Distribution Of Micro (Fe, Zn, Cu, and Mn) and Risk (Al, As, Cr, Ni, Pb, and Cd) Elements in the Organs of *R. Alpinus* L.

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Abstract

Background and aims

Rumex alpinus is a native plant in the mountains of Europe, whose distribution is affected by its utilization as a vegetable and medicinal herb. The distribution of micro and risk elements in its organs and the possibility for phytoremediation are not well-known. We aimed to examine the safety of consuming *R. alpinus* from the Krkonoše Mountains, Czech Republic, and Alps (Austria and Italy).

Methods

We determined the total and plant-available concentration of Fe, Zn, Cu, Mn, Al, As, Cr, Ni, Pb, and Cd in the soil and total concentration in the organs of *R. alpinus* using inductively coupled plasma-optical emission spectrometry.

Results

The uptake and distribution of elements by plants were characterized by bioaccumulation and translocation (TF) factors. The intensity of elements accumulation by *R. alpinus* is considerably different, depending on locality. *R. alpinus* has considerable tolerance to Zn, Cu, As, Cr, Ni, with easy accumulation strategy. High Al and Cd concentration in belowground biomass (rhizome) indicates a defensive mechanism for them. Although the aboveground biomass (emerging, senescent, mature leaves, petiole) has some degree of accumulation of risk elements, *R. alpinus* is potentially suitable for phytoremediation of moderately contaminated soils. The results revealed that *R. alpinus* excludes Al, with high TF for Mn, Zn, Cu, As, Ni, and Pb. Given the accumulation of As and Cr, we recommend caution in its usage.

Conclusion

Detailed elemental analysis of *R. alpinus* organs is recommended before its application as medicinal herb or food, especially in contaminated soils.

1. Introduction

The Monk's Rhubarb (Alpine dock), *Rumex alpinus* L. (*R. alpinus*), is a perennial plant inhabiting nutrient-rich areas, stream banks, spring areas, pastures, and meadows. It is native to the mountains of Central and Southern Europe. The distribution of *R. alpinus* currently results from its utilization as a vegetable and medicinal herb in the past. It is one of the historic food plants in preparing the dish, "farchon"- made of steamed *Chenopodium bonus-henricus*, *Urtica dioica*, and *R. alpinus* in the Alps region (Maude et al. 2005). In Albania, this species is the most quoted and used wild food plants, used as vegetables mainly cooked with dairy products and rice or as filling for homemade savory pies (Pieroni and Quave 2014).

In alpine localities, some organs of this species serve various purposes, e.g., leaves to a surrogate of sauerkraut or spinach, stems peeled and applied instead of rhubarb, or eaten fresh or put into cakes, biscuits, and puddings (Dickson and Dickson 2000; Šťastná et al. 2010). The leaves, seeds, rhizomes, and roots of *R. alpinus* most often are 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* additionally, has emerged suitable for the treatment of inflammation and different bacterial infections (Vasas et al. 2015). Given the benefits of *R. alpinus* to health-related issues, it is pertinent to perform a detailed analysis of the bioaccumulation of elements in the organs of this species. The nutritional status of most plants better reflects in the mineral element concentrations of the leaves (Marschner et al. 1996). However, studies on the distribution of elements (e.g., trace and risk) below- and other above-ground biomass of *R. alpinus* are not well-known.

Atmospheric depositions from anthropogenic activities, e.g., metallurgy, remain one of the crucial sources of trace/risk elements (such as Cu, Zn, Cd, and Pb) in soils. Soil contamination with trace metals represents a risk for crop production, food quality, and

human health because of their high toxicity and the ability of plants to bioaccumulate. Notably, trace metals have high persistency in the environment and relatively high mobility (Mench 1998; Sucharová and Suchara 2004; Wuana and Okieimen 2011). However, *R. alpinus* grows mainly in mountainous areas, especially in protected areas with minimal or no impacts of anthropogenic activities. Hence, trace element concentrations can only result from lithogenic and probably be within permissible limits for soils. Moreover, *R. alpinus* usually grow in nutrient-rich areas, with the tendency of the soils contaminated by risk elements and still use as food and medicine. For example, in the Krkonoše Mountains, Czech Republic, *R. alpinus* have widely distributed in locations with historic mining of Fe ore and arsenic (As) in the 15th – 17th Century AD, with reported high possibility of soil contamination by trace metals (Lokvenc 2007; Tásler 2012).

The possibility of *R. alpinus* use in phytoremediation has been considered as a plant with high biomass encountered in many ecosystems (Klimeš 1992; Šťastná et al. 2010), rendering it suitable for phytoextraction of metal from moderately contaminated soil (Szabó and Fodor 2006; Cui et al. 2004; Turgut et al. 2004). Notwithstanding, studies concerning the bioaccumulation of trace and risk elements by this species are deficient.

A significantly high amount of plant biomass can compensate for a relatively low capacity for metal accumulation, resulting in a large amount of heavy metal removed from the soil (Zhuang et al. 2007). The uptake and transport of microelements by plants can be well-estimated by the bioaccumulation factor (BF); the plant-to-soil concentration ratio of elements (Baker 1981; Klink et al. 2014; Vondráčková et al. 2014). According to Baker (1981), plants that accumulate micro and risk elements are characterized by a BF and translocation factor (TF) > 1, indicator plants by a BF and TF = 1, and plants that exclude these elements by a BF and TF < 1. Thus, this study adopted these indices in estimating bioaccumulation factors of trace metals of *R. alpinus* from different localities in the temperate zone.

In this present study, we aimed to examine the safety of consuming Alpine dock obtained from Krkonoše Mountains. and Alpines localities by asking the following research questions; (1) to what extent can *R. alpinus* accumulate trace metals and whether it is suitable for phytoremediation? and (2) which organs of *R. alpinus* are accumulators of micro and risk elements? This exploratory study seeks to analyze the concentration of elements in the different organs of the studied species to determine their suitability for human consumption and other medicinal purposes. Thus, it is crucial to consider trace metal concentrations of such a plant, especially for environmental quality assessment.

2. Materials And Methods

2.1 Study area

The current study was conducted in typical stands of *R. alpinus* in the Krkonoše Mountains of the Czech Republic and the alps of Austria and Italy, all in the temperate zone (Fig. 1). The selected sites were studied according to their wide distribution of the studied species (see Table 1 for a detailed description of each studied locality).

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

2.2 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 layer with a soil probe (Purchhauer type, core-diameter: 30 mm). In each sampled locality (LB, VT, PC, HM, DCH, and MD), we randomly collected ten soil samples around the *R. alpinus* stands. In total, 60 soil samples were collected for further analysis.

All the soil samples were air-dried and subsequently, oven-dried at 70 °C for 48 hours. The samples were grounded in a porcelain mortar and homogenized by sieving through a 2 - mm sieve after removal of roots and other debris.

