</sup>
VT_A	0.61 $\pm$ 0.08 <sup>a</sup>	0.18 $\pm$ 0.01 <sup>a</sup>	2.2 $\pm$ 0.31 <sup>a</sup>	3 $\pm$ 0.92 <sup>ab</sup>	0.13 $\pm$ 0.03 <sup>bc</sup>	5.9 $\pm$ 2.2 <sup>a</sup>	0.3 $\pm$ 0.08 <sup>a</sup>	0.55 $\pm$ 0.1 <sup>ab</sup>	2.2 $\pm$ 0.7 <sup>b</sup>	0.12 $\pm$ 0.02 <sup>a</sup>
PC_S	0.89 $\pm$ 0.18 <sup>ab</sup>	1.4 $\pm$ 0.13 <sup>c</sup>	1.8 $\pm$ 0.14 <sup>a</sup>	0.69 $\pm$ 0.6 <sup>a</sup>	0.65 $\pm$ 0.09 <sup>ac</sup>	1.9 $\pm$ 1.2 <sup>a</sup>	0.47 $\pm$ 0.09 <sup>a</sup>	3.5 $\pm$ 0.7 <sup>c</sup>	1.24 $\pm$ 0.1 <sup>ab</sup>	0.69 $\pm$ 0.04 <sup>a</sup>
PC_A	1.6 $\pm$ 0.3 <sup>bc</sup>	3.7 $\pm$ 0.4 <sup>d</sup>	2.2 $\pm$ 0.36 <sup>a</sup>	4 $\pm$ 0.84 <sup>b</sup>	2.2 $\pm$ 0.09 <sup>d</sup>	2.5 $\pm$ 1.1 <sup>a</sup>	0.34 $\pm$ 0.01 <sup>a</sup>	0.88 $\pm$ 0.14 <sup>ab</sup>	1.3 $\pm$ 0.01 <sup>ab</sup>	0.94 $\pm$ 0.21 <sup>a</sup>
HM_S	0.32 $\pm$ 0.02 <sup>a</sup>	0.9 $\pm$ 0.03 <sup>bc</sup>	1.7 $\pm$ 0.03 <sup>a</sup>	1.1 $\pm$ 0.01 <sup>a</sup>	0.44 $\pm$ 0.08 <sup>abc</sup>	1.8 $\pm$ 0.3 <sup>a</sup>	0.35 $\pm$ 0.16 <sup>a</sup>	0.20 $\pm$ 0.13 <sup>ab</sup>	1.13 $\pm$ 0.3 <sup>ab</sup>	0 $\pm$ 0 <sup>a</sup>
HM_A	2.4 $\pm$ 0.3 <sup>c</sup>	0.74 $\pm$ 0.1 <sup>abc</sup>	2.1 $\pm$ 0.20 <sup>a</sup>	2.9 $\pm$ 0.4 <sup>ab</sup>	0.5 $\pm$ 0.16 <sup>abc</sup>	0.4 $\pm$ 0.01 <sup>a</sup>	0.48 $\pm$ 0.22 <sup>a</sup>	1.5 $\pm$ 0.5 <sup>ab</sup>	2 $\pm$ 0.7 <sup>b</sup>	0.48 $\pm$ 0.1 <sup>a</sup>
DCH_S	0.37 $\pm$ 0.03 <sup>a</sup>	3 $\pm$ 0.16 <sup>c</sup>	1.9 $\pm$ 0.03 <sup>a</sup>	2 $\pm$ 0.01 <sup>ab</sup>	0.94 $\pm$ 0.09 <sup>a</sup>	0 $\pm$ 0 <sup>a</sup>	0.55 $\pm$ 0.13 <sup>a</sup>	1.15 $\pm$ 0.2 <sup>ab</sup>	0.6 $\pm$ 0.1 <sup>ab</sup>	0.55 $\pm$ 0.5 <sup>a</sup>
ZL_S	0.51 $\pm$ 0.1 <sup>a</sup>	2 $\pm$ 0.0 <sup>e</sup>	1.5 $\pm$ 0.29 <sup>a</sup>	1.4 $\pm$ 0.1 <sup>a</sup>	0.92 $\pm$ 0.21 <sup>a</sup>	0 $\pm$ 0 <sup>a</sup>	0.05 $\pm$ 0.01 <sup>a</sup>	0.08 $\pm$ 0.01 <sup>b</sup>	1.2 $\pm$ 0.5 <sup>ab</sup>	0.02 $\pm$ 0.02 <sup>a</sup>
MD_S	0.7 $\pm$ 0.13 <sup>a</sup>	0.4 $\pm$ 0.02 <sup>ab</sup>	1.2 $\pm$ 0.06 <sup>a</sup>	1.1 $\pm$ 0.02 <sup>a</sup>	0.81 $\pm$ 0.02 <sup>a</sup>	0 $\pm$ 0 <sup>a</sup>	4.4 $\pm$ 1.94 <sup>b</sup>	0.83 $\pm$ 0.2 <sup>ab</sup>	0.8 $\pm$ 0.11 <sup>ab</sup>	0.03 $\pm$ 0.03 <sup>a</sup>
Mean	0.86 $\pm$ 0.14	1.3 $\pm$ 0.24	1.7 $\pm$ 0.09	1.9 $\pm$ 0.24	0.73 $\pm$ 0.12	1.7 $\pm$ 0.5	0.81 $\pm$ 0.28	1.1 $\pm$ 0.2	1.17 $\pm$ 0.15	0.28 $\pm$ 0.1
<i>P</i> value	0.001	0.001	0.04	0.011	0.001	0.06	0.031	0.001	0.04	0.032

