CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF ENVIRONMENTAL SCIENCES

DEPARTMENT OF ECOLOGY

Ecology and genetic variability of *Rumex alpinus* L.

Ekologie a genetická variabilita *Rumex alpinus* L.

/PhD Thesis/

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Abstract

Rumex alpinus L. (*R. alpinus*) is a nonnative and invasive plant in the Krkonoše Mountains and other mountain ranges of the Czech Republic. Given the importance of understanding not only the behavior but also the ecology of invasive plants and their genetic variability, it was decided to focus on this topic in the doctoral thesis. The information obtained can then contribute to the establishment of effective management strategies in combating this invasive species. The ecology, or the nutrient requirements and accumulation of micro and macro elements in the organs of *R. alpinus* in relation to its habitat conditions, has not been described thus far. Given that *R. alpinus* is predominantly found in eutrophicated soils, it is crucial to understand its precise nutrient requirements and how to manage them *(Paper 1: Jungová et al. 2023, Journal of Soil Science and Plant Nutrition volume 23, 469–484).* The results showed the contents of total and plant available macroelements in the soils refected high variability with localities. There was a significant effect of locality and plant organs on the element contents, indicating differences in nutrition. *R. alpinus* exhibited higher N and P contents in emerging and mature leaves than in the senescent compared to Ca and Mg. The N:P and N:K ratios in the mature leaves were within the normal range but were indicative of comparatively higher demand for P. The mean resorption efficiency for N, P, and K in mature leaves was 52, 50, and 22%, respectively, lower than values for most terrestrial plants (62, 65, and 70%). The relatively high availability of macroelements in soils and plant organs with low N, P, and K resorption efficiency indicates a high N, P, and especially K-demanding species of *R. alpinus*.

Considering that *R. alpinus* is a plant-primarily occurring on anthropogenically influenced soils, which can be contaminated with risk elements even in mountainous areas, it is important to know whether this plant accumulates them. This is particularly relevant as *R. alpinus* is still utilized as a vegetable or fodder in the Balkan Peninsula and the Carpathians *(Paper 2: Jungová et al. 2022, Plant and Soil 477, 553–575).* The results showed the level of elements accumulation by *R. alpinus* was considerably different, depending on local geological substrates and environmental conditions. *R. alpinus* has considerable tolerance to Zn, Cu, As, Cr, Ni, with an easy accumulation strategy. High Al and Cd content 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, the results showed that *R. alpinus* is an excluder. *R. 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.

Furthermore, historical literature suggests that *R. alpinus* was likely introduced to the Czech mountains by colonists from the Alps in the 16th century. At the same time, it is considered a native species in the Polish Krkonoše Mountains. Therefore, we also raise the question of how *R. alpinus* made its way to the Krkonoše Mountains - from the Alps or from Poland (*Paper 3: Jungová et al. 2023, Ecology and Evolution, 13:e10145*).The results of AMOVA showed a high 60% variation within populations, 27% variation among groups (mountains), and 13% among the population within groups (mountains). The overall unbiased gene diversity was high ($\hat{h} = 0.55$). The higher level of genetic differentiation among populations ($F_{ST} = 0.35$; $p < 0.01$) indicated restricted gene flow between populations. Compared to native populations, limited genetic variability was observed in the nonnative populations. It was concluded that local adaptation, low gene exchange, and genetic drift affected the genetic diversity of nonnative *R. alpinus*. The results support a genetic link between the Alpine and Czech genotypes of *R. alpinus,* while the Carpathians genotypes corresponded to the Balkan genotype.

Keywords: micro and macroelements; nutrient resorption; genetic variability; invasive plant; weed species

Abstrakt

Rumex alpinus L. (*R. alpinus*) představuje nepůvodní a invazní rostlinu v Krkonoších a v dalších pohořích České republiky. Vzhledem k důležitosti porozumění nejen chování, ale i ekologii invazních rostlin a jejich genetické variability, bylo rozhodnuto zaměřit se v disertační práci právě na toto téma. Získané informace pak mohou přispět ke stanovení účinných strategií managementu v boji proti tomuto invaznímu druhu.

Dosud nebyla podrobně popsána ekologie *R. alpinus*, včetně resorpce a využití živin, akumulace mikro a makroprvků v rostlinných orgánech, a to vše v souvislosti s místními podmínkami výskytu. Vzhledem k tomu, že se *R. alpinus* převážně vyskytuje na eutrofních půdách, je důležité porozumět jeho přesným nutričním požadavkům a způsobům jejich využití (*Výsledek 1: Jungová et al. 2023, Journal of Soil Science and Plant Nutrition volume 23, 469–484*). Výsledky ukázaly vysokou variabilitu obsahu totálních a rostlinami přístupných makroprvků v půdách a mezi lokalitami. Byl pozorován významný vliv lokalit na obsah makroprvků v různých orgánech *R. alpinus*, což naznačuje rozdíly ve výživě. *R. alpinus* vykazoval vyšší obsah N a P v mladých a dospělých listech než v senescentních, ve srovnání s Ca a Mg. Poměry N:P a N:K v mladých listech se pohybovaly v normálním rozmezí, ale naznačovaly relativně vyšší poptávku po fosforu. Průměrná účinnost resorpce N, P a K byla u mladých listů 52 %, 50 % a 22 %, což je nižší než u většiny suchozemských rostlin (62 %, 65 % a 70 %). Relativně vysoká dostupnost makroelementů v půdě a nízká účinnost resorpce N, P a K naznačuje, že *R. alpinus* je druh s vysokou poptávkou po N, P a zejména pak po K.

Vzhledem k tomu, že se *R. alpinus* vyskytuje převážně na antropogenně ovlivněných půdách, které mohou být kontaminovány rizikovými prvky i v horských oblastech, bylo důležité zjistit, zda tato rostlina tyto prvky akumuluje. To je zvláště relevantní v souvislosti s tím, že se *R. alpinus* stále využívá jako zelenina či krmivo na Balkánském poloostrově a v Karpatech (*Výsledek 2: Jungová et al. 2022, Plant and Soil 477, 553–575*). Výsledky ukázaly, že úroveň akumulace rizikových prvků u *R. alpinus* byla výrazně odlišná v závislosti na místním geologickém podloží a environmentálních podmínkách. *R. alpinus* projevoval vysokou toleranci vůči Zn, Cu, As, Cr a Ni a snadnou akumulační strategii. Vysoký obsah Al a Cd v podzemní biomase (oddenky) naznačuje obranný mechanismus pro tyto prvky. Přestože nadzemní biomasa (vzcházející, zralé, senescentní listy a řapíky) vykazuje určitou míru akumulace rizikových prvků, výsledky ukázaly, že *R. alpinus* je druhem s excluder strategií. *R. alpinus* neakumuluje rizikové prvky v orgánech (vzcházející,

zralé listy a řapíky), které se konzumují v souladu s přípustným limitem stanoveným Světovou zdravotnickou organizací (WHO 2001) a tyto orgány tedy mohou být používány bez obav.

Historická literatura uvádí, že *R. alpinus* byl pravděpodobně introdukován do Krkonoš v 16. století kolonisty z Alp. Na druhou stranu je tato rostlina považována za domácí druh v polských Krkonoších. Byla položena hypotéza, odkud se tedy *R. alpinus* do Krkonoš dostal - z Alp nebo z Polska (*Výsledek 3: Jungová et al. 2023, Ecology and Evolution, 13:e10145*).Výsledky analýzy molekulární variance (AMOVA) ukázaly vysokou variabilitu (60 %) uvnitř populací, 27 % variabilitu mezi skupinami (horami) a 13 % mezi populacemi uvnitř skupin (hor). Celková genetická rozmanitost byla vysoká ($\hat{h}$ = 0,55). Vyšší úroveň genetické diferenciace mezi populacemi ($F_{ST} = 0.35$; $p < 0{,}01$) naznačovala omezený genetický tok mezi populacemi. V porovnání s původními populacemi byla pozorována omezená genetická variabilita v nepůvodních populacích *R. alpinus*. Bylo závěrem konstatováno, že místní adaptace, nízká genová výměna a bottleneck efekt ovlivnily genetickou rozmanitost nepůvodního druhu *R. alpinus*. Výsledky dále podporují genetickou spojitost mezi alpskými a českými genotypy *R. alpinus*, zatímco genotypy z Karpat odpovídaly balkánskému genotypu.

Klíčová slova: mikro a makroprvky; absorpce živin; genetická variabilita; invazní rostliny; plevelné druhy

CONTENT

1. GENERAL INTRODUCTION

INTRODUCTION IN ENGLISH

The Polygonaceae family's Alpine dock (*Rumex alpinus* L.) is native to Western, Central, and Eastern European high mountains, including the Iberian and Apennine Peninsula, the Alps, the Carpathians, and the Balkan Peninsula. In the Krkonoše (Giant) mountains of the Czech Republic, the introduction of *R. alpinus* was associated with German-speaking colonists from the Alps in the sixteenth century AD, used for the treatment of different diseases, such as diarrhea, dysentery, stomach problems, and kidney disorders. It has also been cooked as a vegetable, such as salad, spinach, rhubarb, or as a forage crops. Today, *R. alpinus* is considered a troublesome weedy species which infests abandoned grasslands, roads margins, and nutrient-rich sites: typically livestock resting areas, surroundings of mountain farms, and downslope of mountain chalets grasslands, with deposits of animal feces, and nutrient-rich wastewater.

As part of this doctoral thesis, it has been decided to study the ecology of the invasive plant *R. alpinus* in the Krkonoše Mountains. Since *R. alpinus* is an invasive species, all available information about this plant and its behavior can be very useful for proposing measures for its eradication or proper utilization. *R. alpinus* is a plant associated with human settlements and areas influenced by human activities. Considering that it occurs in mountainous areas, which are often protected as nature reserves or national parks, it is important to determine if its spread could become more significant than it has been so far. *R. alpinus* also grows on mountain meadows that have been historically used for grazing or are still influenced by grazing. Due to its expansive spread, there are concerns that it may significantly reduce the biological variability of habitats and, especially, biodiversity. We were interested in whether it can expand to habitats with lower nutrient availability, as many rare mountain plant species occur specifically in these oligotrophic soils. This led us to the question of how *R. alpinus* processes macroelements (N, P, K, Ca, and Mg), how it manages them, and whether it stores them or wastes them unnecessarily. For these reasons, an article titled "**Distribution and Resorption Efficiency of Macroelements (N, P, K, Ca, and Mg) in Organs of *Rumex alpinus* L. in the Alps and the Giant (Krkonoše) Mountains**" (*Jungová et al. 2023, Journal of Soil Science and Plant Nutrition volume 23, 469–484*) was created, which provided us with answers to these questions. We were also interested in whether *R. alpinus*, growing on soils influenced by human activities that may be contaminated with hazardous elements, accumulates these microelements in its organs. This question is particularly relevant because it is still consumed in the Balkans and Carpathians. The possibility of using *R. alpinus* as a phytoremediation plant has also been considered, considering its biomass size if it accumulates risk elements. Based on these questions, a second article titled "**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**" (*Jungová et al. 2022, Plant and Soil 477, 553–575*) was created. After two articles focusing mainly on the micro and macroelements contained in the organs of *R. alpinus*, it was also decided to explore the genetic aspect of this plant. Information obtained through molecular methods, such as genetic variability, which reflects, among other things, the plant's reproductive modes, can be useful in determining suitable management strategies. We were particularly interested in whether *R. alpinus* originates from the Alps and how it spread to the Krkonoše Mountains. Historical literature suggests that settlers from the Alps arrived in the Krkonoše Mountains in the 16th century and brought *R. alpinus* as a vegetable plant. Since the plant spread throughout Europe mainly through human intervention, the question arose whether it could have reached the Czech side of the Krkonoše Mountains through the Polish side. In Poland, where the Krkonoše Mountains and the Carpathians are located, *R. alpinus* is considered a native plant. Therefore, it was decided to use SSR markers to determine this species' true origin and genetic variability. The result of these considerations was a third article titled "**Origin and Genetic Variability of Populations of the Invasive Plant *Rumex alpinus* L. in the Giant (Krkonoše) Mountains**" (*Jungová et al. 2023, Ecology and Evolution, 13:e10145*).

ÚVOD V ČESKÉM JAZYCE

Šťovík alpský (*Rumex alpinus* L.) z čeledi Polygonaceae, má původ ve vysokých horách západní, střední a východní Evropy, včetně Pyrenejského a Apeninského poloostrova, Alp, Karpat a Balkánského poloostrova. Do Krkonoš v České republice byl zavlečen německy mluvícími kolonisty z Alp v 16. století a používal se k léčbě různých onemocnění, jako jsou onemocnění ledvin, střevní a žaludeční potíže. Byl též připravován jako zelenina, například ve formě salátu, špenátu, či jako náhražka rebarbory do koláčů. Dále byl využíván jako krmivo pro dobytek v sušené formě. V současnosti je *R. alpinus* považován za obtížný plevel, který expanduje na opuštěných loukách, okrajích cest a místech bohatých na živiny, jako jsou odpočívadla dobytka, okolí horských pastvin a horských chat se zbytky zvířecího trusu a odpadní vodou.

V rámci této disertační práce bylo rozhodnuto zkoumat ekologii invazní rostliny *R. alpinus* v Krkonoších. Vzhledem k tomu, že *R. alpinus* je invazní druh, veškeré dostupné informace o této rostlině a jejím chování mohou být velmi užitečné při navrhování opatření k její likvidaci nebo k jinému využití. *R. alpinus* je rostlinou spojenou s lidským osídlením a oblastmi ovlivněnými lidskou činností. Vzhledem k tomu, že se vyskytuje v horských oblastech, které jsou často chráněny jako přírodní rezervace nebo národní parky, je důležité zjistit, zda se jeho šíření může stát významnějším, než bylo doposud. Šťovík alpský roste na horských loukách, které byly historicky využívány k pastvě dobytka nebo jsou stále ovlivňovány pastvou. Kvůli svému expanzivnímu šíření existují obavy, že může významně omezit biologickou rozmanitost stanovišť a zejména biodiverzitu. Zajímalo nás, zda může proniknout na lokality s nižší dostupností živin, protože v těchto oligotrofních půdách se vyskytuje mnoho vzácných horských druhů rostlin. To nás následně přivedlo k otázce, jak *R. alpinus* zpracovává živiny (N, P, K, Ca a Mg), jak s nimi nakládá a zda je ukládá nebo s nimi plýtvá. Z těchto důvodů byl sepsán článek s názvem "**Distribution and Resorption Efficiency of Macroelements (N, P, K, Ca, and Mg) in Organs of *Rumex alpinus* L. in the Alps and the Giant (Krkonoše) Mountains**" (*Jungová et al. 2023, Journal of Soil Science and Plant Nutrition, svazek 23, 469–484*), který nám poskytl odpovědi na tyto otázky. Zajímali jsme se také o to, zda *R. alpinus*, když roste na půdách ovlivněných lidskou činností, které mohou být kontaminovány rizikovými prvky, akumuluje tyto mikroprvky v orgánech. Tato otázka je zvláště relevantní, protože je jako zelenina stále konzumován na Balkáně a v Karpatech. Byla také zvažována možnost využití *R. alpinus* jako rostliny pro fytoremediaci, s ohledem na velikost její biomasy a pokud je akumulátorem rizikových prvků. Na základě těchto otázek vznikl druhý článek s názvem "**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**" (*Jungová et al. 2022, Plant and Soil 477, 553–575*).