2.3 Plant organs sampling and preparation

Organs of *R. alpinus* were collected in a mono-dominant stand that covered 100 m² in all the localities (Fig. S2). Samples from above (stem, emerging, mature, and senescent leaves, and petiole) and below-ground (rhizomes) biomass were collected in all the sampled localities (Fig. 2).

In each locality, we collected ten emerging semi-developed leave blades (E), ten fully developed mature leave blades (M), ten senescent (yellow, red, or brown semi-dry) leave blades (S), ten petioles from mature leaves (Pe), ten stems without seeds (St), and three rhizomes (R) developed in the last two years: with enough energy storage (Araki et al. 2020, Fig 2). The samples from Krkonoše Mountains were collected in July during the summer (S) and in October 2018 in the autumn (A). Samples from the Alps localities were collected only once in July 2018, approximately the same time as Krkonoše mountains samples. The collected samples were kept in paper bags and transported to the laboratory. All the plant organs initially were cleaned from soil and other residues in a distilled H₂O and dried for 48 hours at 70 °C. The organs were ground and homogenized in an IKA A11 basic analytical mill (IKA®-Werke GmbH & Co. KG, Germany).

2.4 Chemical analyses of soils and plant organ samples

The total concentrations 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 (USEPA, 1996) – extraction mixture of HNO₃, HCl, and HF.

Usage- a mass of 0.25 g of homogenized *R. alpinus* individual organs was mineralized in a mixture of 9mL HNO₃ and 3mL 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 hours 1 ml of peroxide 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% HNO₃ for two hours and filtrated. Total concentration was determined by Inductively coupled plasmaoptical emission spectrometry (ICP-OES, 720 Series, Agilent Technologies, USA). The plant organs and soil samples were measured in three replicates. In the case of the determination of the total concentration of elements in the soil samples, we used the same approach as in the case of the plant organs.

The plant-available fraction of Fe, Zn, Cu, Mn, Al, As, Cr, Ni, Pb, and Cd in the soil was analyzed by Mehlich-III reagent (Mehlich, 1984) and determined by ICP-OES (Varian VistaPro, Mulgrave, Australia). The extractant composition is as follows: 0.2 M CH₃COOH + 0.25 M NH₄NO₃ + 0.013 M HNO₃ + 0.015 M NH₄F + 0.001 M EDTA, usage: 25 cm³ reagent per 2.5 cm³ soil. The plant-available concentration of the studied elements in the soil samples was analyzed in an accredited national laboratory, Eko-Lab Žamberk (www.ekolab.zamberk.cz). Soil pH (H₂O) was measured in two replicates of all soil samples using voltcraft PH-100 ATC pH meter (pH 212) produced by I & CS spol sr.o., Czech Republic.

2.5. Statistical analyses

Data on pH, the elemental concentrations 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 obtained data. Factorial ANOVA was used to determine the significant difference among the concentration of elements in different organs of *R. alpinus* from all the localities. One-way ANOVA was used to determine the significant difference among the concentration of elements in the organs and soil overall localities.

In all cases, *post-hoc* comparison using the Tukey HSD test was applied to identify significant differences between the concentration of elements in different organs and soils. All statistical analyses were performed using the STATISTICA 13.3 program (www.statsoft.com).

2.5.1. Estimation of bioaccumulation (BF) and translocation factors (TF)

The BF was calculated by the following equation,

$$BF = leaf \div soil$$

$$BF = Petiole \div soil$$

$$BF = rhizome \div soil \quad (1)$$

The BF of the leaf was estimated for total element concentration in emerging and mature leaves by the available in the soil, without senescent (degenerative part).

The TF was calculated by the following equation,

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

We used the mean total concentration of elements in the mature leaf and the rhizome. One-way ANOVA was used to determine the significant difference between BF and TF of the studied elements in all the localities.

3. Results

3.1. pH and concentration of elements in soils

We recorded a significant effect of locality on pH [H₂O] (Table 2). The pH of the soil samples in all the analyzed localities ranged from 5.2 - 6.1. Except for a slightly acidic reaction in DCH, soils in all other localities were moderately acidic, which can result in increased availability of elements to plants.

The statistical description of total and plant-available concentrations of the studied elements is in Tables 2 and 3, respectively. There was a significant effect of locality on the total concentrations of the micro (Fe, Zn, Cu, and Mn) and risk (Al, As, Cr, Ni, Pb, and Cd) elements (Table 2). The total concentration of Fe ranged from 13.6 - 27.5 g kg⁻¹ in DCH and HM, respectively. The total concentration of Zn ranged from 48 in DCH to 182 mg kg⁻¹ in PC. The concentration of total Cu ranged from 4.7 in DCH to 39.8 mg kg⁻¹ in LB. The total Mn ranged from 178 in MD to 693 mg kg⁻¹ in VT. Moreover, the total Al concentration ranged from 11.1 in LB to 27.1 g kg⁻¹ in VT. The concentration of total As was from 3.9 in DCH to 70.9 in HM. The total Cr concentration ranged from 20.6 in MD to 53.2 mg kg⁻¹ in HM. The concentration of Ni ranged from 11.65 in DCH to 28 mg kg⁻¹ in HM. The total concentration of Pb ranged from 13.2 in DCH to 70.8 mg kg⁻¹ in HM. The concentration of total Cd ranged from 0.43 in DCH to 1.35 mg kg⁻¹ in HM.

Except for Cr, there was a significant effect of locality in the concentration of plant-available elements (Table 3). The available fraction of Fe ranged from 204 - 657 mg kg⁻¹ in DCH and HM, respectively. The plant-available concentration of Zn ranged from 5.1 in DCH to 62 mg kg⁻¹ in PC. The plant-available Cu ranged from 0.7 in HM to 7.9 mg kg⁻¹ in MD. The available Mn concentration ranged from 43 in MD to 189 mg kg⁻¹ in PC. The plant-available concentration of Al ranged from 648 - 1720 mg kg⁻¹ in PC and HM, respectively. The available As concentration was from 0.35 in DCH to 3.64 mg kg⁻¹ in LB. The plant available Cr ranged from 0.12 in LB and HM to 0.31 mg kg⁻¹ in MD. The concentration of available Ni was from 0.34 in DCH to 1.85 mg kg⁻¹ in LB. The plant-available concentration of Pb ranged from 2.15 in HM to 21 mg kg⁻¹ in PC. Finally, the available concentration of Cd ranged from 0.06 in MD to 0.51 mg kg⁻¹ in LB.