*Abbreviations:* LB\_S Libuše hut\_Summer, LB\_A Libuše hut\_Autumn, VT\_S Vítkovice v Krkonoších\_Summer, VT\_A Vítkovice v Krkonoších\_Autumn, PC\_S Pec pod Sněžkou\_Summer, PC\_A Pec pod Sněžkou\_Autumn, HM\_S Horní Mísečky\_Summer, HM\_A Horní Mísečky\_Autumn, DCH\_S Ramsau am Dachstein\_Summer, ZL\_S Zillertal\_Summer, MD\_S Madesimo\_Summer, TF Translocation factor

Zn. In this case, this species excluded Zn in localities with high availability in the soil (e.g., in PC) and vice versa in localities with the lowest available Zn (Zhao et al. 2003). In localities with a low content of Zn in the soil, Zn levels in the plant organs were lower than those in localities with higher content; a similar result was reported by Barrutia et al. (2009). *R. alpinus* seemingly exhibited an accumulation strategy for Cu, but the trend was not the same in all localities. Notwithstanding localities LB and PC, *R. alpinus* accumulated Cu only in the leaves during summer. However, accumulation occurred in almost all organs in the remaining localities, except for petioles in VT and MD. Specifically, the BF of Cd in emerging leaves possesses exclusion strategies and, thus, supports accumulation in the rhizome. Hence, this is a strategy to protect most metabolic organs before toxicity, which can reduce biomass production and cause inhibition of cell elongation and division (Anton and Mathe-Gaspar 2005; Chen and Wong 2006; Barrutia et al. 2009; Vondráčková et al. 2014). In localities HM and VT, *R. alpinus* accumulated As in the organs during both seasons, although the BF was not significantly affected by the soil. A similar trend occurred in DCH. Moreover, accumulation occurred only during summer in the petiole at LB, which recorded the highest soil As content. *R. alpinus* has tolerance for a wide range of soil chemical properties with generally high As availability (Lorestani et al. 2011). According to the As content in individual organs, As moved the most in the petiole and senescent leaves. Hence, plants can absorb and transport metals and store them in their senescent-aboveground biomass (Baker 1981; Lorestani et al. 2011). Given that differences in the uptake of elements by plants concerning different soil contents in the soil did not always have the same pattern in all localities, we believe that this is probably related to the plant genotype (Al-Hiyaly et al. 1993). In connection with the results of Vondráčková et al. (2015), where *R. obtusifolius* is a hyperaccumulator of Al, we expected a similar strategy in *R. alpinus*. However, the results indicated that *R. alpinus* is an excluder of Al and can exude chelating ligands, form a pH barrier in the rhizosphere, immobilize the cell wall, and selectively permeabilize the plasma membrane.