Po dvou článcích se zaměřením především na mikro a makroprvky obsažené v orgánech *R. alpinus* bylo také rozhodnuto prozkoumat genetický aspekt této rostliny. Informace získané prostřednictvím molekulárních metod, jako je genetická variabilita, která odráží mimo jiné i reprodukční způsoby rostlin, mohou být užitečné při určování vhodných strategií managementu. Zvláště nás zajímalo, zda skutečně *R. alpinus* pochází z Alp a zda se z nich dostal do Krkonoš. Historická literatura naznačuje, že kolonisté z Alp dorazili do Krkonoš v 16. století a přinesli si *R. alpinus* jako užitkovou rostlinu. Avšak, vzhledem k tomu, že se rostlina šířila po celé Evropě především prostřednictvím lidské činnosti, vznikla otázka, zda se nemohla dostat na českou stranu Krkonoš z Polska. V Polsku, je totiž *R. alpinus* považován za původní rostlinu. Přesněji je to dáno tím, že v Polsku se vyskytují též Karpaty, kde je šťovík alpský původní, a proto by mohlo být možné, že se do Krkonoš dostal z Karpat. Bylo rozhodnuto, prostřednictvím dostupných molekulárních markerů, určit skutečný původ a genetickou variabilitu této rostliny. Výsledkem těchto úvah byl třetí článek s názvem " **Origin and Genetic Variability of Populations of the Invasive Plant *Rumex alpinus* L. in the Giant (Krkonoše) Mountains** " (*Jungová et al. 2023, Ecology and Evolution, 13:e10145*).

2. AIMS OF THE THESIS

The dissertation's primary focus is investigating the ecology of the invasive plant species *R. alpinus*, known for being a problematic weed. The objective is to enhance our understanding of the species' ecological behavior in permanent grasslands and other areas where it is found. Additionally, the dissertation aims to contribute new knowledge regarding the accumulation of risk elements in *R. alpinus* organs across various mountainous regions in Europe. Furthermore, it seeks to explore the utilization of macro-elements by the species in nutrient-rich mountain habitats, providing valuable insights in this regard. It also focuses on determining its origin in the Czech Republic and the genetic variability in European mountains.

Paper 1 Distribution and Resorption Efficiency of Macroelements (N, P, K, Ca, and Mg) in Organs of *Rumex alpinus* L. in the Alps and the Giant (Krkonoše) Mountains

This paper focuses on the distribution and resorption of nutrients (N, P, K, Ca, and Mg) by *R. alpinus* in its different organs and at different mountain sites in Europe and answers the following research questions.

Q1 How can the variability of soil chemical properties affect *R. alpinus* stand from different localities?

Q2 How different are the contents of N, P, K, Ca, and Mg and the N:P, N:K, and P:K ratios in various organs of *R. alpinus* in different localities?

Q3 How intensive is the resorption of N, P, K, Ca, and Mg from senescent to young leaves in *R. alpinus*?

Paper 2 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

This paper investigated the safety of consuming *R. alpinus* from the Krkonoše Mountains (Czech Republic), and the Alps (Austria and Italy). Because in the Carpathians and Balkan Mountains, some organs of this species have been and recently are used as vegetables, addressed the following research questions.

Q1 To what extent can accumulate micro-and risk elements?

Q2 Which organs of *R. alpinus* are accumulators of micro-and risk elements?

Paper 3 Origin and Genetic Variability of Populations of the Invasive Plant *Rumex alpinus* L. in the Giant (Krkonoše) Mountains

This paper main aim was to verify whether *R. alpinus* was introduced into the Krkonoše Mountains by alpine colonists or whether it was anthropogenically introduced from the Polish Carpathians Mountains. Furthermore, the genetic structure of native and introduced populations of *R. alpinus* was determined and answered the following research questions.

Q1 *R. alpinus,* whose European distribution is determined by human activity, was introduced into the Czech part of the Krkonoše Mountains via the Polish part of the Krkonoše Mountains (Karkonosze Mountains) from the Carpathians or the Austrian regions of Alp?

Q2 Do differences in genetic diversity exist within native and nonnative habitats?

Q3 Does population structure reflect geographic distances?

OTÁZKY V ČESKÉM JAZYCE

Primárním zaměřením disertační práce je zkoumání ekologie invazního druhu *R. alpinus* v Krkonoších, což je současně v Evropě problematický plevel. Cílem této práce je zlepšit naše chápání ekologického chování tohoto druhu v trvalých travních porostech a na dalších místech, kde se vyskytuje. Disertační práce si rovněž klade za cíl přinést nové poznatky týkající se například akumulace rizikových prvků v orgánech *R. alpinus* na různých horských lokalitách v Evropě. Kromě toho zkoumá využití makroprvků (N, P, K, Ca a Mg) tímto druhem na horských lokalitách s bohatými zdroji živin a přináší cenné poznatky v tomto ohledu. Dalším zaměřením práce je určení genetického původu *R. alpinus* v České republice a analýza jeho genetické variability v horách Evropy.

Článek 1 Distribution and Resorption Efficiency of Macroelements (N, P, K, Ca, and Mg) in Organs of *Rumex alpinus* L. in the Alps and the Giant (Krkonoše) Mountains

Tento článek se zaměřuje na distribuci a resorpci živin (N, P, K, Ca a Mg) *R. alpinus* v jeho různých orgánech a na různých horských lokalitách v Evropě a odpovídá na následující výzkumné otázky.

Q1 Jak může variabilita půdních chemických vlastností ovlivnit obsah prvků v rostlinných orgánech *R. alpinus* z různých lokalit?

Q2 Jak se liší obsahy N, P, K, Ca a Mg a poměry N:P, N:K a P:K v různých orgánech *R. alpinus* a z různých lokalit?

Q3 Jak intenzivní je resorpce N, P, K, Ca a Mg ze senescentních a mladých listů u *R. alpinus*?

Článek 2 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

Tato práce zkoumala bezpečnost konzumace *R. alpinus* z Krkonoš (Česká republika) a Alp (Rakousko a Itálie). Vzhledem k tomu, že na karpatských a balkánských lokalitách některé orgány tohoto druhu byly a neustále jsou využívány jako zelenina, byly položeny následující výzkumné otázky.

Q1 Do jaké míry se mohou akumulovat mikro- a rizikové prvky?

Q2 Které orgány *R. alpinus* jsou akumulátory mikro- a rizikových prvků?

Článek 3 Origin and Genetic Variability of Populations of the Invasive Plant *Rumex alpinus* L. in the Giant (Krkonoše) Mountains

Hlavním cílem této práce bylo ověřit, zda byl *R. alpinus* zavlečen do Krkonoš alpskými kolonisty nebo zda byl zavlečen antropogenně z polských Karpat. Dále byla stanovena genetická struktura původních a introdukovaných populací *R. alpinus* a zodpovězeny následující výzkumné otázky.

Q1 *R. alpinus*, jehož evropské rozšíření je dáno lidskou činností, byl do české části Krkonoš zavlečen přes polskou část Krkonoš (Karkonosze) z Karpat nebo z rakouských oblastí Alp?

Q2 Existují rozdíly v genetické rozmanitosti v původních a nepůvodních populacích?

Q3 Odráží struktura populace geografické vzdálenosti?

3. LITERATURE REVIEW

Rumex alpinus L. (Polygonaceae) is a perennial species consisting of a horizontal rhizome, aboveground vegetative shoots with three to five large leaves, and fertile stems bearing smaller leaves with several thousand flowers and fruits (Klimeš 1992; Šťastná et al. 2010). It grows along riverbanks, on nutrient-rich soil, and in moderately moist abandoned pastures and meadows. The plant is native to the mountains of Central and Southern Europe, and its spread to other regions has been partly influenced by its historical use as a utility plant and medicinal herb (Maude and Moe 2005; Šťastná et al. 2010).

3.1. Taxonomy

Rumex alpinus L., Sp. Pl.: 334. 1753.

Syn.: *Lapathum alpinum* (L.) Lam., Fl. Franc-. 3: 7. 1779. – *Acetosa alpina* (L.) Moench, Menth. Pl. 357. 1794. – *Rumex pseudoalpinus* Hofft, Catal. Pl. Kursko: 26. 1826.

Names in other languages:

German: Alpen-Ampfer, Mönchs-Rhabarber

English: Monk's Rhubarb, Alpine dock

French: Rumex des Alpes, Rhubarbe des moines, Rhapontique des moines

Italian: Rabarbaro alpino

Spanish: Ruibarbo de monjes, Rapontico

Polish: Szczaw alpejski

Czech: Šťovík alpský

Kingdom: Plantae - plants

Subkingdom: Tracheobionta - vascular plants

Division: Magnoliophyta - flowering plants

Class: Rosopsida - higher dicotyledons

Order: Caryophyllales - carnation-like

Family: Polygonaceae – smartweed-buckwheat family

Genus: *Rumex* L. - dock

Species: *Rumex alpinus* L. - alpine dock, šťovík alpský

3.2. Rhizome

Monopodial branching rhizomes are dark brown and consist of basal parts of shoots, from which rootlets anchor into the ground. The rhizomes typically grow at a depth of 5 cm (Rudyshin and Tsarik 1982; Klimeš 1992), occasionally up to 10 (-12) cm (Kliment 1988). The average depth is less than 20 cm, as *R. alpinus* struggles to regenerate when located below 20 cm (Klimeš et al. 1993). The rhizomes are evenly segmented and have a dark orange, oval, and flattened cross-section in the horizontal plane. Each segment corresponds to one vegetative season (Figure 1), which is also reflected in annual growth increments (Šmarda 1963; Klimeš 1992). This enables a straightforward estimation of the precise age of individual senesced rhizome segments. However, this applies only to plants that originate from seeds and have the oldest part of the rhizome containing the primary taproot preserved (Klimeš 1992).

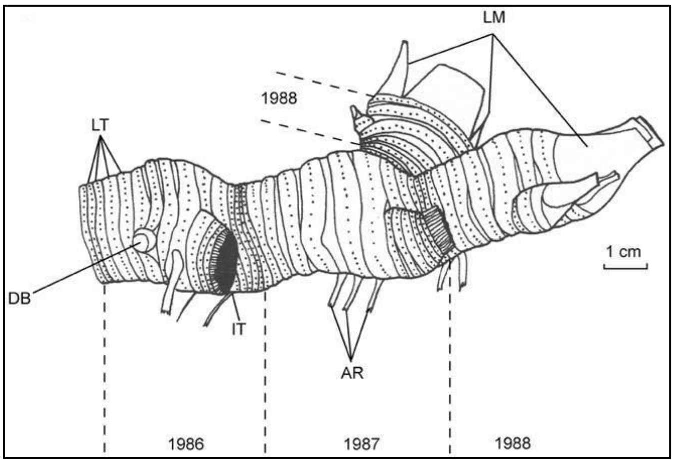

Figure1: Individual rhizome segments (Klimeš, 1992)

Most of these segments develop in the middle of the growing season (summer), while the tapered parts are formed in spring and autumn (Šmarda 1963). The length of rhizome segments is approximately 4 cm, and the width is 2-3 cm (Klimeš 1992). Up to six large lateral buds form on each rhizome segment, but only one to two produce leaves in the current year. In the following year, these buds either remain dormant or continue their growth, giving rise to lateral branches bearing leaves or flowers. If the apical meristem of the rhizome is damaged, one or several lateral buds, typically the youngest ones located next to the former apical meristem, become activated (Klimeš 1992).

Rhizome branching typically occurs from the second year of plant life, more intensively at the edge rather than within dense stands of *R. alpinus* (Klimeš 1992). The branching angle is usually right-angled (Šmarda 1963). In the case of a branched rhizome, the gradual decay of the oldest parts leads to fragmentation into physically independent ramets and the formation of clonal fragments (Figure 2).

Individual rhizome segments are horizontally marked by leaf scars. As the rhizomes initiate petiole attachment, leaf scars with remnants of vascular bundles are preserved around the rhizome segments, forming distinctly visible rings. The widest leaf scars are found in the strongest part of the rhizome segment, gradually narrowing towards the segment's tapered ends. Each segment bears a specific number of scars, corresponding to the number of leaves initiated within a particular year (Klimeš 1992).

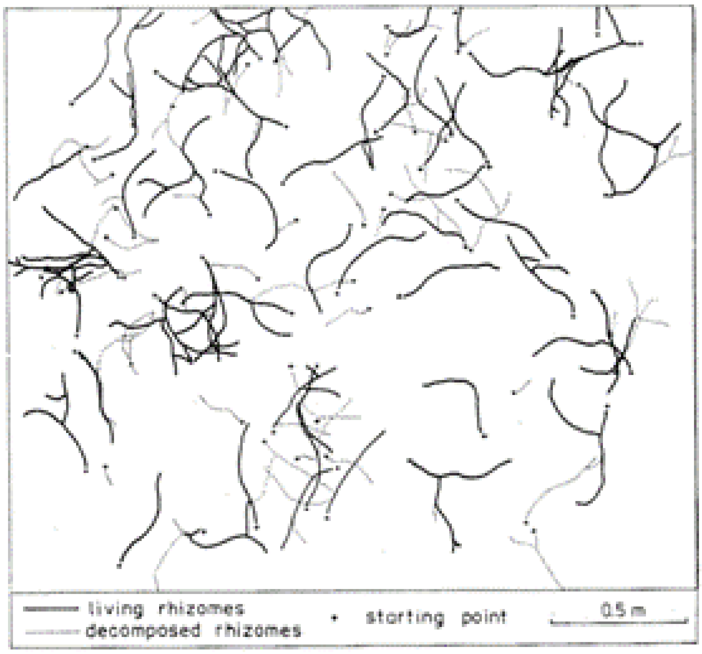

Figure 2: Spatial distribution of rhizomes of *R. alpinus* according to Klimeš (1992).

Remnants of leaf bases are usually preserved for several weeks to months (Šmarda 1963). If the rhizomes overlap each other, their internodes can elongate by several centimeters (Klimeš et al. 1993). Scars indicating the previous positions of flowering and fruiting shoots are preserved on the side of the rhizome throughout its life. According to Klimeš (1992), this "recorded" information on the rhizome can be used for retrospective studies of plant populations. Based on data from the Krkonoše Mountains, Low Tatras, and the Alps, segments persist in the soil for an average of 13 years (Grabherr and Mucina 1993). An exception was recorded by Šmarda (1963) from the High Tatras, which reported an average rhizome age of only eight years. The maximum persistence of a segment Klimeš (1992) observed in the Krkonoše Mountains was 20 years.

Šťastná et al. (2012) reported finding a 35-year-old rhizome in the Low Tatras (at the highest location - 1900 m above sea level). Longer persistence of segments in the soil is generally observed at higher elevations. The length of the rhizome depends on the plant's age and local conditions. Šmarda (1963) recorded an average length of living rhizomes of about 45 cm and nearly 20 cm for preserved dead rhizome parts. The length of a 35-year-old rhizome from the Low Tatras was approximately 120 cm (Šťastná et al. 2010).

3.3. Roots

The primary root, which has a diameter of 0.15-0.25 mm in seedlings, loses its dominant role in the second year of plant life but often remains preserved for more than ten years. Adventitious roots develop in the upper third of the rhizome segment in a given year, with a density of 5-6 (7) per 1 cm. At the branching point, up to 15 or more rhizomes, each 1 cm long, may grow, but only some survive the following year. The roots of adult plants mostly emerge from the lateral and lower parts of the rhizomes, with a diameter of approximately 10 mm (Kutschera and Lichtenegger 1992). The total reported length of roots by Rudyshin and Tsarik (1982) is up to 100 cm, while according to Kutschera and Lichtenegger (1992), it can reach up to 300 cm. The highest underground biomass is typically found at a depth of 20-40 cm (Šmarda 1963). The width of the root system was reported to be up to 229 cm in a plant from the Karawanken region (Kutschera and Lichtenegger 1992).

The roots are fleshy and tough, ranging in color from creamy white (young) to medium brown, sometimes slightly reddish at the base (Kubát 1990). Young roots are among the main nutrient absorbers (Klimešová and Klimeš 1997).