3.2. Concentration of total elements in plant organs

The concentration of the elements in all analyzed organs' overall localities is given in Figs. 3 – 6 and Table S1. There was a significant effect of organ, locality, and organ/locality interaction on the concentration of all the analyzed elements. The mean total concentrations of the elements in different organs are in Table S1. In the Krkonoše Mountains, there was a significant effect of organ and terms (summer and autumn) in the concentration of Fe, Cu, Mn, Al, As, Cr, and vice versa in the case of Zn, Ni, Pb, and Cd (Figs. 7 and 8). The concentration of Fe ranged from 15 in the stem from LB during summer (LB_S) to 818 mg kg⁻¹ in senescent leaves at HM in autumn (HM_A). The mean Fe concentration in organs' overall localities was $Pe < St < E < M < R < S$ (Fig.

3a). Except for mature and senescent leaves, the concentration of Fe was higher in summer than autumn (Fig. 7a). The concentration of Zn ranged from 6 in stem from PC_S to 212 mg kg⁻¹ in rhizome from VT_A. The mean Zn concentration in organs' overall localities and collection terms was Pe<St<S<M<E<R (Fig. 3b). The concentration of Zn was higher in autumn than in summer (Fig. 7b). The Cu concentration was from 0.7 in stem from PC_A to 13.1 mg kg⁻¹ in emerging leaves from VT_S. The mean Cu concentration in organs' overall localities and collection terms was St<Pe<S<R<M<E (Fig. 4a). The concentration of Cu was higher in summer than in autumn (Fig. 7c). The Mn concentration ranged from 4.5 in the stem from ZL to 322 mg kg⁻¹ in senescent leaves in MD. The mean Mn concentration in organs' overall localities and collection terms was St<R<Pe<E<M<S (Fig. 4b). Except for rhizome, Mn concentration was higher in autumn than in summer (Fig. 7d).

The concentration of Al ranged from 15 in emerging leaves to 1590 mg kg⁻¹ in the petiole from LB_A and VT_S, respectively. The mean of Al concentration in organs in all localities and collection terms was E<St<M<R<S<Pe (Fig. 5a). Except for stem and rhizome, Al concentration was higher in summer than in autumn (Fig. 8a). The concentration of As ranged from 0.009 in emerging leaves in ZL to 5 mg kg⁻¹ in senescent leaves in PC_A. The mean of As concentration in organs' overall localities and collection terms was St<M<E<R<S<Pe (Figure 5b). Except for emerging leaves, the concentration of As was higher in autumn than in summer (Fig. 8b). The level of Cr ranged from 0.06 in mature leaves in DCH to 6.6 mg kg⁻¹ in rhizome in ZL, and the mean concentration in organs' overall localities and collection terms was Pe<E<M<S<St<R (Fig. 5c). The Cr concentration was higher in autumn than in summer (Fig. 8c). The concentration of Ni was from 0.01 in stem from PC_S to 6.6 mg kg⁻¹ in rhizome in VT_A. The mean of Ni concentration in organs' overall localities and collection terms was Pe<St<M<S<E<R (Fig. 6a). The level of Ni in all organs was higher in autumn than in summer (Fig. 8d). Pb concentration ranged from 0.001 in petiole and rhizome in LB_S to 8.2 mg kg⁻¹ in senescent leaves in HM_A. The mean Pb concentration in organs' overall localities and collection terms was R<M<Pe<St<S<E (Fig. 6b). The level of Pb was higher in autumn than in summer (Fig. 8e). The concentration of Cd in organs across the Alps localities was mostly below detection except for rhizome, which recorded 0.8 - 1 mg kg⁻¹ in ZL and MD, respectively. In Krkonoše Mountains, Cd concentration was from 0.1 in emerging leaves in VT_S to 3.9 mg kg⁻¹ in rhizome from VT_A. The mean of Cd concentration in organs' overall localities and collection terms was R<M<Pe<St<S<E (Fig. 6c). The concentration of Cd was higher in autumn than in summer (Fig. 8f).

3.3. Bioaccumulation and translocation factor

3.3.1 BF (leaf ÷ soil)

The results of BF of micro and risk elements are in Table 4. There was a significant effect on locality for all the elements. BF for Fe in all localities was < 1, with a mean value of 0.28. The BF for Zn was < 1 only in two localities (LB_A, PC_S, and PC_A) and a mean value of 2.8. Similar results (BF<1) were obtained for Cu in LB_A, LB_S, and PC_A, with a mean value of 4.5. BF for Mn in all localities, except for HM_A and MD_S, was below 1, and the mean value of 0.74. BF of Al in all localities again was < 1, the mean value was 0.08. BF of As was < 1 in two localities (LB_S, LB_A, PC_S, and MD_S), the mean value was 1.96. BF for Cr was in all the localities > 1 and the mean value was 4.07. In LB_S, PC_S, and HM_S, BF for Ni was < 1, with a mean value of 1.8. However, BF for Pb was > 1 only in one locality (HM_A), and the mean value was 0.64. Similar results were obtained for Cd, where BF was mostly < 1, except for VT_A, PC_A, and HM_A, with a mean value of 0.76.

3.3.2 BF (petiole ÷ soil)

In all the localities, BF for Fe was < 1, and the mean value of 0.11. Again, the BF of Zn was below 1 only in two localities (LB_S and LB_A, PC_S and PC_A), with a mean value of 1.98. The BF in petiole for Cu was > 1 only in VT_S, HM_S, HM_A, and DCH_S, with a mean value of 1.96. The BF of Mn was > 1 only in two localities (HM_A and MD_S), with a mean value of 0.74. Similar results were recorded for BF of Al, with only two localities (VT_S and DCH_S) > 1 and a mean value of 0.36.

Additionally, in all the localities, except for MD_S, BF of As was > 1, with a mean value of 2.6. A similar pattern was recorded for BF of Cr in all the localities, except MD_S was > 1 and the mean value was 3.7. The BF for Ni was in four localities (VT_S, VT_A, HM_A, DCH_S, and MD_S) > 1, with the mean value of 1.3. We recorded BF of Pb only in VT_A, HM_A, and DCH_S > 1. *R. alpinus* was an indicator of Pb in one locality (HM_S), and the mean value was 0.55. In only LB_A, *R. alpinus* was Cd indicator and in two localities (VT_A and HM_A) BF was > 1, with a mean value of 1.3.