In general, TF can be used to evaluate the ability of plants to transport risk elements between aboveground and belowground biomass. In our study, the mean TF found from all localities was higher than 1 for Zn, Cu, Mn, As, Ni and Pb. However, the TF values

differed between localities and seasons. For example, Zn was mostly in belowground parts of *R. alpinus*, except in localities PC\_S, PC\_A, DCH\_S, and ZL\_S, where the plants moved Zn to the aboveground organs, probably depending on the plant genotype in the locality (Al-Hiyaly et al. 1993). Meanwhile, this was different in the case of Mn, which in most cases moved more in the aboveground part; only in localities LB\_S, VT\_S, and PC\_S where it was located in the underground biomass, which was probably related to seasonal changes (Vaněk et al. 2012). While BF for Cu in most of the localities was in aboveground biomass (VT\_S, VT\_A, PC\_S, HM\_S, HM\_A, DCH\_S, and MD\_S), TF results indicate an accumulation strategy as Cu moved to aboveground organs in all localities—probably remains an essential plant nutrient. The translocation of As was also not the same in all the localities.

In all localities, Arsenic was in belowground biomass. However, in VT\_S, VT\_A, PC\_S, PC\_A, and HM\_S, As was translocated to the aboveground parts. The observed differences between plants, terms, and localities may likely relate to the collection of plant tissues from different individuals. In comparison, sampling in summer versus autumn was different. Physiologically driven differences in plant elemental composition relate to the plant's natural life cycle, multiple environmental variables, etc.

The BF results suggest an accumulation strategy for Cr, which accumulated in both terms in all localities, except for petiole and rhizome in MD. Moreover, the TF for Cr was below 1, following the higher content of these risk elements in the rhizome. The high tolerance of *R. alpinus* to Cr did not confirm an accumulation strategy in all localities, except for MD. Additionally, in the case of Al and Cd, the TF was below 1, indicating an exclusion strategy by retaining risk elements in the underground part of the plant protecting the metabolizing organs, which is consistent with observations for *R. acetosa* (Barrutia et al. 2009; Gaweda 2009).

## Conclusions

According to this study, the level of micro- and risk element bioaccumulation is considerably site-specific. And this is connected to both anthropogenic, lithogenic sources, and soil biogeochemical properties.



The study revealed that *R. alpinus* has considerable accumulation strategies for Cr, Zn, Cu, and Ni compared to Fe, Mn, Al, and Pb. Additionally, the studied species is tolerant to As indicated by the high BF of rhizome (belowground biomass), and content of As according to the above permissible limit in most of the soils. Nevertheless, the values of these elements in the consumed plant tissue (leaves and petiole) do not pose a risk to human health, as stated by the World Health Organization.

Additionally, the accumulation of As, Cr, Ni, Pb, and Cd by *R. alpinus* was affected by seasonal changes. For example, precipitation during the autumn can contribute to the release of elements in the soil, which was evident in the higher contents in the organs compared with those in summer in the localities of the Krkonoše Mountains. Although the aboveground biomass (emerging and mature leaves and petioles) has some degree of accumulation of other elements, e.g., Cu), however, Al and Cd accumulate in the belowground biomass (rhizome) and only in the stem in the case of Cd. However, the content of As, Cr, Ni, Pb, and Cd in the emerging, mature, senescent leaves and petiole are in the range of typical concentrations for plants. This study also showed the translocation of Zn, Cu, Mn, As, Ni, and Pb into aboveground biomass by *R. alpinus*. Hence, we recommend great caution while consuming this vegetable from contaminated soils, especially in the case of Pb, these values exceeded allowable limits in some localities and may pose a risk to human health, as stated by the World Health Organization. Therefore, we recommend a detailed elemental analysis of the organs of this species before its application as medicinal herbs and food.

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#### Declarations

**Conflict of interest** The authors declare no conflicts of interest whatsoever.

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