3.4. Leaves

Basal leaves are round-heart-shaped with wavy margins, reaching up to 50 cm in length and 20 cm in width, with 70-80 cm long petioles (Šťastná et al. 2010). The leaf apex is rounded, and the margin is flat or slightly wavy, with a broad and deeply ovate base and rounded basal lobes. Leaves also occur on flowering shoots but remain alive only during the flowering period, and later, during seed maturation, they wither. These leaves vary in size and shape: the leaves from the basal part of the flowering shoot are similar to those arising from the apical meristem on the rhizome, the leaves from the middle part are smaller and rounded at the base, and the leaves in the upper part are wedge-shaped and reduced to a few centimeters (Kubát 1990; Šťastná et al. 2010). The youngest leaves have a transparent intricate, and mossy membrane to which the base of the previous leaf adheres. The membrane remains attached to the leaf until it grows to about 20 cm (Šmarda 1963) or less, depending on the final size of the leaf. Leaf bases protect the apical rhizome bud, and axillary buds occur in the axils of supporting leaves (Šťastná et al. 2010).

3.5. Reproductive Organs

3.5.1. Flowers

Flowering shoots arise from lateral branches (dicyclic shoots) in the previous year, and their apical meristem changes from vegetative to flowering in autumn (Šťastná et al. 2010). Usually, one or two, occasionally four, flowering shoots grow from the rhizome segment. The flowering shoots are robust, leafy but without a basal rosette, longitudinally ribbed, approximately 30-200 cm tall, erect, shortly papillose to glabrous. The inflorescence arising at the stem tips is a vigorous, leafless, densely branched spike about half the length of the stem. The flowers with filiform stalks occur in numerous clusters. Usually, the flowers are hermaphroditic, but some plants are polygamous, with male sterile flowers developing at the base of the inflorescence and female sterile flowers at the top (Šťastná et al. 2010). According to Klimeš (1994), functional polygamous flowers, andromonoecy, gynomonoecy, and even dioecy can strongly prevail in some inflorescences. The flowers near the base of the inflorescence bloom first, while the flowers at the top develop last (Šťastná et al. 2010).

The size of the flower ranges from 4 to 5.5 mm, the petals are green and without a callus, and the outer perianth is elongated, adjacent to the inner perianth (Šťastná et al. 2010). The flowers are wind-pollinated, and for cross-pollination with foreign pollen, the stigmas mature two to three days earlier than the anthers. Three outer tepals wither, and three inner ones transform into heart-shaped, pointed, entire, or slightly toothed along the edges, about 5 mm long and 4 mm wide nectaries, mostly without papillae. They contain seeds that are 3 mm long, dark ochre-colored achenes. The fruit stalks beneath the nectaries are inflated like a bulb. The plants start flowering in June and fruiting in July. According to Bucharová (2003), a robust plant can produce 3.200 seeds, while Handlová (2003) reported 6.000-18.000 seeds, and Malinowskij et al. (1982) mention 11.500 seeds (in the Ukrainian Carpathians), gradually falling off the stems.

3.5.2. Seeds

R. alpinus seeds do not have primary dormancy and can germinate at different times with sufficient light (Šťastná et al. 2010). Seeds of species that thrive in highly humid environments often demand a higher germination temperature. However, an experiment conducted by Jungová contradicted this notion, as *R. alpinus* seeds exhibited a 90% germination rate even at lower temperatures (Jungová, unpublished data, Figure 3).

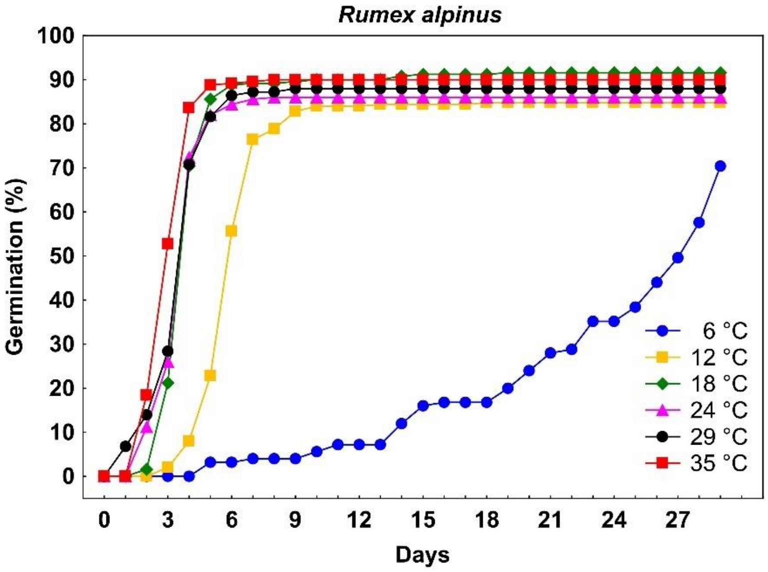

Figure 3: Germination of *R. alpinus* at different temperatures

According to Klimeš (1992), seed germination of *R. alpinus* is significantly promoted by grazing domestic animals, primarily cattle, whose hooves disturb the cover and whose excrement creates an ideal space for germination. Young seedlings of *R. alpinus* are rarely found in dense grassland communities, likely due to low shade tolerance (Klimeš 1992) and interspecific competition with grass species (Červenková and Münzbergová 2009). Furthermore, the results of Červenková and Münzbergová (2009) revealed that *R. alpinus* seeds have a pronounced dispersal potential. They observed a significant increase in new *R. alpinus* plants along streams, and their invasion progresses up to 100 m each year, facilitated by the downstream spread. Moreover, the study by Červenková and Münzbergová (2009) showed that new seedlings are more commonly found along stream banks rather than in meadows. Demographic measurements of *R. alpinus* populations demonstrated a higher generative growth rate for plants along streams, as seeds along streams germinate better due to moisture and less competition from surrounding vegetation (Červenková and Münzbergová 2009). Seed dispersal in the Krkonoše Mountains occurs up to a distance of 20 m from watercourses. Nathan (2006) defined the boundary of long-distance dispersal as the absolute distance within which plants can spread based on their anatomical and morphological characteristics. Similar results (26 m) were obtained from seed release experiments in the field, simulating the spread of docks under altered conditions (Bucharová

2003). However, based on these results, it cannot be universally concluded that dock seeds disperse up to approximately 25 m and no further. This is contradicted by the findings of Kubátová's (1994) thesis, where the related species *Rumex longifolius* was found up to 50 m away from the source in Kopecký's ramparts.

3.6. Distribution of *Rumex alpinus*

3.6.1. Worldwide

3.6.1.1. Indigenous Occurrence

R. alpinus is distributed in high mountains of Western, Central, and Eastern Europe, including the Apennine Peninsula, Balkan Peninsula, the Caucasus, Armenia, and northern Anatolia (Figure 4). In Slovakia, it occurs in the Krivánska Malá Fatra, Veľká Fatra, Nízké Tatry, Západné Tatry, Vysoké Tatry, Oravská Magura, Oravské Beskydy, and Bukovské vrchy (Šťastná *et al.* 2010).

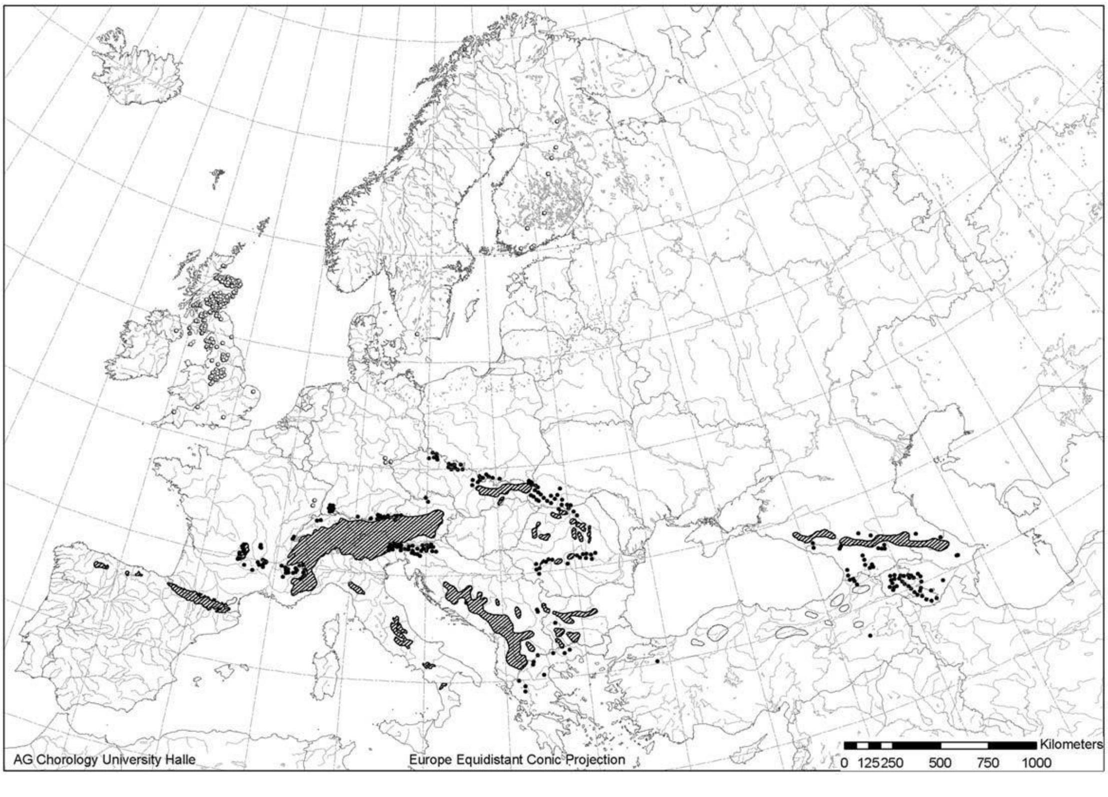

Figure 4: Distribution of *R. alpinus* (Šťastná et al. 2010)

3.6.1.2 Allochthonous occurrence (invasion)

Tutin et al. (1993) stated that *R. alpinus* was introduced to Great Britain (Scotland) from lowlands up to 375 m above sea level during the Middle Ages. It is also found in the USA

(Vermont and Maine) from lowlands up to 1500 m above sea level. Additionally, it occurs in Canada (Nova Scotia since 1921). Isolated introduced populations of *R. alpinus* have been present in Finland since 1923, where it was introduced from Central and Southern Europe as a medicinal plant (Piirainen 1996). Furthermore, Dickson and Dickson (2000) introduced it to Sweden in 1997 and to Norway (Tromsø) as an escapee from a botanical garden.

3.6.2. Occurrence in the Czech Republic

R. alpinus is not an indigenous plant in the mountainous areas of the Czech Republic (Krkonoše, Jizerské Hory, Orlické hory), where it was introduced in the second half of the 16th century (Kopecký 1973; Kubát 1990; Šťastná et al. 2010). Its current distribution (Figure 5) largely coincides with the distribution of past and present settlements in many areas (Kopecký 1973). *R. alpinus* is a strongly nitrophilous species that grows in moist soil and is most commonly found where there is manure and waste from domestic animals near farm buildings, roads, and human-disturbed areas. It can also be considered a pasture weed that dominates pastures with its biomass, thus limiting the chances of rare species to spread.

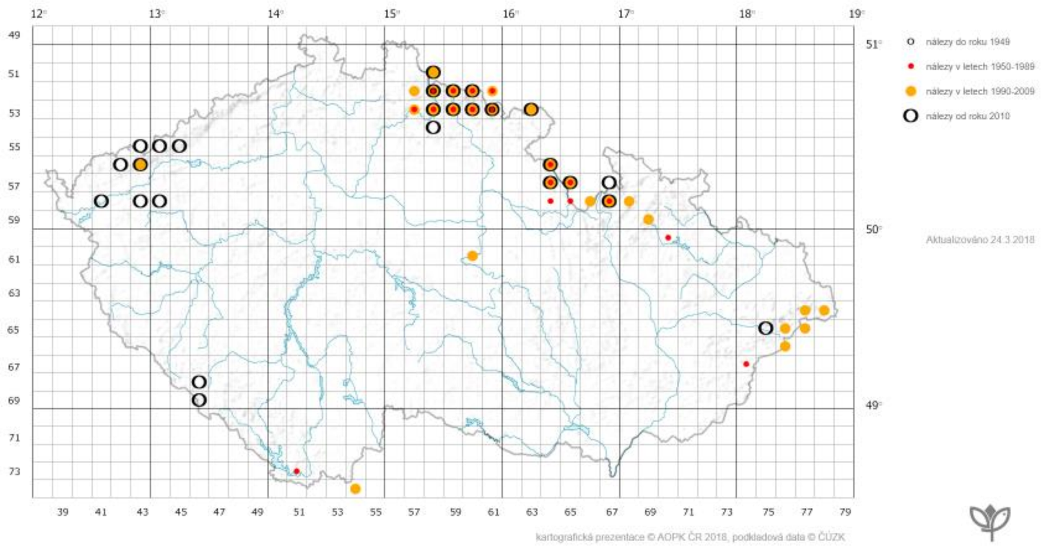

Figure 5: Distribution of *R. alpinus* in the Czech Republic (source: AOPK)

Ellenberg et al. (1992) and Pott (1996) report records of *R. alpinus* occurrence in mountainous, subalpine, and alpine zones (Bernina Alps: up to 2640 m). According to Rechinger (1957), it can descend along streams in the Alps to lower elevations (600 m). Localities with the lowest altitudes (except for Northern Europe) have been reported from the Czech Republic, where it was found at an elevation of 230 m near Mnichovo Hradiště (Kubát 1990), which is contrary to our observation (Jungová 2017-2023). The altitudinal gradient of *R. alpinus* extends over an area exceeding 1000 m (900 to 1900 m above sea level), indicating that the species is capable of adapting to large differences in the length of the growing season and temperature. Although the plants originally inhabited wet sections along streams, the strongest growth was observed in secondary deforested areas within the forest zone (Šťastná et al. 2012). Its peak distribution is (880) - 1300-2400 m in Spain, (800) - 1600-2300 m in Italy, 1500-2450 m in Macedonia, 1400-2200 m in Bulgaria, and 1600-2250 m in Greece; (750) - 950-1610 m in Slovakia (Jarolímek and Kliment 2004; Šťastná et al. 2010).

3.7 Uses of *R. alpinus*

3.7.1. Food

Currently, *R. alpinus* is considered one of the most widespread weeds (Bohner 2005; Šťastná et al. 2010; Delimat and Kiełtyk 2019). However, historically, it has been recognized as a valuable plant cultivated in special gardens, Figure 6 (Maude and Moe, 2005; Vasas et al. 2015). Although animals do not consume *R. alpinus* in its fresh state, probably due to its laxative effects (Hejcman, oral discussion), it has been used as fodder for domestic animals in the past, according to Roth et al. (1994). Maude and Moe (2005) have the same opinion and mention that the leaves were harvested and dried on a large scale (Graubünden) and mainly used as feed for pigs and goats.

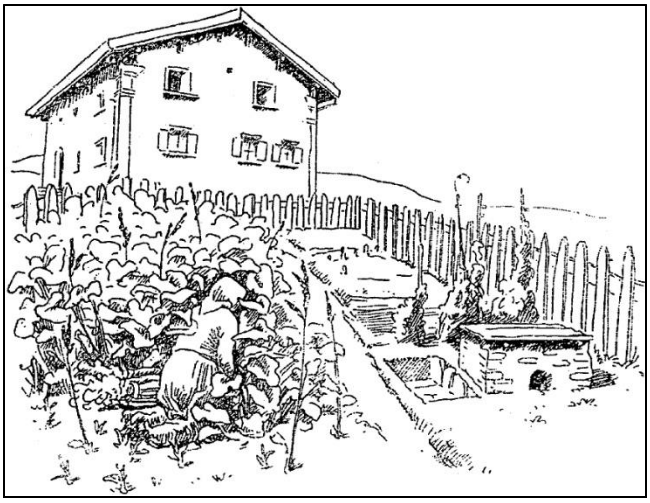

Figure 6: Farmer growing *R. alpinus* (Maude and Moe 2005).