3.3.3 BF (rhizome ÷ soil)

In all localities except for DCH_S, BF for Fe was < 1 , with a mean value of 0.44. In only one locality (PC_S and PC_A), BF for Zn was < 1 , with a mean value of 5.7. Two localities (LB_S and LB_A, PC_S and PC_A) recorded BF for Cu < 1 , the mean value was 2.5. The BF for Mn in all localities, except (MD_S) was < 1 , and a mean value of 0.45. In all localities, the BF of Al was below 1, with a mean value of 0.17. For As, we recorded BF in three localities (LB_S and LB_A, PC_S and PC_A, DCH_S) below 1, and the mean value was 1.25. Moreover, in all localities except for MD_S, recorded BF > 1 for Cr and the mean value was 9.3. Opposite results were recorded for BF of Mn, in all localities, except (MD_S), were < 1 , with a mean value of 0.45. BF for Ni in only two localities (LB_S and PC_S) was below 1, the mean value was 2.2. Only one locality (HM_A) had BF of Pb above 1, the mean value was 0.47. The BF of Cd in only PC_S and DCH_S were < 1 , and a mean value of 5.6.

3.3.4 TF (leaf ÷ rhizome)

The mean TF of Al, Cr, and Fe was < 1 (Table 5). Only in PC_A was TF of Al above 1 and in MD Cr was above 1. TF of Cd was < 1 in all the localities. The TF for Fe was above 1 in autumn in localities LB, PC, and HM. The TF of Cu, Mn, Ni, and Pb were mostly above 1.

4. 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 intensity of elements accumulation by *R. alpinus* is considerably site-specific. The release of the trace and risk elements relates to the lithogenic and anthropogenic (e.g., metallurgy) sources resulting in the subsequent accumulation in different departments of *R. alpinus*. Moreover, the accumulation of risk elements such as As, Cr, Ni, Pb, and Cd was affected by season. In addition to acidic soil, the dissolution by precipitation H₂O during the autumn contributed to the release of elements (Truog 1947), reflected in higher concentrations in the organs during autumn compared to summer in localities of the Krkonoše Mountains.

4.1. Chemical characterization of soil

The reduced soil acidity in all the localities was due to the high concentrations 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 concentrations of total Zn and Cu were below the permissible limits (220 for Zn and 70 mg kg⁻¹ for Cu) for most agricultural soils (Kabata-Pendias and Pendias 2001). Meanwhile, the average value of Zn in agricultural soils in the Czech Republic ranges from 105 - 120 mg kg⁻¹ (Poláková et al. 2016). Thus, the limit exceeded all the localities of the Krkonoše Mountains. The high total concentration of Zn in the soils from the Krkonoše Mountains relates to the historic mining and smelting of Zn (Kafka 2003). The average concentration of Cu for agricultural soils ranges from 1 - 50 mg kg⁻¹ (Adriano 2001) and corresponds with the investigated localities. Additionally, a higher total concentration of Mn was recorded in the localities of the Krkonoše Mountains and DCH. According to Kabata-Pendias and Pendias (2001), the average total Mn concentration in podzols is 270 mg kg⁻¹ and this exceeded more than twice. The high Mn is due to the acidic conditions and redox potential of the soils. Limited uptake of Mn by plants can occur after organic fertilization, where Mn is oxidized in the soil, thereby reducing its solubility and availability to plants. Moreover, the reduction in the solubility of Mn and its availability can result from a lack of H₂O precipitation (Vaněk et al. 2012, Fig. 7d). The total concentrations of Al, Cr, Ni, Pb, and Cd were within the reported range in most agricultural soils (Kabata-Pendias and Pendias 2001). In the localities of the Krkonoše Mountains, the concentrations of these elements were higher compared to the Alps, which relate to mining influence from the past (Lokvenc 2003, 2007, Tásler 2012). Cr concentration in soils is directly related to parent rocks (geogenic elements), with an average value of 50 mg kg⁻¹ (Adriano 2001). The average total concentration in the Czech Republic is approximately 41 mg kg⁻¹ (Tásler 2012), which indicates that the limit was exceeded in VT, PC, and HM, resulting from the anthropogenic activities. The average Ni concentration in the soil is generally considered to be about 20 mg kg⁻¹ (Adriano 2001), the results show that it was again exceeded in the Krkonoše localities. Notably, contaminated agriculture areas in the Czech Republic have values of Ni > 200 mg kg⁻¹ (Poláková et al. 2016). The permissible limit of Pb in agricultural soils is 100 mg kg⁻¹: thus, there was no exceedance of Pb in the examined localities (Kabata-Pendias and Pendias 2001). According to Pugh et al.

(2002), Cd concentration in uncontaminated soils ranges from 0.05 – 2 mg.kg⁻¹, thus, in VT and LB, Cd exceeded this range in rhizome during autumn. The concentrations of As, was above Czech legislative limits (20 mg kg⁻¹) at LB, VT, HM, and MD, indicating a risk to the safety of food or feed, direct danger to human or animal health in contact with soil, and a negative impact on the production function of agricultural land (Kabata-Pendias and Pendias 2001). In the Krkonoše localities, the soils remain contaminated by As probably due to arsenopyrite mining in the past (Tásler 2012).

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

The selection of adequate plant species with accumulative characteristics is crucial for successful phytoextraction processes. Hyperaccumulating plants frequently reach low biomass, pest management, or harvesting practices as main drawbacks for their utilization for metal phytoextraction (Wenzel et al. 1999). The amount of risk elements extracted depends on the concentrations in the harvestable parts of plants and other plant biomass. Mean concentrations of Fe, Zn, Cu, and Mn in all organs of *R. alpinus* were found to be relatively low and within ranges of geochemical background values given by Kabata-Pendias and Pendias (2001). The variability in the concentrations of microelements in the organs of the *R. alpinus* relates to the 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 (Lu et al., 2009, She et al. 2018). The concentration of Zn was highest in the rhizome, consistent with the finding of Bohner (2005). Moreover, the concentration of Fe was lowest in the stem, which is consistent with the studies by Gaweda (2009) and Vondráčková et al. (2014). The highest concentration of Fe was in senescent leaves, and this finding corresponds with the study by Bohner (2005). The same pattern as Fe exhibited in the case of Mn compared to Bohner (2005), who reported elevated concentration of Mn in mature leaves of *R. alpinus*. We recorded the lowest concentration of Mn in mature leaves and highest in senescent leaves. Gaweda (2009) reported the highest concentration of Fe and Mn in *Rumex acetosa* in the below-ground biomass. Nonetheless, Fe and Mn concentrations in the senescent leaves were higher than the other organs, which suggests that the plant is probably supersaturated (Baker 1981) by these elements due to the high level in the soil. However, the Fe and Mn concentrations in the leaves (emerging and mature leaves) are within the normal recorded range according to Levy et al. (1999) and Mahler (2004) (see Table S1). A different distribution of Cu was in emerging and mature leaves, which is not consistent with the distribution of Cu in below-ground organs of *Rumex acetosa* (Gaweda 2009). The high concentration of Cu in the emerging and mature leaves explains why the higher part of Cu localize in the growing section of the tissues and chloroplasts, respectively (Vaněk et al. 2012).

Several studies have investigated plants with the mechanisms to tolerate high concentration of Al (Tolrà et al. 2004, Arunakumara et al. 2013, Vondráčková et al. 2015). In this study, the mean total concentration of Al was lowest in emerging leaf and highest in the petiole, followed by the rhizome, due to low transport from below-ground organs to leaf: a defensive mechanism against high concentration of Al in plants (Poschenrieder et al. 2008). In comparison with the experiment by Vondráčková et al. (2015), concerning *R. obtusifolius*, the recorded values in the organs of this study were low, which indicates that *R. alpinus* prevents the intake of Al. The mean total concentration of As in individual organs ranged from 1-1.8 mg kg⁻¹, which slightly exceeds the limit according to Kabata-Pendias and Pendias (2001). Arsenic concentration in the leaves was lower and higher in petiole and senescent leaves, which indicates that the plant tried to get rid of it. The mean total concentration of Cr in *R. alpinus* organs of this study was lower compared to the results of *Rumex obtusifolius*, according to Vondráčková et al. (2014). While their results showed the highest Cr concentration in the leaves, we obtained the lowest in emerging, mature leaves and the highest in stem and rhizome. However, these results are comparable to Gaweda (2009), who studied *Rumex acetosa*. Notably, the different results in varying degrees relate to the species, anthropogenic activities, and the soil chemical properties.

Meanwhile, Pb usually is taken up by plants from air pollution because of anthropogenic activities (Kabata-Pendias and Pendias 2001). The mean total concentration of Pb and Ni in all the organs was approximately the same and in the normal range (Allen 1989, Pugh et al. 2002). Compared to *Rumex acetosa*, *Rumex crispus*, and *Rumex K-1* (Zhuang et al. 2007, Gaweda 2009), where the highest concentration of Pb was in below-ground organs, we recorded Pb higher in above-ground organs. According to Pawlak et al. (2007), Pb accumulates mainly in roots with an affinity to galacturonic acid. The concentration of Cd was lower in above-ground organs and highest in the below-ground organ. Zn and Fe may have an antagonistic effect on the absorption of Cd in plants, leading to the inhibition of Cd absorption (Li et al. 2004). Zinc and Fe compete with Cd for plant surface absorption sites, thereby affecting the absorption of Cd in the plant tissue. Fei et al. (2018) reported that the young leaf protection mechanism with

Cd preferentially distributed to the senescent leaves to avoid Cd toxic effect in the emerging at mature leaves (She et al. 2018, Wang et al. 2019). However, the highest concentration was in the rhizomes, which indicates that *R. alpinus* save the above-ground organs against Cd (Zhuang et al. 2007, Gaweda 2009).

4.3 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 concentration of some of the elements was high (e.g., Fe) in the soil. *R. alpinus* accumulated Mn only in MD and HM, even with the lowest plant-available concentration in the soil, perhaps because the plants used this element as essential. The situation was similar for Zn. In this case, the studied species exclude Zn in localities with high availability in the soil (e.g., in PC) and vice versa in localities with the lowest available Zn, rendering them accumulators (Zhao et al. 2003). In localities with a low concentration of Zn in the soil, Zn levels in the plant organs were lower than localities with higher concentration: a similar result reported by Barrutia et al. (2009). *R. alpinus* seemingly exhibits accumulating strategy for Cu, but the trend was not the same in all localities. Notwithstanding, in LB and PC *R. alpinus* are accumulators only in the leaves during summer. However, in the remaining localities, accumulation occurred almost in all the organs, except for petioles in VT and MD. Precisely, BF of Cd in the emerging leaves have excluding strategy supporting accumulating strategy in the rhizome. Hence, this is a strategy to protect the most metabolic organ before toxicity, which can reduce biomass production, 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 HM and VT, *R. alpinus* accumulated As in the organs in both terms, although BF was not significantly affected by the soil. A similar trend occurred in DCH. Meanwhile, at LB with the highest soil As concentration, accumulation occurred only during summer in the petiole. *R. alpinus* has tolerance for a wide range of soil chemical properties with generally a high As availability (Lorestani et al. 2011). According to the As concentration in individual organs, As moved the most in the petiole and senescent leaves. Hence, the plant can absorb and transport metals and store them in their senescent (above-ground biomass), the declining part (Baker 1981, Lorestani et al. 2011). In connection with the results of Vondráčková et al. (2015), where *R. obtusifolius* is a hyperaccumulator of Al, we expected a similar strategy with *R. alpinus*. However, the results showed that *R. alpinus* is an excluder of Al and can exude chelating ligands, form a pH barrier at the rhizosphere, cell wall immobilization, and selective permeability of the plasma membrane.

The TF represents a phytoextraction parameter used to evaluate the capacity of each accession to translocate metals from rhizome to leaves. In our study, the mean TF found from all localities was higher than 1 for Zn, Cu, Mn, As, Ni and Pb. The BF results suggest an accumulation strategy for Cr, which accumulated in both terms at all localities except the petiole and rhizome at MD. However, TF for Cr was below 1, following the high concentration of these risk elements in the rhizome. Probably, high tolerance by *R. alpinus* to Cr has not confirmed accumulation strategy in all localities except MD.

5. Conclusions

According to this study, the level of micro and risk elements bioaccumulation is considerably site-specific. Our analysis strongly revealed that *R. alpinus* has considerable tolerance to As, Cr, Zn, Cu, and Ni, but easy bioaccumulation of these elements. Thus, considered a plant for phytoremediation. The high concentration of elements such as Al and Cd in the below-ground biomass (rhizome) indicates a defensive mechanism (excluder) for high concentrations of risk elements.

Additionally, the accumulation of As, Cr, Ni, Pb, and Cd by *R. alpinus* was affected by seasonal changes. For example, precipitation H₂O during the autumn can contribute to the release of elements in the soil, evident in higher concentrations in the organs compared to summer in localities of the Krkonoše Mountains. *R. alpinus* has also revealed the accumulation ability (accumulator) for As and Cr. Although the above-ground biomass (emerging, mature leaves, and petiole) has some degree of accumulation of other elements (e.g., Cu), *R. alpinus* is potentially suitable for the phytoremediation of moderately contaminated soils. The study also showed a significant translocation for Zn, Cu, Mn, As, Ni, and Pb by *R. alpinus*. Hence, we recommend great caution while consuming this vegetable on contaminated soils.

Therefore, we recommend, detailed elemental analysis of the organs of the studied species before its application as medicinal herb and food, especially in contaminated soils.

Declarations

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Conflict of interest

The authors declare no conflicts of interest whatsoever.