At the same time, *R. alpinus* has been utilized by humans as a vegetable, where the stalks are peeled and used as rhubarb in pies (Ali-Shtayeh et al. 2008; Misra et al. 2008; Dreon and Paoletti 2009) or consumed raw (Łuczaj 2010). The leaves of *R. alpinus* have been eaten as a spinach substitute or prepared as sauerkraut (Dickson and Dickson 2000). In alpine regions, a special dish called "Farchon" is known, which is made from plant species, including *Chenopodium bonus-henricus*, *Urtica dioica*, and *R. alpinus*. The leaves were also used for wrapping fresh butter to facilitate transportation and preservation (Maude and Moe 2005).

The leaves of *R. alpinus* were occasionally used for making sauces and soups (Guerra et al. 2008). They could also be eaten raw, drizzled with olive oil (Łuczaj et al. 2013), and sometimes mixed with boiled potatoes (Dénes et al. 2013). In the eastern part of Turkey, young leaves of *Rumex* species were used as a vegetable or used to stuff meat and in the preparation of herbal cheese as a preservative and aromatic source (Ozturk et al. 2000).

3.7.2. Medicinal plant

In Turkey, where *R. alpinus* is native, it has been widely used in traditional medicine to treat various disorders such as constipation, diarrhea, and eczema (Ulukanli et al. 2005). Many compounds present in the plant have antimicrobial, antioxidant, antifungal, and antibacterial properties. For centuries, plants have been used in herbal medicine to treat various ailments. The plant's aerial parts, leaves, and roots were used in traditional medicine to treat several health disorders, including infections, diarrhea, constipation, edema, jaundice, and being an antihypertensive diuretic and analgesic.

Flowers, stems, leaves, and roots are typically prepared and consumed to treat various illnesses, including pain, edema, digestive problems, arthritis, cold and flu, fevers, and irritability (Baytop 1996; Beuchat and Golden 1989). They are also used for kidney and liver diseases, gallbladder inflammation, swelling, ulcers, rashes, skin diseases, and wound healing (Alvarez-Castellanos et al. 2001; Tepe et al. 2005; Dénes et al. 2013). *R. alpinus* has also been utilized as a laxative, diuretic, antipyretic, and antiinflammatory wound healing agent (Baytop 1996; Suleyman et al. 1999). In traditional Austrian medicine, the leaves and roots of *R. alpinus* were used internally to treat viral infections (Bogl et al. 2013). Various parts of the plant, such as flowers, stems, and rhizomes, have been applied in treating different types of tumors, such as poultices, ointments, and powders (Hartwell 1970).

It is known that plants belonging to the Polygonaceae family produce a large number of biologically important secondary metabolites, such as anthraquinones, naphthalenes, stilbenoids, steroids, flavonoid glycosides, leucoanthocyanidins, and phenolic acids (Gescher et al. 2011). *Rumex* species are rich in anthraquinones, especially in the roots, including emodin, chrysophanol, and physcion (Vasas et al. 2015).

On the other hand, *R. alpinus* contains higher levels of oxalic acid, which can cause serious problems such as kidney stone formation and reduced iron absorption if consumed in large quantities (Vasas et al. 2015).

3.8. Control and Management

Suppression of *R. alpinus* is often desirable, especially in protected areas where its extensive spread can reduce species diversity and threaten rare plants. An experiment conducted in the Ukrainian Carpathians (Tsarik 1987) used three cutting intensities, trampling and cuttings removal, to evaluate plant survival, biomass, and vegetation cover. The greatest reduction of *R. alpinus* was achieved in plots cut every ten days, which resulted in decreased sorrel cover. As a result, new individuals sprouted from the seed bank. Cutting twice a year increased seedling withering by approximately 30% compared to the control. Seed bank limitation until the next year was 20% compared to the uncut plot. Similarly, treatments involving rhizome fragmentation induced vegetative regeneration of Rumex plants. Unfortunately, the vegetation response was monitored for only one season (Tsarik 1987). *R. alpinus* displays remarkable vitality and regenerative capability after leaf removal, as evidenced by leaf development observed as soon as one day after cutting (Šmarda 1963). Within the following days, the plants increased their height by 2 cm per day and fully reestablished the site within ten days. Rapid regeneration of cut stands was also reported by Antosiak (1987), who observed *R. alpinus* reaching a height of 40 cm after 20 days following complete defoliation. Moreover, Bohner (2005) states that *R. alpinus* tolerates defoliation quite well. Successful suppression can only be achieved through regular cutting and removal of cut biomass, preventing *R. alpinus* from becoming dominant in pastures (Ellenberg 1996; Bucharová 2003). Bucharová (2003) and Jiřiště and Mládková (1998) reported results of chemical control of *R. alpinus* in the Krkonoše Mountains. Treatment with a 3-5% solution of the herbicide Roundup Biaktiv was applied three times during the season (July-September). After damaging the *R. alpinus* stands, grasslands were established by sowing seeds of several grass species (*Alopecurus pratensis, Agrostis capillaris,* and *Festuca rubra*). Despite a large number of *R. alpinus* seeds germinating from the seed bank in treated areas, the young plants were quickly suppressed by grasses. Only a few small *R. alpinus* plants remained on the experimental plots three years later, while grasses became dominant. Utilizing hay containing mature grass seeds as mulch, rather than sowing grass seeds or using geotextiles to cover the area, was the most effective method (Jiřiště 2000). Regular cutting is necessary to promote grass growth, resulting in dense grass carpets where *R. alpinus* cannot thrive (Kutschera and Lichtenegger, 1992; Bucharová 2003).

Due to the biological characteristics of *R. alpinus*, its eradication is quite challenging. After mowing, it quickly regenerates from reserves in its rhizomes, and after herbicide treatment, the vegetation promptly recovers from a significant seed bank in the soil (Bucharová 2003). There aren't many options or instructions available for eradication. Among the possibilities that were considered, such as removing the topsoil, mowing to deplete the rhizome reserves, or removing the basal material along roadsides (methods described in more detail in Naglová's bachelor's thesis in 2012), the combination of methods proposed by Jiřiště (2000) and Bucharová (2003) proved to be the most effective. This combination involves the spot or blanket application of herbicide spray, using Roundup herbicide, which Janata (2010) and Mládková (1992) found to be the most efficient when applied repeatedly and spot-wise. Bachtík also states in his work that after spraying with Roundup, 95% of *R. alpinus* clusters were destroyed, and after one vegetation season, the first monitored area was covered by 50-55% of local vegetation, while the second area was covered by only 30-40%. However, multiple herbicide applications can be relatively expensive, and when using chemical substances, caution must be exercised in protected areas (Bucharová 2003). The first spraying should be done from mid-May to June. After three weeks, the area needs to be revisited, and any overlooked or insufficiently affected plants or newly emerged seedlings should be treated. This procedure requires ongoing attention, often for more than five years. It is crucial not to let any inflorescence mature during the eradication process. Therefore, if, for any reason, spraying is skipped, it is necessary to remove at least and burn maturing inflorescences. Creating a competitive environment with native grasses and herbs is also beneficial. This can be achieved by overseeding with a regional mixture of herbs and grasses (Janata 2010).

Competition between *R. alpinus* and grasses and herbs alone is not enough to restrict its growth (Zaller 2004). It should be combined with other management practices. It is important to mow the docks at a height of 10 cm to allow other plants to regenerate earlier (Šilc et al. 2016).

3.8.1 Biological Control

Rumex alpinus is a challenging weed, especially in protected areas or Natura 2000 sites where only non-chemical measures are employed. Šilc et al. (2016) investigated the effectiveness of various non-chemical methods through a field experiment: mowing, manual removal, heating, covering with plastic sheeting, and grazing by cattle and pigs. Growth changes, coverage, number of shoots, and biomass were monitored at 14-day intervals over three consecutive years. The results indicated that manual removal of plants and plastic sheeting were the most successful, nearly eliminating biomass and reducing coverage to

50%. Other methods were less effective. *R. alpinus* was avoided by animals for grazing, and heat only temporarily suppressed its growth.

Plastic sheeting (or any other covering material) to reduce light availability for weeds or invasive species is a common practice (Bond and Grundy 2001). It has been successfully employed with *R. alpinus* (Bechtold and Machatschek 2011). Light availability is crucial for *R. alpinus* and contributes to its competitive ability (Zaller 2004). Within one year, docks are destroyed due to reduced light and high temperatures under the plastic sheeting during the summer. However, plastic sheeting is less suitable for large areas, particularly mountainous regions with adverse climatic conditions (Šilc et al. 2016).

Animals rarely graze docks, and livestock and horses tend to avoid *R. alpinus*. Occasionally, goats graze on it (Bohner 2005; Ellenberg 1996; Hejcman et al. 2014). In the past, it was also used as feed for pigs (Wendelberger 1971). Šilc et al. (2016) employed cattle and pigs for only one season, resulting in some suppression of *R. alpinus*. However, this was likely due to trampling by livestock rather than actual grazing. Trampling can effectively reduce the aboveground biomass of *R. alpinus* (Tsarik 1987). Grazing by goats, sheep, or in combination with cattle can effectively remove plants from grassland communities (Hejcman et al. 2014). Still, in the case of mountain pastures, this is not common in Central Europe and is even restricted in protected areas. Introducing grazing by these animals would require a change in grazing policies associated with Natura 2000 (Šilc et al. 2016).

3.9 Pests and diseases

3.9.1 Herbivores

Gastrophysa viridula (*Chrysomelidae*, Coleoptera) is a natural enemy of docks in Europe (Martínková and Honěk, 2004), and its subspecies *caucasica* Jolivet, was found in Turkey. It is an important potential biological agent (Aslan and Ozbek 1999; Hatcher et al. 1997). Although *Gastrophysa viridula* (*G. Viridula)* reduces plant biomass, it cannot eradicate the plants, especially at higher elevations (Tsarik 1986). A potential disadvantage of *G. viridula* as a biological control agent is its short flight distance, usually less than 10 meters (Martínková and Honěk 2004).

Trophic relationships in *R. alpinus* habitats were studied by Rudyshin and Tsarik (1982) in the Ukrainian Carpathians. They found that the most important leaf-feeding herbivores on *R. alpinus* were the larvae and adults of *G. viridula, Otiorhynchus niger,* and *Otiorhynchus* sp. On average, they found 5 to 7 adult *Gastrophysa* beetles and 1 to 2 adult *Otiorhynchus* beetles per plant. The beetles were most active in June and July, reducing leaf area by approximately 20% (*Gastrophys*a) and 75% (*Otiorhynchus*). It was also found that two species of mammals feed on the aboveground parts of *R. alpinus*. Leaves were consumed by *Microtus subterraneus*, and *Microtus agrestis* consumed flowering stem*s*.

Among other herbivores that consume *R. alpinus* are several species of *Syrphidae*: *Mygatropus florea* (Rudyshin and Tsarik 1982), *Syrphus torvus, Parasyrphus lineola* (Klimeš 1994), and the bumblebee *Bombus terrestris* (Klimeš 1994). The average percentage of pollen recorded in the guts of *Syrphus torvus* flies was nearly 90%. For *Parasyrphus lineola*, it was 67%, indicating that these flies may prefer this type of food if it is available in excess. Similarly, pollen was repeatedly recorded in the species *Bombus terrestris*. The percentage of recorded *R. alpinus* pollen in pollen baskets ranged from 93% to 96% (Klimeš 1994).

Seeds of *R. alpinus* are consumed by several mammal species, including *Apodemus sylvaticus, A. flavicollis, Muscardinus avellanarius, Dyromys nitedula*, *Sicista betulina*, as well as omnivores *Aeanthis cannabina, Fringilla coelebs, Emberiza citrinella, and Prunella modularis* (Rudyshin and Tsarik, 1982). Rhizomes and roots are consumed by larvae of beetles *Otiorhynchus niger, Otiorhynchus* sp., and mammals (*Rattus norvegicus* and *Arvicola terrestris*). Other mammals, *Microtus subterraneus* and *M. agrestis*, specialize in consuming underground organs of *R. alpinus* during winter (Rudyshin and Tsarik 1982).

3.9.2 Insect pests on *R. alpinus*

Coleoptera: *Chrysomelidae*: *Oreina elongata* Suffrian (larvae). However, their survival on *R. alpinus* as a potential host plant is limited (Ballabeni and Rahier 2000).

Coleoptera: *Curculionidae: Otiorhynchus corvus* Boheman. Larvae of this polyphagous species feed on young roots of *R. alpinus* in the Beskids and western Carpathians (Knutelski 2005). *Plinthus tischeri* Germar in the western Carpathians and Sudetes. *Plinthus findeli* Boheman has been found only on *R. alpinus* in the Czech Republic, Pyrenees, Alps, and the Balkans (Smreczynski 1968). *Plinthus sturmii* Germar was found in the Austrian Alps, Bosnia, Albania, Carpathians, and Sudetes (Knutelski 2005). *Donus comatus* Boheman is found only in the Carpathians, Sudetes, Alps, and the Balkans (Knutelski 2005). *Rhinoncus pericarpius* L. - this oligophagous species occurs on several *Rumex* species, including

R. alpinus, and is distributed in the Palearctic part of North America up to an altitude of 1500 m (Smreczynski 1974).

Lepidoptera: Hepialidae: Larvae of *Hepialus humuli* L. have been found in the rhizomes of *R. alpinus*. The genus *Hepialus* generally includes polyphagous species, so other species of mountain habitats, such as *Hepialus fuscomelubosus* DeGeer and *Hepialus carna* Denis and Schiffermuller, may also feed on *R. alpinus* (Šťastná et al. 2010).

3.9.3 Diseases

The impact of herbivory on *R. alpinus* may be exacerbated by other factors, such as rust or mold. Rust caused by *Uromyces rumicis* from the *Pucciniaceae* family commonly affects the leaves of *Rumex obtusifolius* and *Rumex crispus*. However, no data is available regarding its colonization of *R. alpinus* (Št'astná et al. 2010).

In an unpublished experiment by Jungová exploring the growth of *R. alpinus* seedlings, it was noted that several *R. alpinus* seedlings perished due to infestations of aphids and powdery mildew. This experiment underscored the potential of these pests to significantly impact the health of *R. alpinus* (Jungová, data not yet published).

3.10 Effect of macro and microelements on *Rumex* species

The growth of *R. alpinus* is influenced by nutrient availability, as documented by Bohner (2005) and Šťastná et al. (2012). Like other *Rumex* species in early developmental stages, *Rumex obtusifolius and Rumex crispus* are sensitive to excessive nitrogen (N) supply, as observed by Křišťálová et al. (2011). Adult plants require nitrogen and phosphorus (P), as P is an essential nutrient crucial for early plant growth (Bohner 2005). The growth of *Rumex* species is also impacted by the availability of calcium (Ca) and magnesium (Mg) in the soil. Hann et al. (2012) reported a negative correlation between plant-available Ca and Mg concentrations in the soil and the density of *R. obtusifolius* in grasslands. Furthermore, both adult plants and seedlings are affected by high Ca availability in the soil, as recorded by Hejcman et al. (2012). *Rumex obtusifolius*, as observed by Vondráčková et al. (2014), is a calciphobic plant that reduces excessive concentrations of Ca ions in its tissues through the precipitation of insoluble Ca-oxalate crystals (Bohner 2001). Similar results were reported by Bohner (2005) for *R. alpinus*. According to Barker and Pilbeam (2007), high concentrations of Ca and Mg in the soil can restrict plants' potassium (K) uptake, resulting in limited K availability for highly K-demanding species such as *Rumex*. Consequently, this can reduce competitiveness and increase mortality for Rumex species in Ca-rich soils (Hann et al. 2012).

The influence of trace elements on the biomass production of *Rumex* species and the distribution of these elements were studied by Gaveda et al. (2009) and Vondráčková et al. (2014). The findings of Vondráčková et al. (2014) indicated a negative relationship between biomass production and the concentrations of Cd, Ni, Pb, and Zn in the plant biomass, highlighting the toxicity of these elements to plants. The presence of these trace elements led to a reduction in biomass production due to the inhibition of cell elongation and division (Anton and Mathe-Gaspar 2005; Barrutia et al. 2009), as well as visible changes in leaf shape and color (Hejcman et al. 2012).