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Tables

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, CZ	Bank of Úpa river	50°41'19"N 15°46'43"E	700	850	6.5	Podzol	Phyllite
Vítkovice v Krkonoších, CZ	Ditch close to the road	50°41'56"N, 15°31'41"E	650	900	5.5	Podzol	Phyllite
Pec pod Sněžkou, CZ	Ruderal area under the hotel	50°41'46"N, 15°44'8"E	815	850	5.5	Podzol	Phyllite
Horní Mísečky, CZ	Eutrofied grassland	50°44'2"N, 15°34'5"E	1050	1000	4.5	Podzol	Phyllite
Ramsau am Dachstein, A	Cattle pasture	47°27'1"N, 13°37'1"E	1650	1100	3.8	Calcaric Cambisols	Limestone
Zillertal, A	Cattley/horse pasture	47°14'21"N 12°7'39"E	1650	933	3.9	Luvic Cambisol	Granite gneiss
Madesimo, 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

Table 2. Mean total concentration ($\pm$ Mean absolute deviation) 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, localities with the same letter were not significantly different.

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

The values above the permissible limit for agricultural soils are in bold, according to Kabata-Pendias and Pendias (2001).

Table 3. Mean (± SE) plant-available concentration of elements in the upper 10 cm soil layers of the studied localities.

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

Table 4. Bioaccumulation factor (Mean ± 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, localities with the same letter were not significantly different. The mean values indicating accumulators are in bold. The asterisk indicates * *p* < 0.01.

Locality	Fe*	Zn*	Cu*	Mn*	Al*	As*	Cr*	Ni*	Pb*	Cd*
LB_S_leaves	0.17 ± 0.003 ^a	1.01 ± 0.001 ^a	0.7 ± 0.02 ^a	0.24 ± 0.01 ^{abc}	0.07 ± 0.01 ^a	0.66 ± 0.01 ^a	6.09 ± 0.31 ^{ab}	0.64 ± 0.03 ^a	0.04 ± 0.01 ^a	0.06 ± 0.02 ^{ab}
LB_S_petiole	0.07 ± 0.01 ^{ab}	0.95 ± 0.05 ^{ab}	0.35 ± 0.01 ^a	0.31 ± 0.01 ^{ab}	0.1 ± 0 ^{ab}	1.2 ± 0.1 ^a	9 ± 2.4 ^{ab}	0.27 ± 0.09 ^a	0 ± 0 ^a	0.31 ± 0.1 ^a
LB_S_rhizome	0.37 ± 0.01 ^{ab}	2.4 ± 0.1 ^{ab}	0.58 ± 0.04 ^{ac}	0.27 ± 0.004 ^a	0.11 ± 0.02 ^{acd}	0.87 ± 0.33 ^a	8.1 ± 2.2 ^{abcd}	0.36 ± 0.04 ^{ac}	0 ± 0 ^a	2.2 ± 0.1 ^a
LB_A_leaves	0.17 ± 0.01 ^a	0.85 ± 0.02 ^{ab}	0.4 ± 0.03 ^a	0.34 ± 0.001 ^b	0.04 ± 0.01 ^a	0.29 ± 0.1 ^a	5.25 ± 1.2 ^{abc}	1.3 ± 0.13 ^{ab}	0.21 ± 0.03 ^a	0.62 ± 0.41 ^{abc}
LB_A_petiole	0.04 ± 00.04 ^a	0.52 ± 0.003 ^a	0.23 ± 0.05 ^a	0.16 ± 0.03 ^a	0.12 ± 0.01 ^{ab}	0.52 ± 0.12 ^a	2.3 ± 0.7 ^{bc}	0.58 ± 0.13 ^a	0.19 ± 0.04 ^{abc}	1± 0.04 ^a
LB_A_rhizome	0.17 ± 0.01 ^{bc}	2.6 ± 0.2 ^{ab}	0.33 ± 0.01 ^a	0.17 ± 0.04 ^a	0.35 ± 0.03 ^{bc}	0.73 ± 0.18 ^a	15 ± 3.2 ^{bcd}	1.8 ± 0.15 ^{abc}	0.2 ± 0.01 ^a	4.5 ± 0.1 ^a
VT_S_leaves	0.3 ± 0.01 ^{bc}	3.9 ± 0.06 ^c	2.8 ± 0.03 ^a	0.18 ± 0.01 ^{ac}	0.05 ± 0.003 ^a	4.91 ± 1.4 ^{bc}	1.32 ± 0.04 ^c	1.1 ± 0.3 ^{ab}	0.34 ± 0.1 ^a	0.24 ± 0.02 ^{ab}
VT_S_petiole	0.13 ± 0.01 ^{bcd}	2.2 ± 0.2 ^{bcd}	1.4 ± 0.22 ^a	0.13 ± 0.02 ^a	1.1 ± 0.04 ^c	0.01 ± 0 ^a	4 ± 1 ^{abc}	1.5 ± 0.15 ^{ab}	0.14 ± 0.03 ^{ab}	0 ± 0 ^a
VT_S_rhizome	0.6 ± 0.08 ^{de}	6 ± 0.2 ^c	1.4 ± 0.1 ^b	0.11 ± 0.01 ^a	0.08 ± 0 ^{acd}	1.4 ± 0.57 ^a	1.83 ± 0.7 ^a	1.6 ± 0.35 ^{abc}	0.26 ± 0.04 ^a	4.7 ± 1 ^a
VT_A_leaves	0.25 ± 0.02 ^{abc}	3.6 ± 0.2 ^c	2.3 ± 0.15 ^a	0.92 ± 0.01 ^d	0.03 ± 0.004 ^a	5.42 ± 0.97 ^c	4.63 ± 0.8 ^{abc}	2.7 ± 0.16 ^c	0.86 ± 0.03 ^b	1.7 ± 0.22 ^c
VT_A_petiole	0.06 ± 0.01 ^a	3.1 ± 0.6 ^{cd}	0.82 ± 0.3 ^a	0.57 ± 0.09 ^b	0.02 ± 0.002 ^a	10 ± 1.6 ^c	7 ± 0.6 ^{ab}	3.5 ± 0.36 ^c	1.08 ± 0.14 ^{cd}	5 ± 0.9 ^b
VT_A_rhizome	0.41 ± 0.02 ^{ad}	20 ± 0.01 ^d	1.1 ± 0.1 ^{bc}	0.35 ± 0.12 ^{ab}	0.23 ± 0.03 ^{bc}	1.01 ± 0.21 ^a	15 ± 1.4 ^{bcd}	5 ± 0.12 ^d	0.42 ± 0.12 ^a	14 ± 0.1 ^b
PC_S_leaves	0.31 ± 0.03 ^c	0.5 ± 0.02 ^b	1.5 ± 0.11 ^a	0.16 ± 0.01 ^a	0.14 ± 0.02 ^{bc}	0.65 ± 0.2 ^a	2.24 ±0.11 ^{ac}	0.5 ± 0.01 ^a	0.21 ± 0.03 ^a	0.001 ± 0 ^a
PC_S_petiole	0.13 ± 0.01 ^{cd}	0.35 ± 0.01 ^a	0.68 ± 0 ^a	0.11 ± 0.01 ^a	0.2 ± 0.002 ^{ab}	0.2 ± 0 ^a	2.4 ± 0.5 ^c	0.36 ± 0.16 ^a	0.11 ± 0.04 ^{ab}	0 ± 0 ^a
PC_S_rhizome	0.35 ± 0.04 ^{ab}	0.35 ± 0.04 ^e	0.86 ± 0.13 ^{abc}	0.24 ± 0.03 ^a	0.22 ± 0.06 ^{abc}	0.45 ± 0.18 ^a	5 ± 1.16 ^{abc}	0.14 ± 0.02 ^a	0.17 ± 0.01 ^a	0± 0 ^a
PC_A_leaves	0.59 ± 0.03 ^d	0.95 ± 0.05 ^a	0.74 ± 0.1 ^a	0.22 ± 0.01 ^{ac}	0.08 ± 0.02 ^{ab}	1.20 ± 0.3 ^a	6.03 ± 0.9 ^{ab}	2.1 ± 0.4 ^{bc}	0.25 ± 0.04 ^a	1.29 ± 0.3 ^{bc}
PC_A_petiole	0.18 ± 0.02 ^{de}	0.36 ± 0.04 ^a	0.26 ± 0.02 ^a	0.09 ± 0.02 ^a	0.12 ± 0.02 ^{ab}	3.7 ± 0.96 ^{ab}	3.3 ± 0.3 ^{bc}	0.92 ± 0.46 ^a	0.21 ± 0.04 ^{abc}	0.39 ± 0.3 ^a
PC_A_rhizome	0.37 ± 0.04 ^a	0.25 ± 0.01 ^e	0.35 ± 0.02 ^a	0.06 ± 0.01 ^a	0.04 ± 0.01 ^d	0.53 ± 0.12 ^a	18 ± 3 ^d	2.4 ± 0.1 ^{abc}	0.2 ± 0.03 ^a	1.4 ± 0.01 ^a
HM_S_leaves	0.2 ± 0.001 ^{abc}	2 ± 0.02 ^d	14 ± 0.56 ^b	0.67 ± 0.03 ^e	0.08 ± 0.004 ^{ab}	2.40 ± 0.2 ^{abc}	2.53 ± 0.5 ^{abc}	0.27 ± 0.1 ^a	0.87 ± 0.15 ^b	0.01 ± 0.003 ^a
HM_S_petiole	0.09 ± 0.02 ^{abc}	1.6 ± 0.2 ^{ab}	7 ± 0.9 ^b	0.47 ± 0.06 ^b	0.32 ± 0.03 ^b	0.01 ± 0 ^a	1.13 ± 0.3 ^c	0.54 ± 0.31 ^a	1.27 ± 0.01 ^d	0.32 ± 0.3 ^a
HM_S_rhizome	0.62 ± 0.05 ^e	2.2 ± 0.04 ^a	8 ± 0.2 ^d	0.62 ± 0.03 ^{bc}	0.2 ± 0.04 ^{abc}	1.37 ± 0.32 ^a	8.3 ± 2.4 ^{abcd}	1.8 ± 0.6 ^{abc}	0.85 ± 0.33 ^{ab}	7 ± 1.3 ^{ab}

HM_A_leaves	0.19 ± 0.01 ^{ab}	2.58 ± 0.09 ^e	13 ± 1.26 ^b	2.5 ± 0.04 ^f	0.03 ± 0.004 ^a	1.98 ± 0.5 ^{ab}	6.99 ± 1.71 ^b	3.9 ± 0.04 ^d	2.9 ± 0.02 ^c	3.01 ± 0.21 ^d
HM_A_petiole	0.06 ± 0.01 ^a	2.1 ± 0.1 ^{bc}	4.7 ± 0.6 ^c	1.33 ± 0.08 ^c	0.03 ± 0.01 ^{ab}	3.5 ± 0.9 ^{ab}	5 ± 0.8 ^{abc}	2.9 ± 0.4 ^{bc}	1.01 ± 0.4 ^{bcd}	6 ± 0.3 ^b
HM_A_rhizome	0.08 ± 0.01 ^c	3.5 ± 0.33 ^b	6 ± 0 ^f	0.88 ± 0.12 ^c	0.07 ± 0.01 ^{ad}	4.8 ± 1.1 ^b	16.4 ± 3.8 ^{cd}	3 ± 1.1 ^{bd}	1.6 ± 0.5 ^b	7 ± 1.6 ^{ab}
DCH_S_leaves	0.42 ± 0.03 ^e	5.5 ± 0.06 ^f	7.9 ± 0.6 ^c	0.27 ± 0.01 ^{bc}	0.16 ± 0.02 ^c	2.06 ± 0.5 ^{abc}	1.88 ± 0.04 ^{ac}	2.9 ± 0.2 ^{cd}	0.38 ± 0.11 ^a	0.23 ± 0.22 ^{ab}
DCH_S_petiole	0.23 ± 0.01 ^e	3.4 ± 0.2 ^d	3.5 ± 0.24 ^c	0.46 ± 0.004 ^b	1.5 ± 0.2 ^d	6 ± 1.4 ^b	2 ± 1.1 ^{bc}	1.1 ± 0.6 ^{ab}	1.06 ± 0.25 ^{cd}	0 ± 0 ^a
DCH_S_rhizome	1.13 ± 0.01 ^e	2.1 ± 0.2 ^a	4 ± 0.3 ^f	0.13 ± 0.005 ^a	0.02 ± 0 ^{acd}	0.02 ± 0 ^a	3.6 ± 0.7 ^{ab}	2.6 ± 0.32 ^{bcd}	0.61 ± 0.1 ^{ab}	0.4 ± 0.1 ^a
MD_S_leaves	0.23 ± 0.02 ^d	7.3 ± 0.06 ^g	1.2 ± 0.1 ^a	1.9 ± 0.01 ^g	0.17 ± 0.01 ^c	0.02 ± 0.01 ^a	3.74 ± 0.8 ^{abc}	2.7 ± 0.5 ^c	0.32 ± 0.05 ^a	0.35 ± 0.34 ^{ab}
MD_S_petiole	0.06 ± 0.01 ^a	5.2 ± 0.2 ^e	0.62 ± 0.02 ^a	1.7±0.08 ^d	0.14 ± 0.04 ^d	0.41 ± 0.19 ^a	0.5 ± 0.12 ^c	1.3 ± 0.3 ^{ab}	0.39 ± 0.1 ^{abcd}	0.25 ± 0.2 ^a
MD_S_rhizome	0.32 ± 0.02 ^{ab}	18 ± 0.54 ^f	1.04 ± 0.03 ^{bc}	1.7 ± 0.03 ^d	0.2 ± 0.01 ^{abc}	1.3 ± 0.2 ^a	0.95 ± 0.23 ^a	3 ± 0.01 ^{bd}	0.42 ± 0 ^a	15 ± 4 ^b
Mean_leaves	0.28 ± 0.03	2.8 ± 0.5	4.5 ± 1.14	0.74 ± 0.18	0.08 ± 0.01	1.96 ± 0.4	4.07 ± 0.48	1.8 ± 0.3	0.64 ± 0.2	0.76 ± 0.2
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).