Rumex obtusifolius and similar plant species exhibit high sensitivity to the elevated availability of trace elements in the soil, making them effective indicators for field mapping of contaminated soils based on the symptoms of toxicity observed in their aboveground organs (Hejcman et al. 2012; Vondráčková et al. 2014). Additionally, some *Rumex* species have been investigated as a potential plants for phytoremediation (Hejcman et al. 2012; Vondráčková et al. 2014). However, Vondráčková et al. (2014) found that *Rumex obtusifolius* restricted the translocation of trace elements from belowground organs to leaves, indicating its behavior as an excluder of As, Cd, Pb, and Zn. As a result, it is not suitable for phytoremediation of heavily contaminated soils. Nevertheless, further research demonstrated the plant's high ability to accumulate Al in its organs, particularly in alkaline soils (Vondráčková et al. 2015).

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4. RESULTS OF THE THESIS

4.1 CHAPTER 1 (PAPER 1)

Distribution and Resorption Efficiency of Macroelements (N, P, K, Ca, and Mg) in Organs of *Rumex alpinus* L. in the Alps and the Giant (Krkonoše) Mountains

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(Photo: Michaela Jungová)

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ORIGINAL PAPER

Distribution and Resorption Efficiency of Macroelements (N, P, K, Ca, and Mg) in Organs of *Rumex alpinus* L. in the Alps and the Giant (Krkonoše) Mountains

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Abstract

Rumex alpinus, an alpine nitrophilous species, is a troublesome weed in pastures. Nutrient uptake and distributions in the organs of *Rumex alpinus* are not well-studied. We aimed to determine the distribution of macroelements in organs of *Rumex alpinus* together with the nitrogen-phosphorus potassium ratio (NPK) and the resorption efficiency of N, P, K, calcium (Ca), and magnesium (Mg) in the Alps of Austria, Italy, and the Krkonoše (Giant) Mountains, Czech Republic. The pseudo-total and plant-available N, P, K, Ca, and Mg in soils and organs of *Rumex alpinus* (emerging, mature, and senescent leaves, petioles, stems, and rhizomes) were extracted with *aqua regia* and Mehlich-3 procedures, respectively, followed by inductively coupled plasma-optical emission spectrometry. The contents of total and available macroelements in the soils reflected high variability with localities. There was a significant effect of locality and organs on the element contents, indicating differences in nutrition. *Rumex alpinus* exhibited higher N and P contents in emerging and mature leaves than in the senescent compared to Ca and Mg. The N:P and N:K ratios in the mature leaves were within the normal range but were indicative of comparatively higher demand for P. The mean resorption efficiency for N, P, and K was 52, 50, and 22%, respectively, lower than values for most terrestrial plants (62, 65, and 70%). The relatively high availability of macroelements in soils and plant organs with low N, P, and K resorption efficiency indicates a high N, P, and especially K-demanding species of *Rumex alpinus*.

Keywords Alpine dock · Plant organs · Resorption · Weed · Nutrients

1 Introduction

The Alpine dock (*Rumex alpinus* L) of the Polygonaceae family is native to the high mountains of Western, Central, and Eastern Europe, including the Iberian and Apennine peninsula, the Alps, the Carpathians, and the Balkan Peninsula. Additionally, *R. alpinus* is native to the Caucasus (Nakhutsrishvili et al. 2017), the mountains of northern Anatolia, Armenia, and Iran.

In the Giant mountains of the Czech Republic, the introduction of *R. alpinus* was associated with German-speaking colonists from the Alps in the sixteenth century AD, used for the treatment of different diseases, such as salad and forage crop (Lokvenc 1978; Šťastná et al. 2010). For example, 2-acetyl-3-methylnaphthalene-1, 8-diol, a bioactive compound found in the leaves and roots of *R. alpinus*, possesses an antibacterial characteristic that serves as a laxative and cures jaundice, astringent, constipation, diarrhea, and eczema (Ozturk and Ozturk 2007; Grieve 2013; Mishra et al. 2018).

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Today, *R. alpinus* is considered a troublesome weedy species which infests abandoned grasslands, roads margins, and nutrient-rich sites: typically livestock resting areas, surroundings of mountain farms, and downslope of mountain chalets grasslands, with deposits of animal feces, and nutrient-rich wastewater (Bucharová 2003; Šťastná et al. 2010; Šilc and Gregori 2016). This species, however, is a perennial nitrophilous and calciphobous species, with high nitrogen (N, 66 g kg^{-1}), phosphorus (P, 7.3 g kg^{-1}), and potassium (K, 47 g kg^{-1}) contents in young leaves and an N:P ratio of about 9.1 (Bohner 2005). Another characteristic trait of this species is its ability to accumulate nitrate (NO_3^-) in young leaves (Rehder 1982; Bohner 2005; Kołodziejek 2019). *Rumex alpinus* develops on soils ranging from acid to alkaline, usually humus- and nutrient-rich, mesic-moist-wet and fine sandy, dusty, and well-ventilated throughout the year (Stachurska-Swakoń 2009; Doležal et al. 2020). As a nitrophilous plant, *R. alpinus* is well-adapted to temporal surpluses of NO_3^- and K and dis-harmonic nutrient supplies in the soil solution. Due to the absence of high-yielding, competitive fodder grasses due to unfavorable climatic conditions in mountainous regions and its competitive ability (Klimeš 1992), *R. alpinus* forms a stable but species-poor, productive permanent community dominated by a few nitrophilous herbs (Bohner 2005). Nitrophilous plants grow on nutrient-rich sites and can waste nutrients (Opačić 2022).

Indications of nutrient wasting include low nutrient-use efficiency, high nutrient contents in different organs compared to species adapted to nutrient-poor conditions, and low re-translocation of nutrients from senescent to the most photosynthetically active young leaves (Delgado et al. 2018). Except for the study by Bohner (2005), there is no scientific research on the contents and distribution of N, P, K, calcium (Ca), and magnesium (Mg) in different organs of *R. alpinus.* However, this species can be considered an excellent example of alpine nitrophilous species. So far, there are no studies on the resorption efficiency of this species and variability in its nutrition in localities with different geological substrates on a large geographical scale and at different sampling intervals.

Additionally, as a mountainous species with diverse vegetative regeneration, such as clones and seeds (Bohner 2005), knowledge of its nutrient absorption is pertinent in making inferences on *R. alpinus's* adverse implications on mountain ecosystems. Nutrient resorption is a mechanism of plant species' response to nutrient-limiting conditions, which play a pertinent role in efficient nutrient cycling—thus reducing the dependence on soil-available nutrients (Brant and Chen 2015; Thapa et al. 2020). Foliar nutrient concentrations play a vital role in nutrient resorption. However, many factors affect nutrient resorption, which directly obscures the relationships with soil nutrient availability (Yan et al. 2018; Thapa et al. 2020), e.g., variation in available content of nutrients and seasonal changes.

To fill the gap of knowledge, we determined macro element (N, P, K, Ca, and Mg) contents of both soils and organs of *R. alpinus* from four localities in the Krkonoše (Giant) Mountains (Czech Republic) and alps of Austria and Italy.

This study aimed to answer the following research questions: (i) How can the variability of soil chemical properties affect *R. alpinus* stand from different localities? (ii) How different are the contents of N, P, K, Ca, and Mg and the N:P, N:K, and P:K ratios in various organs of *R. alpinus* in different localities? (iii) How intensive is the resorption of N, P, K, Ca, and Mg from senescent to young leaves in *R. alpinus*?

2 Materials and Methods

2.1 Study Area

Rumex alpinus plants were well-studied according to their wide distribution in four localities of the Krkonoše Mountains, Czech Republic, two in Austria, and one in Italy (Fig. 1). The localities of the Krkonoše Mountains (Horní Mísečky- HM; Vítkovice v Krkonoších- VT; Libuše hut- LB; and Pec pod Sněžkou- PC; Figure S1) are characterized by podzols located on phyllite geological substrate (Němeček and Kozák 2005). In Ramsau am Dachstein (DCH) and Zilllertal (ZL), Austria, the localities are well-characterized by Calcaric Cambisol and Luvic Cambisol on limestone and Granite gneiss parent bedrock, respectively. And in Madesimo (MD), Italy, the soil is Vertic Cambisol on a sandstone geological bedrock (Jones et al. 2005). Additionally, the localities represent different altitudes and environmental conditions—precipitation, temperature, and altitudes (Table 1).

As an invasive species in the Krkonoše Mountains (Kopecký et al. 1973; Lokvenc 1978; Pyšek et al. 2012), *R. alpinus* covers approximately 70% of the area expelling indigenous plants and preventing species diversity (Náglová 2014).

2.2 Sampling and Preparation of Soils

To maximize sample collection, we adopted a judgemental approach (Frey 2018), covering the variability of soils in the locations of *R. alpinus*. In this case, we sampled the upper 10-cm soil layer with a soil probe (Purchhauer type, core diameter: 30 mm) in LB, VT, PC, HM, DCH, and MD in July 2018. In each locality, we randomly collected ten soil samples in the surroundings of *R. alpinus* stands. And these samples were mixed to form a representative sample from the localities of the Krkonoše Mountains in the Czech Republic, DCH, and MD. Soil samples were air-dried and

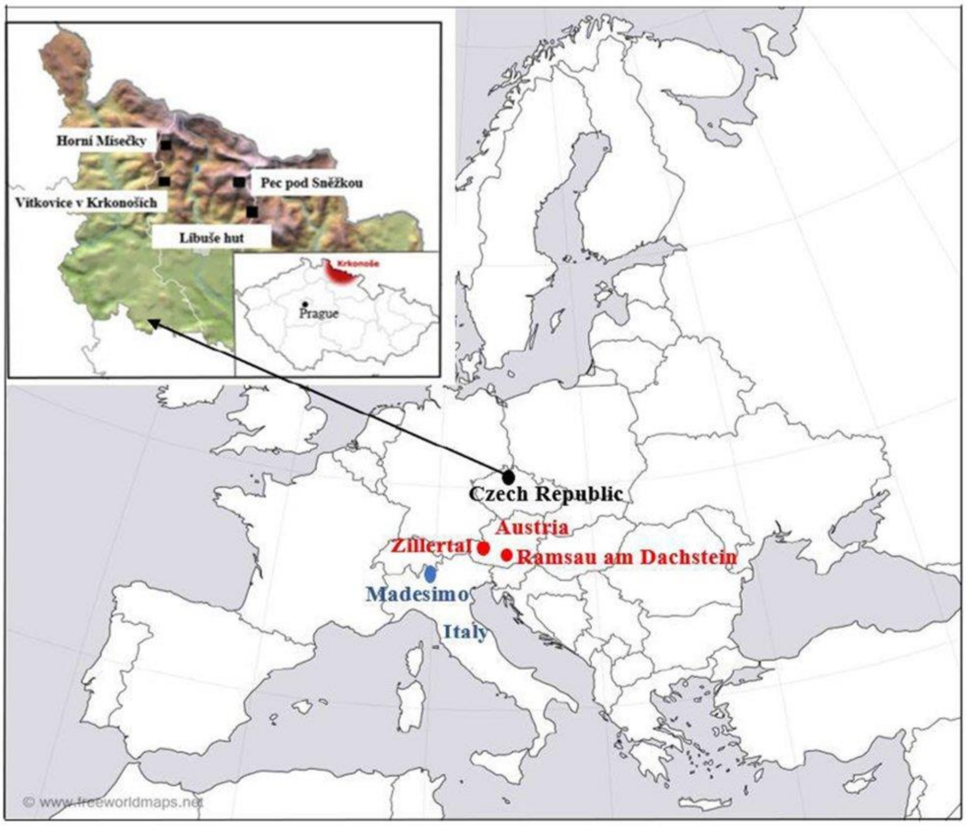

Fig. 1 Location of studied localities in the Czech Republic (Horní Mísečky—HM; Vítkovice v Krkonoších—VT; Libuše hut—LB; and Pec pod Sněžkou—PC), Ramsau am Dachstein and Zillertal in Austria, and Madesimo, Italy

oven-dried at 60 °C for 48 h (Francová et al. 2017). For the homogenous fraction under 2 mm, we analyzed for macroelement contents. The representative soil samples were divided to obtain three replicates (sub-samples) per locality and sent to the laboratory for chemical analysis.

2.3 Sampling and Preparation of Plant Organs

Organs of *R. alpinus* (Fig. 2) were collected in a mono-dominant stand covering 100 m^2 in all localities. At each locality, 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 seeds (St), and three rhizomes developed in the last two years (Fig. 3). All collected samples were put into paper bags and transported to the laboratory.

Laboratory protocol: Plant organs were cleaned from soil and other residues using distilled H_2O and then dried at 70 °C for 48 h. Each organ sample immediately was mixed to obtain one representative sample per locality. Next, we ground the representative organ samples per locality with an IKA® A11. The representative organ sample of each locality was homogenized and divided into three replicates for further elemental analysis.

The samples from the Krkonoše Mountains were collected twice in 2018, in July (Summer—S) and October (Autumn—A). Samples from the Alps were collected only in July 2018 (summer), approximately at the same time as in the Krkonoše Mountains.

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

Locality	Geographical location	Altitude [m a.s.l.]	Mean annual precipitation [mm]	Mean annual temperature [ºC]	Soil type	Geological substrate
Czech Republic						
Libuše hut, Velká Úpa (LB)	50°41′19″N 15°46′43″E	700	850	6.5	Podzol	Phyllite
Vítkovice v Krkonoších (VT)	50°41′56″N, 15°31′41″E	650	900	5.5	Podzol	Phyllite
Pec pod Sněžkou (PCS)	50°41′46″N, 15°44′8″E	815	850	5.5	Podzol	Phyllite
Horní Mísečky (HM)	50°44′2″N, 15°34′5″E	1050	1000	4.5	Podzol	Phyllite
Austria						
Ramsau am Dachstein (DCH)	47°27′1″N, 13°37′1″E	1650	1100	3.8	Calcaric Cambisols	Limestone
Zillertal (ZL)	47°14′21″N 12°7′39″E	1650	933	3.9	Luvic Cambisol	Granite gneiss
Italy						
Madesimo MD)	46°26′13″N, 9°21′27″E	1600	2000	2.0	Vertic Cambisols	Sandstone

2.4 Chemical Analyses and Analytical Methods

The total content of P, K, Ca, and Mg in plant organ and soil samples were extracted with USEPA 3052 (International Organization for Standardization, USEPA 1996) procedure using a mixture of nitric (HNO_3), hydrochloric (HCl), and hydrofluoric (HF) acids.

Procedure: A mass of 0.25 g of homogenized *R. alpinus* individual organs was mineralized in a mixture of 9 mL of HNO_3, 3 mL of HCl, and 1 mL of 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 hydrogen peroxide (H_2O_2) was added to each sample and evaporated on a hot plate at 50 °C for 24 h. Evaporated samples were diluted in 20 mL of 2% HNO_3 for 2 h and filtered. The content of the total element of each plant organ sample was determined with inductively coupled plasma-optical emission spectrometry (ICP-OES; 720 Series, Agilent Technologies, USA). We used the same procedures and analytical device to obtain the total content of macroelement for all the soil samples. The total content of N in plant organ and soil samples was determined by the Dumas method with the DUMATHERM® Nitrogen determination system (http://www.gerhardt.de).

The plant-available fractions of P, K, Ca, and Mg in soil samples were extracted by Mehlich-3 reagent (Mehlich 1984) and determined with ICP-OES (Varian VistaPro, Mulgrave, Australia). The plant-available content of the studied elements was determined in an accredited laboratory EKO-Eko-Lab Zamberk (www.ekolab.zamberk.cz). Soil pH $_{[H2O]}$ was measured in all soil samples in three replicates at a ratio of 1:2 (soil: water) with a Voltcraft PH-100 ATC pH meter manufactured by I & CS spol s r.o. (Czech Republic).

2.5 Statistical Analyses

Data was tested by the Shapiro–Wilk W-test for normality and met assumptions for parametric tests. Factorial analysis of variance (ANOVA) was used to evaluate the effect of locality, organ, and their interaction on the content of different elements in biomass samples. One-way ANOVA was used to evaluate the effect of locality on the elemental composition of soils and organs and terms on the element contents of biomass samples. Additionally, we applied the ANOVA model to evaluate the effect of locality and season on the N:P, N:-K, and K:P ratios and on *NuR*. In the case of significant ANOVA, a post hoc comparison using the Tukey higher significance difference (HSD) test was applied. Moreover, we used a correlation analysis to evaluate the relationship between the total and plant-available content of elements in the soil and content elements in the soil and mature leaves. All statistical analyses were performed using the STATISTICA 13.3 program (www.statsoft.com).

2.6 Estimation of N, P, K Ratio and Nutrient Resorption (Nur)

To characterize the nutritional status of the plants, we estimated the N:P, N:K, and K:P ratios in mature and senescent leaves (aboveground biomass) after the critical values for vascular plants (Olde Venterink 2003), with the following values and interpretations.

i. N-limited N:P $<$ 14.5 and N:K $<$ 2.1
ii. P-limited or P + N-limited N:P $>$ 14.5 and K:P $>$ 3.4
iii. K-limited or K + N-limited, N:K $>$ 2.1 and K:P $<$ 3.4

NuR for N, P, K, Ca, and Mg were calculated after Vergutz et al. (2012) as

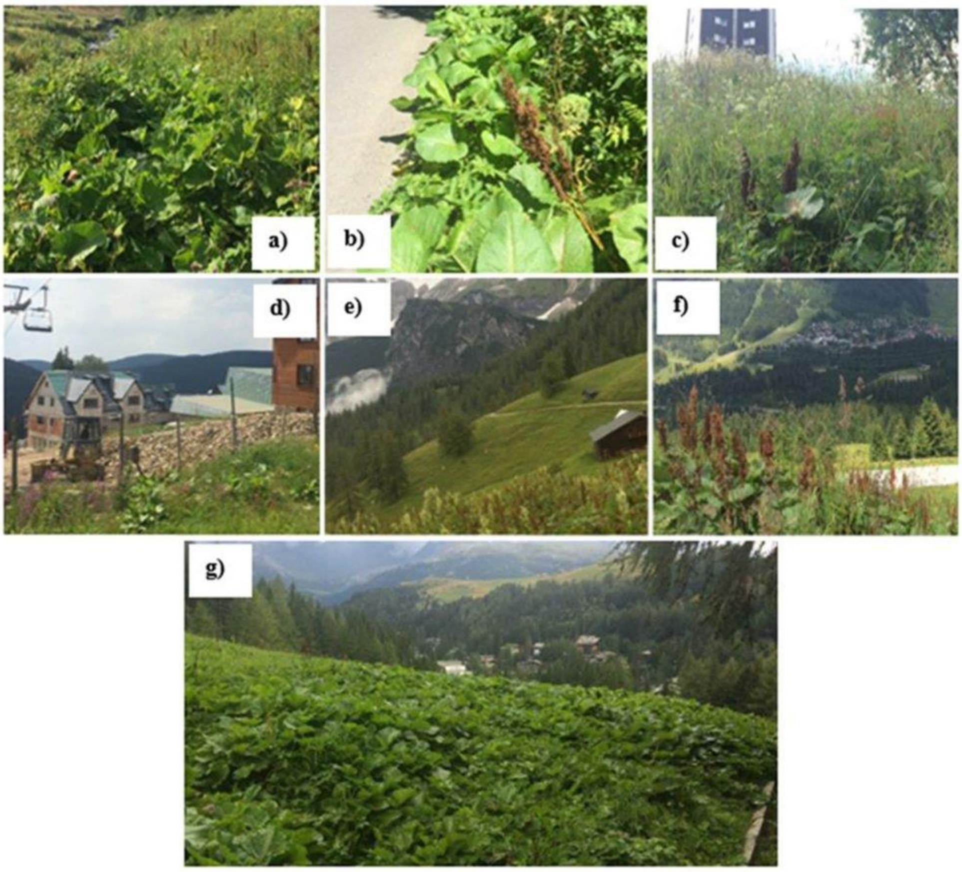

Fig. 2 *Rumex alpinus* stands in (**a**) Libuše hut, (**b**) Vítkovice v Krkonoších, (**c**) Pec pod Sněžkou, (**d**) Horní Mísečky, (**e**) Ramsau am Dachstein (DCH), (**f**) Zillertal (ZL), and (**g**) Madesimo (MD)

$$NuR = 1 - \left(\frac{content\ of\ elements\ in\ senescent\ leaves}{contents\ of\ elements\ in\ mature\ leaves}\right) \times 100$$

3 Results

3.1 Soil Chemical Properties

There was a significant effect of locality on soil reactions ($pH_{[H2O]}$) and the total and plant-available contents of all analyzed elements (Tables 2 and 3). Except for the slightly acidic reaction in DCH, soils in all other localities were moderately acidic. The pH ranged from 5.2 to 6.1 in LB and DCH, respectively (Table 2). The content of total N was from 1.33 in DCH to 9.02 g kg^{-1} in HM. The content of total P ranged from 0.42 to 1.01 g kg^{-1} in DCH and HM, respectively. The total K ranged from 11.8 in LB to 22.5 g kg^{-1} in MD, while Ca ranged from 1.04 in HM to 42.25 g kg^{-1} in DCH. Moreover, the total Mg content was from 1.11 in LB to 9.46 g kg^{-1} in DCH.

Available P content was from 20 to 51 mg kg^{-1} in MD and DCH, respectively (Table 3). Meanwhile, available K content ranged from 33 in VT to 129 mg kg^{-1} in MD, with Ca ranging from 547 in VT to 5133 mg kg^{-1} in DCH. The plant-available Mg content ranged from 116 in LB and

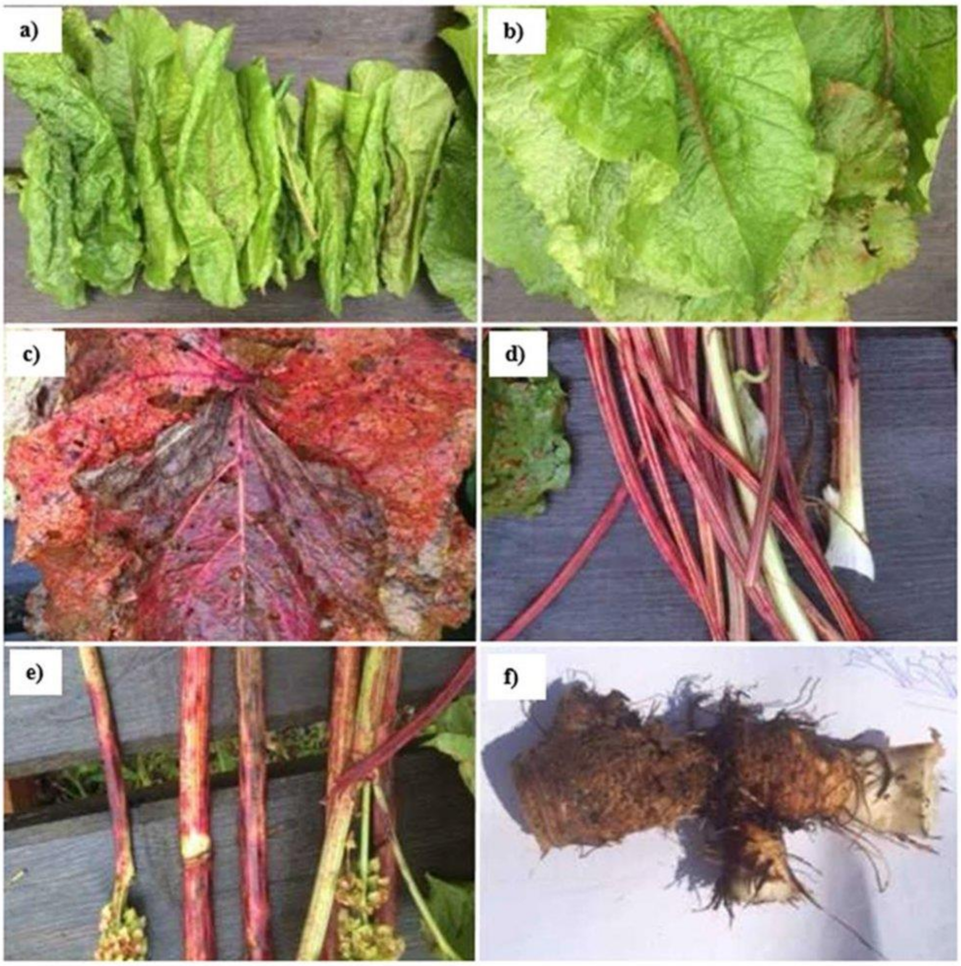

Fig. 3 Sampled organs of *Rumex alpinus*: (**a**) emerging, (**b**) mature, (**c**) senescent leaf blades, (**d**) petioles from mature leaves, and (**e**) stems from flowering plants, and (**f**) 2-year-old rhizome

VT to 207 mg kg^{-1} in MD. There was no significant correlation between total and plant-available P, K, Ca, and Mg in the soils (Table 4a).

3.2 Elemental Content of Plant Organs

There was no significant correlation between total N, P, K, Ca, and Mg in the soils and the same elements in the mature leaves (Table 4b). Meanwhile, this was similar in the case of plant-available P, K, and Mg except for Ca ($r = 0.91$, $p = 0.01$; Table 4c). The total content of macroelements in all organs' overall localities and terms (autumn and summer) are in Figs. 4 and 5.

We recorded a significant effect on the content of elements in organs in each locality. The overall mean contents of N, P, K, Ca, and Mg in organs across all the localities were significantly different (Table S1). Total N ranged from 3 in stems from HM_A to 67 g kg^{-1} in emerging

Table 2 The pH and total content (mean ± SE) of elements in the upper 10-cm soil layer from six localities. The p value was obtained by one-way ANOVA. Using the Tukey post hoc test, the mean values of each element with the same letter among localities were not significantly different. * $p < 0.01$ ** $p < 0.001$

Locality		LB	VT	PC	HM	DCH	MD
pH (H_2O)*		5.2 ± 0.3c	5.7 ± 0.03abc	5.9 ± 0.1ac	5.6 ± 0.1abc	6.1 ± 0.4a	5.3 ± 0.2c
N **	(g kg^{-1})	2.92 ± 0.03e	3.0 ± 0.04d	2.98 ± 0.02e	9.02 ± 0.02c	1.33 ± 0.02b	2.36 ± 0.03a
P **	(g kg^{-1})	0.77 ± 0.06ab	0.76 ± 0.10ab	0.75 ± 0.02ab	1.01 ± 0.27a	0.42 ± 0.01b	0.63 ± 0.06ab
K *	(g kg^{-1})	11.8 ± 0.4c	17.3 ± 2.1a	14.4 ± 0.7ab	10.9 ± 0.9c	16.0 ± 0.1ab	22.5 ± 1.7c
Ca *	(g kg^{-1})	2.51 ± 0.49ab	3.47 ± 1.5ab	5.69 ± 1.04b	1.04 ± 0.16c	42.25 ± 0.68a	1.17 ± 0.14c
Mg *	(g kg^{-1})	1.11 ± 0.29b	3.76 ± 2.34b	1.62 ± 0.43b	1.54 ± 0.61b	9.46 ± 0.23a	2.10 ± 0.09b

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

Table 3 Mean content (± SE) of plant-available (Mehlich-III) P, K, Ca, and Mg in the upper 10-cm soil layer from six studied localities. The p value was obtained by one-way ANOVA. Using the Tukey post hoc test, the mean values of each element with the same letter among localities were not significantly different

Locality	LB	VT	PC	HM	DCH	MD	p-value
P (mg kg^{-1})	38 ± 2.2b	37 ± 3.7b	41 ± 3.1ab	49 ± 6.3a	51 ± .7a	20 ± 1.7c	< 0.001
K (mg kg^{-1})	121 ± 5.1b	33 ± 2.8c	128 ± 5.7a	127 ± 3.3a	124 ± 3b	129 ± 4.6a	< 0.001
Ca (mg kg^{-1})	1689 ± 24.2c	547 ± 11.6e	4454 ± 20.4b	1130 ± 17.9d	5133 ± 16.8a	1465 ± 23c	< 0.001
Mg (mg kg^{-1})	116 ± 2.9d	116 ± 5.8d	395 ± 6.7a	120 ± 5.6d	153 ± 7c	207 ± 4.1b	< 0.001

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

Table 4 The relationship between the content of (a) Total and plant-available elements in soil, (b) total elements in soil and mature leaves, and (c) plant-available in soil and total elements in mature leaves

(a) Total and plant-available in soil					
Parameter	P	K	Ca	Mg	
Regression equation	y = 0.04 + 0.003*x	y = 0.13 − 0.002*x	y = 1.57 + 0.09*x	y = 0.21 − 0.01*x	
Correlation coefficient (r)	r = 0.10	r = − 0.17	r = 0.75	r = − 0.20	
p value	p = 0.68	p = 0.74	p = 0.08	p = 0.71	
(b) Total in soil and mature leaves					
Parameter	N	P	K	Ca	Mg
Regression equation	y = 47.31 + 0.76*x	y = 3.76 − 0.39*x	y = 16.32 + 0.28*x	y = 1.78 + 0.02*x	y = 2.11 − 0.003*x
Correlation coefficient (r)	r = 0.29	r = − 0.11	r = 0.63	r = 0.64	r = − 0.06
p value	p = 0.58	p = 0.83	p = 0.18	p = 0.17	p = 0.90
(c) Plant-available in soil and total content in mature leaves					
Parameter	P	K	Ca	Mg	
Regression equation	y = 3.37 + 2.80*x	y = 23.48 − 25.88*x	y = 1.45 + 0.20*x	y = 2.15 − 0.24*x	
Correlation coefficient (r)	r = 0.05	r = − 0.54	r = 0.91	r = − 0.20	
p value	p = 0.93	p = 0.27	p = 0.01	p = 0.71	

leaves from VT_S (Fig. 4a). The total N content in ascending order in organs' overall localities and terms was St < R < Pe < S < M < E (Table S1).