Table 5 Translocation (TF) (Mean ± 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, localities with the same letter were not significantly different.

Locality	Al	As	Cr	Fe	Zn	Cu	Mn	Ni	Pb	Cd
LB_S	0.61 ± 0.02 ^{abc}	0.88 ± 0.34 ^a	0.8 ± 0.18 ^a	0.46 ± 0.01 ^a	0.43 ± 0.01 ^{ab}	1.2 ± 0.05 ^a	0.88 ± 0.05 ^a	1.8 ± 0.3 ^{ab}	0 ± 0 ^a	0.03 ± 0.01 ^a
LB_A	0.12 ± 0.03 ^b	0.38 ± 0.05 ^a	0.38 ± 0.16 ^a	1 ± 0.14 ^{ab}	0.32 ± 0.03 ^{ab}	1.3 ± 0.11 ^a	2.2 ± 0.51 ^{ab}	0.72 ± 0.01 ^{ab}	1.1 ± 0.2 ^{ab}	0.14 ± 0.1 ^a
VT_S	0.66 ± 0.03 ^{ac}	4.7 ± 2.9 ^a	0.83 ± 0.29 ^a	0.5 ± 0.08 ^a	0.7 ± 0.04 ^{ab}	2.1 ± 0.12 ^a	0.68 ± 0.02 ^a	0.72 ± 0.2 ^{ab}	1.3 ± 0.2 ^{ab}	0.05 ± 0.01 ^a
VT_A	0.13 ± 0.03 ^{bc}	5.9 ± 2.2 ^a	0.3 ± 0.08 ^a	0.61 ± 0.08 ^a	0.18 ± 0.01 ^a	2.2 ± 0.31 ^a	3 ± 0.92 ^{ab}	0.55 ± 0.1 ^{ab}	2.2 ± 0.7 ^b	0.12 ± 0.02 ^a
PC_S	0.65 ± 0.09 ^{ac}	1.9 ± 1.2 ^a	0.47 ± 0.09 ^a	0.89 ± 0.18 ^{ab}	1.4 ± 0.13 ^c	1.8 ± 0.14 ^a	0.69 ± 0.6 ^a	3.5 ± 0.7 ^c	1.24 ± 0.1 ^{ab}	0.69 ± 0.04 ^a
PC_A	2.2 ± 0.09 ^d	2.5 ± 1.1 ^a	0.34 ± 0.01 ^a	1.6 ± 0.3 ^{bc}	3.7 ± 0.4 ^d	2.2 ± 0.36 ^a	4 ± 0.84 ^b	0.88 ± 0.14 ^{ab}	1.3 ± 0.01 ^{ab}	0.94 ± 0.21 ^a
HM_S	0.44 ± 0.08 ^{abc}	1.8 ± 0.3 ^a	0.35 ± 0.16 ^a	0.32 ± 0.02 ^a	0.9 ± 0.03 ^{bc}	1.7 ± 0.03 ^a	1.1 ± 0.01 ^a	0.20 ± 0.13 ^{ab}	1.13 ± 0.3 ^{ab}	0 ± 0 ^a
HM_A	0.5 ± 0.16 ^{abc}	0.4 ± 0.01 ^a	0.48 ± 0.22 ^a	2.4 ± 0.3 ^c	0.74 ± 0.1 ^{abc}	2.1 ± 0.20 ^a	2.9 ± 0.4 ^{ab}	1.5 ± 0.5 ^{ab}	2 ± 0.7 ^b	0.48 ± 0.1 ^a
DCH_S	0.94 ± 0.09 ^a	0 ± 0 ^a	0.55 ± 0.13 ^a	0.37 ± 0.03 ^a	3 ± 0.16 ^e	1.9 ± 0.03 ^a	2 ± 0.01 ^{ab}	1.15 ± 0.2 ^{ab}	0.6 ± 0.1 ^{ab}	0.55 ± 0.5 ^a
ZL_S	0.92 ± 0.21 ^a	0 ± 0 ^a	0.05 ± 0.01 ^a	0.51 ± 0.1 ^a	2 ± 0.0 ^e	1.5 ± 0.29 ^a	1.4 ± 0.1 ^a	0.08 ± 0.01 ^b	1.2 ± 0.5 ^{ab}	0.02 ± 0.02 ^a
MD_S	0.81 ± 0.02 ^a	0 ± 0 ^a	4.4 ± 1.94 ^b	0.7 ± 0.13 ^a	0.4 ± 0.02 ^{ab}	1.2 ± 0.06 ^a	1.1 ± 0.02 ^a	0.83 ± 0.2 ^{ab}	0.8 ± 0.11 ^{ab}	0.03 ± 0.03 ^a
Mean	0.73 ± 0.12	1.7 ± 0.5	0.81 ± 0.28	0.86 ± 0.14	1.3 ± 0.24	1.7 ± 0.09	1.9 ± 0.24	1.1 ± 0.2	1.17 ± 0.15	0.28 ± 0.1
<i>P</i> -value	0.001	0.060	0.031	< 0.001	< 0.001	< 0.040	0.011	0.001	0.040	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).

Figures

Figure 1

Locations of studied sites in the Krkonoše (Giant) Mountains, Czech Republic, Ramsau am Dachstein and Zillertal in Austria, and Madesimo, Italy, where *Rumex aphinus* samples were collected. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Studied organs of *Rumex alpinus*: (a) emerging (b) mature, and (c) senescent leaf blades, (d) petioles from mature leaves, (e) stems from flowering plants, and (f) two years old rhizome.

Figure 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 concentration of elements in individual organs' overall localities was evaluated by One-way ANOVA. Using the Tukey post hoc test, 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).

Figure 4

Effect of locality on the concentration (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, 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).

Figure 5

Effect of locality on the concentration (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 concentration of elements in individual organs' overall localities was evaluated by One-way ANOVA. Using the Tukey post hoc test, 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).

Figure 6

Effect of locality on the concentration (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 concentration of elements in individual organs' overall localities was evaluated by One-way ANOVA. Using the Tukey post hoc test, 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).

Figure 7

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

Figure 8

Effect of term on the concentration (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.

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