The pattern of P recorded by *R. alpinus* was variable depending on the locality and terms (Fig. 4b). The content of P ranged from 0.2 g kg^{-1} in stems at MD to 6.2 g kg^{-1} in emerging leaves at VT_S. The P content in ascending order in organs' overall localities and terms was St < R < S < Pe < M < E (Table S1). The K content ranged from 7 in the rhizome at MD_S to 44 g kg^{-1} in the petiole at VT_S (Fig. 4c). The K content in ascending order in organs' overall localities and collection terms was R < St < S < M < E < Pe (Table S1). The content of Ca ranged from 0.8 in emerging leaves at MD_S to 11.7 g kg^{-1} in senescent leaves at VT_A. The Ca content in ascending order in organs' overall localities and

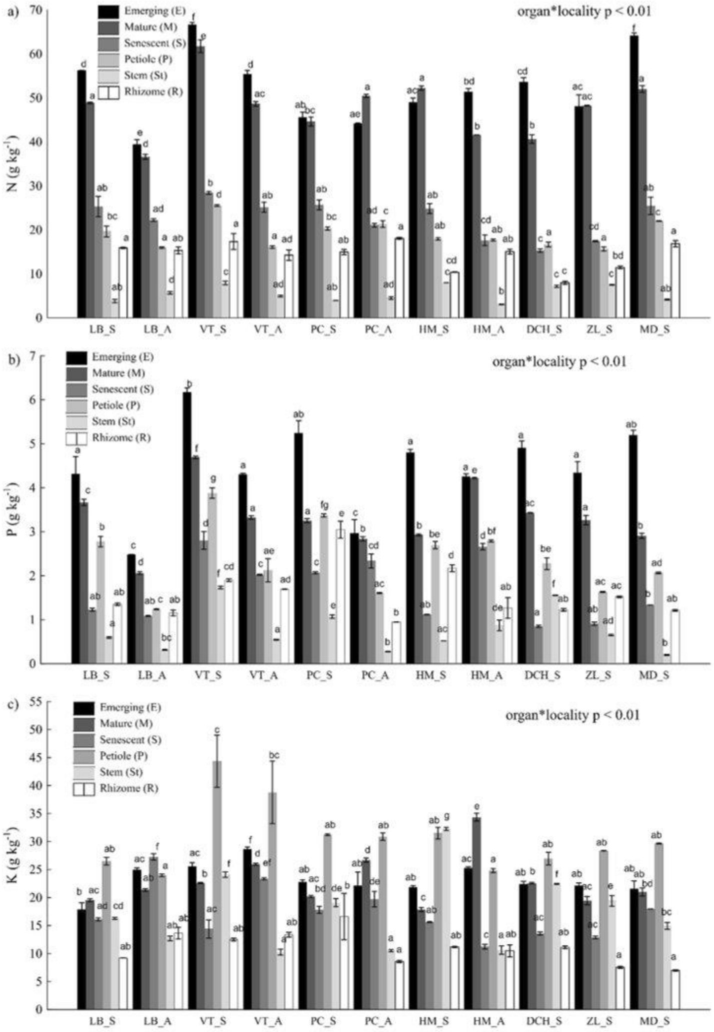

Fig. 4 Effect of locality on the content (mean ± SE) of (**a**) N, (**b**) P, and (**c**) K in different organs of *R. alpinus*. The *p* value for organ, locality, and organ*locality was obtained by factorial ANOVA. The content of elements in individual organs ' overall localities was evaluated by one-way ANOVA. Using the Tukey post hoc test, overall mean values 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)

terms were E < M < St < Pe < R < S (Table S1). There was the highest Mg content in senescent leaves and the lowest in stems for all the localities (Fig. 5b). There was a similar pattern of Mg and Ca in organs' overall localities and terms (Figs. 5a, b). The content of Mg ranged from 0.3 in stems at LB_A to 5 g kg^{-1} in senescent leaves at VT_S. Again, Mg content in ascending order in organs' overall localities and terms was St < R < Pe < E < M < S (Table S1).

In the Krkonoše Mountains, there was a significant effect among different organs and terms for the elements in all localities (Fig. 6). The content of N was slightly higher in summer than in autumn (Fig. 6a). Except for senescent leaves, the content of P was significantly higher in summer than in autumn Fig. 6b). In all three categories of leaves, the K content was higher in autumn, while in stems, in summer (Fig. 6c). The Ca content was significantly lower in emerging and mature leaves and stems in summer than in autumn

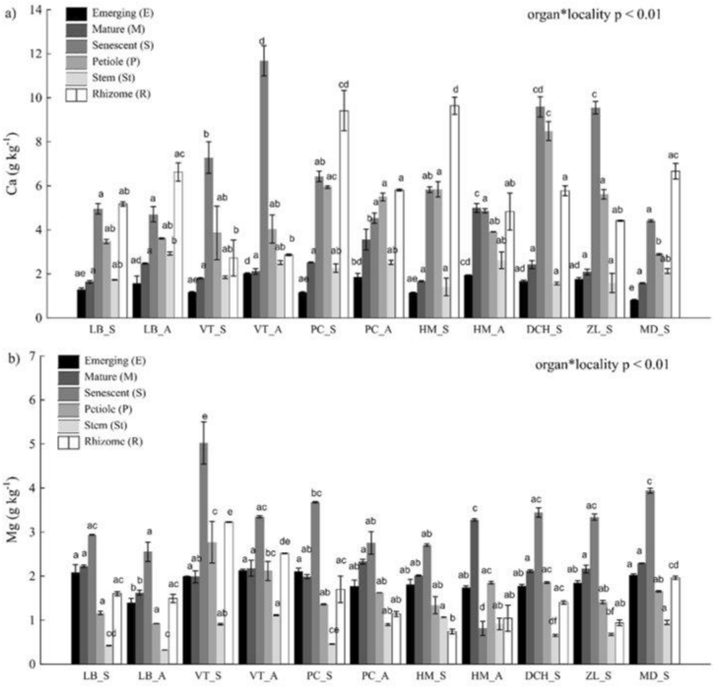

Fig. 5 Effect of locality on the content (mean ± SE) of (**a**) Ca and (**b**) Mg in different organs of *R. alpinus*. The *p* value for organ, locality, and organ*locality was obtained by factorial ANOVA. The content of elements in individual organs ' overall localities was evaluated by one-way ANOVA. Using the Tukey post hoc test, overall mean values of organs 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_Summer), DCH_S (Ramsau am Dachstein_Summer), ZL_S (Zillertal_Summer), and MD_S (Madesimo_Summer)

(Fig. 6d). The content of Mg was significantly higher in summer than in autumn only in senescent leaves (Fig. 6e).

The relationship between each of the studied elements among the overall dataset of biomass samples (above- and below-ground biomass) is given in Figures S2 and S3. Except for the strong positive relationship between N and P ($r = 0.85$, $p < 0.001$; Figure S2a), all the other elements recorded weak correlations ($r = 0.12 – 0.43$), while Ca content was negatively correlated with N, P, and K ($r = -0.17$—0.39; Figures S2d, S2e, and S2f).

3.3 N:P, N:K, and K:P Ratios

The N:P, N:K, and K:P ratios for mature and senescent leaves in each locality are in Table 5. The N:P ratio in mature leaves ranged from 9.8 to 17.9 in HM_A and MD_S. The N:P ratio in senescent leaves ranged from 6.6 to 22.2 in HM_S and autumn, respectively. The mean N:P ratio overall localities were 15 for mature and senescent leaves.

The N: K ratio in mature leaves ranged from 1.2 at HM_A to 2.9 at HM_S. The N: K ratio in senescent leaves ranged from 0.82 to 2.0 in LB_A and VT_S. The mean N:K ratios were 2.2 and 1.4 in mature and senescent leaves, respectively. The K:P ratio in mature leaves ranged from 4.8 to 10.4 in VT_S and LB_A, respectively. The K:P ratio in senescent leaves ranged from 4.2 to 25 in HM_A and LB_A. The mean K:P ratios were 7.1 and 12 in mature and senescent leaves, respectively.

3.4 Resorption of N, P, K, Ca, and Mg

There were varied effects of localities on the resorption of N, P, K, Ca, and Mg (Table 6). The *Nur* of N ranged from 39 to 64%, and the mean N resorption overall localities were 52%. The *Nur* of P ranged from 18 to 75%, with the mean overall localities of 50%, while K was from 10 to 67%. However, we recorded *Nur* of − 28%, indicating more K in senescent than mature leaves. The mean resorption overall localities were 22%.

The *Nur* of Ca and Mg were negative. The only exception was the positive resorption of Ca (2%) and Mg (75%) at HM_A. The negative values for Ca *Nur* ranged from − 22 to − 457%, with mean overall localities of − 211%. The negative values of Mg *Nur* ranged from − 18 to − 153%, with mean overall localities of − 50%.

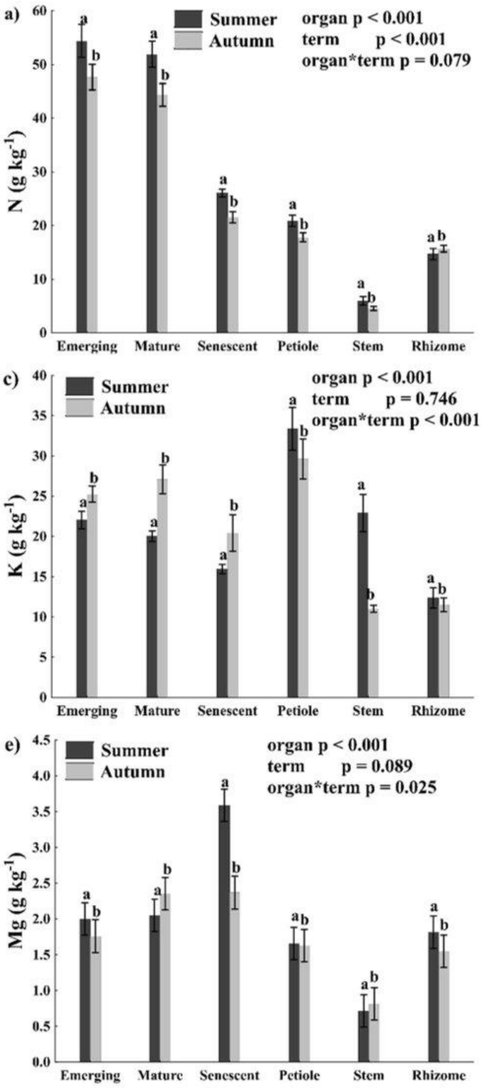

Fig. 6 Effect of terms (summer and autumn) on the content (mean ± SE) of (**a**) N, (**b**) P, (**c**) K, (**d**) Ca, and (**e**) Mg in different organs of *R alpinus* collected from the Giant (Krkonoše) Mountains. The *p* value for organ, locality, and organ*locality was obtained by factorial ANOVA. The *p* value of terms in individual organs was evaluated by one-way ANOVA

4 Discusssion

Different organs of *R. alpinus* reflected varied contents of macroelements. The variability in the contents of N, P, K, Ca, and Mg in the organs directly relates to their contents in the soils. The variation in elemental contents resulted from soil-forming processes, site-specific environmental conditions, and anthropogenic activities per locality

Table 5 The ratio (mean ± SE) between elements N:P, N: K, and K:P in mature and senescent leaves of *R. alpinus*. Differences between localities were obtained by one-way ANOVA—asterisk indicates results significant at $p < 0.001$. Using the Tukey (HSD) post hoc test, mean values with the same letter among localities were not significantly different

Variable	Locality											
	LB_S	LB_A	VT_S	VT_A	PC_S	PC_A	HM_S	HM_A	DCH_S	ZL_S	MD_S	Mean
N:P Mature*	13.3 ± 0.2ab	17.8 ± 0.5a	13.2 ± 0.4ab	14.6 ± 0.3b	13.7 ± 0.1ab	17.8 ± 0.2a	17.8 ± 0.02a	9.8 ± 0.04c	11.9 ± 0.3b	14.8 ± 0.5b	17.9 ± 0.6a	15 ± 0.6
N:P Senescent*	20.5 ± 1.3a	20.5 ± 0.4a	10.2 ± 0.6bc	12.4 ± 0.6b	12.4 ± 0.4b	9.0 ± 0.4bc	22.2 ± 0.9a	6.6 ± 0.6c	18 ± 0.001a	19.2 ± 0.9a	19.1 ± 1.5a	15 ± 1.1
N:K Mature*	2.50 ± 0.03b	1.7 ± 0.04c	2.7 ± 0.1ac	1.8 ± 0.03c	2.2 ± 0.03d	1.9 ± 0.01b	2.9 ± 0.08a	1.2 ± 0.02e	1.8 ± 0.06c	2.48 ± 0.1ad	2.48 ± 0.1ad	2.2 ± 0.1
N:K Senescent*	1.57 ± 0.12ac	0.82 ± 0.03c	2.0 ± 0.20a	1.08 ± 0.06ab	1.44 ± 0.01ac	1.07 ± 0.05ab	1.59 ± 0.08ac	1.57 ± 0.17ac	1.13 ± 0.01ab	1.36 ± 0.03ab	1.42 ± 0.11b	1.4 ± 0.1
K:P Mature*	5.3 ± 0.03ah	10.4 ± 0.04a	4.8 ± 0.05gf	7.8 ± 0.04 cd	6.2 ± 0.03e	9.4 ± 0.04b	6.1 ± 0.16e	8.1 ± 0.13c	6.6 ± 0.03ef	5.96 ± 0.03dh	7.2 ± 0.39 g	7.1 ± 0.4
K:P Senescent*	13 ± 0.15b	25 ± 0.34b	5.1 ± 0.21e	11.5 ± 0.04c	8.6 ± 0.19d	8.4 ± 0.001d	14 ± 0.12b	4.2 ± 0.04e	16 ± 0.08a	14 ± 0.35b	13 ± 0.03b	12 ± 1.2

Abbreviations of localities: *LB* Libuše hut, *VT* Vítkovice v Krkonoších, *PC* Pec pod Sněžkou, *HM* Horní Mísečky, *DCH* Ramsau am Dachstein, *ZL* Zillertal, *MD* Madesimo. *S* summer, *A* autumn, *LB_S* Libuše hut summer)

Table 6 Resorption of elements (*1 − (senescent ÷ mature) × 100)*. Differences between localities (mean ± SE) were evaluated by one-way ANOVA. The asterisk indicates results significant at $p < 0.001$. Using the Tukey post hoc test, the mean values of each element with the same letter among localities were not significantly

Variable	LB_S	LB_A	VT_S	VT_A	PC_S	PC_A %	HM_S	HM_A	DCH_S	ZL_S	MD_S	Mean
N*	48 ± 4.9bc	39 ± 1.7bc	54 ± 1.6acd	48 ± 1.7bc	42 ± 3.8bd	58 ± 0.6ab	52 ± 2.5ac	58 ± 2.9ab	62 ± 0.1ab	64 ± 0.3a	51 ± 3ac	52 ± 8
P*	66 ± 1.5ac	47 ± 1.2bd	40 ± 3.9b	39 ± 0.5b	36 ± 1.7b	18 ± 4.2e	62 ± 0.4af	37 ± 1.9b	75 ± 0.7b	72 ± 2.1ac	54 ± 0.8^{df}	50 ± 4
K*	17 ± 3.4acd	− 28 ± 5.8b	36 ± 10de	10 ± 0.1f	12 ± 5.6f	26 ± 7ad	13 ± 3.2a	67 ± 2.7a	40 ± 2.3e	34 ± 5.2 cd	14 ± 4.1ac	22 ± 5
Ca*	− 204 ± 26abd	− 90 ± 12 cd	− 304 ± 45abf	− 457 ± 65f	− 154 ± 7bcd	− 29 ± 11de	− 249 ± 13abc	2 ± 6a	− 299 ± 49abf	− 359 ± 42af	− 179 ± 0.2ab	− 211 ± 30
Mg*	− 32 ± 2ab	− 57 ± 8abc	− 153 ± 7d	− 55 ± 14abc	− 85 ± 5c	− 18 ± 8b	− 34 ± 2ab	75 ± 5a	− 63 ± 7ac	− 55 ± 9abc	− 72 ± 3ac	− 50 ± 11

The nutrient resorption was done after Vergutz et al. (2012)

Abbreviations of localities: *LB* Libuše hut, *VT* Vítkovice v Krkonoších, *PC* Pec pod Sněžkou, *HM* Horní Mísečky, *DCH* Ramsau am Dachstein, *ZL* Zillertal, *MD* Madesimo. *S* summer, *A* autumn

(Jungová et al. 2022). For example, localities with reduced soil acidity recorded high Ca and Mg contents. The pH range partly supports the dissolution of the elements and subsequent bioaccessibility. In DCH, the comparative low acidity predominantly remained influenced by the Limestone parent rock. Notwithstanding, *R. alpinus* demonstrates tolerance for many soils with diverse chemical properties (Krahulec and Bureš 2019).

The total N content in Krkonoše Mountains localities was similar to other studied sites and corresponds to the long-term fertilizer grassland experiment by Hejcman et al. (2014), except for locality HM, which was 3 times higher. Meanwhile, this resulted from the steep topography of the locality, which accumulated N in the topsoil (Gros et al. 2004; Burt and Rice 2009). Conversely, the low N content in DCH resulted from the inclined position on the North Slope of the Alp, where N losses incur due to leaching and denitrification from low temperatures (Aerts and Chapin 2000; Brant and Chen 2015). Meanwhile, *R. alpinus*, as a nitrophilous plant species, requires a high N supply and adequately high P (Müllerová et al. 2014; Šilc and Gregori 2016; Kołodziejek 2019).

The mean content of N in all the leaves was higher (22.6–52.2 g kg^{-1}) than herbages from different plant communities, e.g., *Utica dioica* (23.2 g kg^{-1}) and many other grassland species—7 to 17 g kg^{-1} (Müllerová et al. 2014; Hejcman et al. 2014; Vondráčková et al. 2014). Consequently, *R. alpinus* utilize large amounts of N in the above-ground biomass (Klimeš et al.1993; Klimešová and Klimeš 1996; Bohner 2005), supported by higher N content in emerging leaves (high metabolic part) than senescent in all the localities (Brant and Chen 2015). The high N content in the emerging leaves is utilized mainly at the cellular level, primarily for enzymatic activities (e.g., increase nitrate reductase activities) and to enable photosynthesis (Bohner 2005; Evans and Clarke 2019) while playing a vital role in the mobility and storage of other nutrients (Canton et al. 2005). Also similar to other alpine plants with high nutrients in the above-ground organs, e.g., *Gentiana rigescens* (Zhang et al. 2020). The relatively similar content of N in the senescent leaves in all the localities is associated with the moderately acidic condition of the soils, which contributes to the dissolution of elements for further bioavailability. The content of N was lowest in the stem and rhizome. The stem serves as a transport conduit from roots to photosynthetic tissues, with lower nutrient contents compared to, e.g., the leaf (Müllerová et al. 2014; Brant and Chen 2015). Nitrogen contents in the rhizomes were lower compared to Bohner (2005). In this study, the rhizomes of *R. alpinus* are comparatively N-rich in the contents of mountain grassroots (Hejcman et al. 2014).

Although the total P content was comparatively lower than most recorded values in the mountain soil ecosystem (1.11 – 1.88 g kg^{-1}; Hejcman et al. 2014; Zhou et al. 2022), the plant-available portion was relatively higher than many other mountain soils, e.g., soils in the community of *Polygono-Trisetion* (Hejcman et al. 2007; Pavlů et al. 2011, 2013). However, the total P content (0.8 g kg^{-1}) in mountain grassland recorded by Semelová et al. (2008) was similar to this study, indicating wide variability of P content in mountain soils. The high P content in emerging and mature leaves supports their high metabolic activities (Vance et al. 2003; Vondráčková et al. 2014; Gao et al. 2019). In the petiole (transport conduit), the relatively high P content in all localities relates to the formation of chelates, with cellular compartments. The contents of individual N and P nutrients in mature and senescent leaves differed at different localities, indicating competitive interactions between species. Localities affected by human activities often result in higher N and P, which may increase competitor organisms for *R. alpinus* seedlings (Zaller 2004; Šilc and Gregori 2016). Additionally, *R. alpinus* can acquire N and P from the soil profile highly facilitated by mycorrhizae (Aerts and Chapin 2000; Bohner 2005). Our results confirm that high leaf N and P contents as a trait for leaf longevity, high photosynthetic, and growth rates are central to *R. alpinus* communities (Vance et al. 2003; Grime et al. 2007; Vergutz et al. 2012; Brant and Chen 2015).

The strategy of plants with nutrient-rich foliage is fast growth and dominance in nutrient-rich ecosystems, which increases eutrophication. Thus, *R. alpinus* is a problematic weed (Stachurska-Swakoń 2009; Delimat and Kiełtyk 2019) and invasive species in the Krkonoše Mountains (Šťastná et al. 2010; Pyšek et al. 2012).

The N:P and N: K ratios in mature leaves of *R. alpinus* are within the range in plant tissues and indicate a comparatively high P demand. The mean resorption of N (52%) and P (50%) was smaller compared to the average percentage of many other vascular plants (62.1 N to 64.9% P) (Vergutz et al. 2012; Brant and Chen 2015; Kong et al. 2020). Compared to senescent leaves, mature leaves have rapid biomass production and thus higher N and P demands, which could be possible drivers of higher N and P resorption efficiency in this study (Minden and Venterink 2019). The significant difference in the resorption of N and P between seasons in the Krkonoše localities indicates that the mature leaves are metabolically active during the summer. The N and P resorption efficiencies in leaves of *R. alpinus* had similar patterns in all the studied localities. The highly effective N and P resorption in *R. alpinus* leave indicate better internal cycling of these elements and the ability of this species to cope with low soil N and P availability (Hejcman et al. 2014). Compared to *Deschampsia caespitosa* (Hejcman et al. 2014), which commonly grows in the *Rumicetum alpini* community, the resorption intensity of P by *R. alpinus* was similar. Thus, the ratio between N and P indicates whether N or P limits plant growth, not the absolute N and P content in plant tissues. Plants with element restrictions in nutrient-rich habitats, despite adequate N content, often have low N:P ratios (Thompson et al. 1997; Ding et al. 2022). The growth of boreal and temperate plants tends to be more N-limited because of N losses due to leaching and denitrification at lower temperatures (Aerts and Chapin 2000; Brant and Chen 2015). This pattern suggests that plant growth might be limited mainly by P availability, particularly in pastures.

The range of total K content in all localities probably was connected with the parent rock (Prášková and Němec 2016). The plant-available K content was relatively similar in all the studied localities except for the VT locality. In VT, the lowest available K resulted from partly taken K via environmental losses, e.g., graminoids, conifers, and forbs that grow in the vicinity (Vergutz et al. 2012). Soils with high organic matter and low clay resist the release of K by weathering clay minerals to compensate for K removal, which is affected by the management regime in the vicinity (Pavlů et al. 2013).

Our study indicated the highest K content in the petiole. Notably, K is a highly mobile element and is re-translocated from an organ to other organs via phloem transport, especially in summer (Chen et al. 2016; de las Heras et al. 2017). Additionally, the high content of H_2O in the petiole is responsible for K enrichment (Tůma et al. 2004; Bohner 2005).

The resorption content of K was 22% compared to the 70% recorded by Vergutz et al. (2012), probably because most of K was in the petiole. Thus, there is K wastage. Plants tend to allocate higher quantities of nutrients to leaves, except for senescent leaves, to facilitate faster growth for competitive advantage during shorter growing seasons (Brant and Chen 2015). Hence, *R. alpinus* recorded high content of K in juvenile and mature leaves. Thus, K deficiency in soil is perhaps more limiting for *R. alpinus* than N and P deficiency (Pavlů, unpublished data). Eventually, this corresponds to the findings of Šťastná et al. (2010) that *R. alpinus* is a plant species with a high absorption capacity for K and is a bioindicator of soils rich in K.

The mature leaves of *R. alpinus* were moderately K co-limited because the N:K ratio was above 2.1 (Olde Venterink et al. 2003; Minden and Olde Ventering 2019), especially during summer in all localities, as they undergo higher N metabolic activities (López-Lefebre et al. 2001). Meanwhile, K metabolic activity is much higher in the petiole. Strong N-limitation in all localities was in senescent leaves. Additionally, sufficient K and P-limitations occurred by a K:P ratio above 3.4 in the mature and senescent leaves (Olde Venterink et al. 2003).

The high availability of Ca and Mg in locality PC is partly from the different compositions of an organic substrate as the locality is on a building plot (Müllerová et al. 2011). The total contents of elements in the soils were higher than plant-available, indicating different patterns of element distributions (Hejcman et al. 2009; Müllerová et al. 2014). The total content of elements is of less value in diagnosing plants' nutrient deficiencies (Cole et al. 2016) as they are relatively insoluble.

Comparatively low contents of Ca and Mg were in the stems as a conduit with low metabolic activity but high mobility of elements, which leads to translocation into plant apices (Anton and Mathe-Gaspar 2005; Gaweda 2009; Vondráčková et al. 2014). The mean contents of Ca and Mg in emergent and mature leaves were low, especially Ca *Rumex alpinus* does not require an increased supply of essential nutrients, e.g., Ca and Mg, during the growing season. Meanwhile, the highest contents of Ca and Mg were recorded in senescent leaves as *R. alpinus* is an "oxalate plant" that regulates excessive Ca in tissues by precipitation of Ca-oxalate (White and Broadley 2003; Vondráčková et al. 2014).

Calciophobic plant species, such as *R. alpinus*, are characterized by a relatively low Ca:Mg and high K:Ca ratio in their leaves (Bohner 2005). The translocation of Ca from senescent leaves and rhizomes to mature and emerging leaves is strongly restricted. The least resorption of Ca and Mg were stored in the mature leaves, as their contents tend to decrease with age (Jones 2012). Thus, negative values show an indication of restricted resorption in senescent leaves. *Rumex alpinus* discriminates Ca and Mg uptake, characterized by relatively low contents (Bohner 2005). Bohner (2005) reported that *R. alpinus* discriminates Ca for nutrient uptake (stored in rhizome) due to the high content of H_2O-soluble oxalate together with a comparatively low content of H_2O-soluble malate in the mature leaves. And this is confirmed by the negative mean resorption of Ca and Mg (-211% and -50%, respectively). Thus, *R. alpinus* discriminates Ca and Mg uptake (van Heerwaarden et al. 2003). Additionally, Ca content cannot be mobilized from older tissues and redistributed through the phloem to other parts of plants and is conserved in the leaves as a structural element (White and Broadley 2003; Kazakou et al. 2007).

Although the resorption of Mg is generally low in all the localities, there was high resorption of Mg (75) during the autumn in HM. Further studies on the resorption efficiency of Mg, considering the effects of many environmental parameters, are required.

5 Conclusions

The comparative study revealed high variability in the distribution and accumulation of phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in different organs of *R. alpinus* in all the localities. Although variation in the content of macroelements in organs indicates their functionality and peculiar characteristic of *R. alpinus*, their availability is associated with environmental conditions, geological substrates, element content in the soil, pH, and seasons. Notwithstanding, there was a clear pattern of high accumulation of nitrogen (N) and P, especially in the emerging and mature leaves, and Ca in the senescent leaves in all localities.

These relatively low resorptions for Ca and Mg indicate low nutrient use efficiency and re-translocation of these nutrients from senescent to young leaves. As a consequence, *R. alpinus* is considered oxalate, according to the high Ca and Mg in their senescent leaves. The resorption content of N and P was higher compared to the average percentage of other terrestrial plants. *Rumex alpinus* exhibit less resorption of K, an indication of nutrient waste, and simultaneously a high K demand.

Rumex alpinus have N:P and N:K ratios within the normal range in plant tissues but show a comparatively high demand for P. Due to the high nutrient demands of *R. alpinus*, it inhabits only nutrient-rich localities, which encourages significant degradation of various vegetation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42729-022-01059-5.

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Declarations

Conflict of interest The authors declare no competing interests.

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4.2 CHAPTER 2 (PAPER 2)

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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RESEARCH ARTICLE

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 belowground 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.

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; Šťastná 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 (Karczewska 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 [ºC]	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 grassland	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^2 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^2 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_2O 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_3, 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_3, 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_2O_2 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_3 for two hours and filtrated. Total content was determined by inductively coupled

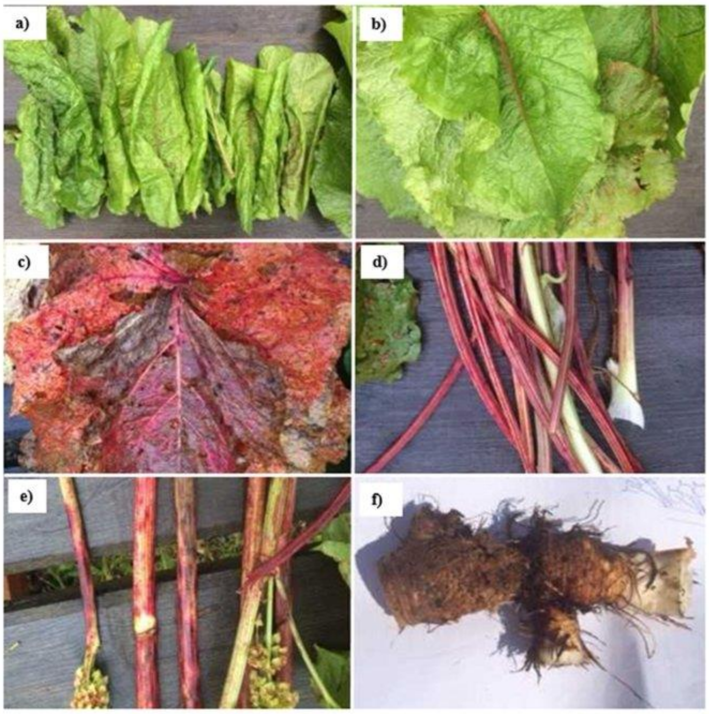


Fig. 2 Studied organs of *Rumex alpinus*: (**a**) emerging- E (**b**) mature- M, and (**c**) senescent leaf blades- S, (**d**) petioles from mature leaves- Pe, (**e**) stems from flowering plants- St, and (**f**) two-year-old rhizome- R

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 $CH_3COOH + 0.25$ M $NH_4NO_3 + 0.013$ M $HNO_3 + 0.015$ M $NH_4F + 0.001$ M EDTA; usage: 25 cm^3 reagent per 2.5 cm^3 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). We determined the soil pH (H_2O) 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).

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

The BF was calculated by the following equation,

$$\begin{array}{rl} i. & BF = leaf \div soil \\ ii. & BF = petiole \div soil \\ iii. & BF = rhzome \div soil \end{array} \qquad (1)$$

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,

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

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_2O] (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^{-1} in DCH and HM, respectively. The total content of Zn ranged from 48 in DCH to 182 mg kg^{-1} in PC. The content 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 content ranged from 11.1 in LB to 27.1 g kg^{-1} 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^{-1} 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	*F*-value	*P* value
pH (H_2O)		5.2 ± 0.17^{d}	5.7 ± 0.04^{bcd}	5.9 ± 0.11^{cd}	5.6 ± 0.13^{abc}	6.1 ± 0.33^{a}	5.3 ± 0.67^{ab}	22.1	0.001
Fe	(g kg^{-1})	21.1 ± 0.6^{ab}	24.2 ± 2.1^{a}	23.8 ± 1.4^{a}	27.5 ± 4.0^{a}	13.6 ± 0.1^{b}	18.2 ± 2.3^{ab}	3.0	0.05
Zn	(mg kg^{-1})	179 ± 12.6^{a}	165 ± 37.3^{a}	182 ± 36.8^{a}	124 ± 19.4^{ab}	48 ± 0.6^{b}	58 ± 0.5^{b}	3.3	0.05
Cu	(mg kg^{-1})	39.8 ± 1.4^{a}	28.3 ± 3.3^{ab}	23.3 ± 1.5^{ab}	31.9 ± 6.2^{ab}	4.7 ± 0.1^{c}	19.8 ± 5.2^{bc}	6.6	0.002
Mn	(mg kg^{-1})	655 ± 6.2^{a}	693 ± 101^{a}	603 ± 29^{ab}	581 ± 216^{ab}	519 ± 19^{ab}	178 ± 79^{b}	1.6	0.24
Al	(g kg^{-1})	11.1 ± 2.1^{b}	27.1 ± 5.2^{a}	14.6 ± 2.0^{b}	14.4 ± 2.4^{b}	13.1 ± 0.5^{b}	18.1 ± 1.3^{ab}	3.4	0.33
As	(mg kg^{-1})	$\mathbf{54.5} \pm 5.9^{ab}$	22.5 ± 2.3^{c}	16.3 ± 2.6^{cd}	$\mathbf{70.9} \pm 6.2^{a}$	3.9 ± 0.4^{d}	$\mathbf{41.9} \pm 1.1^{b}$	29.9	0.001
Cr	(mg kg^{-1})	36.1 ± 1.5^{abc}	46.1 ± 3.5^{a}	43 ± 1.6^{ab}	53.2 ± 6^{a}	22.6 ± 0.4^{bc}	20.6 ± 9.5^{c}	5.4	0.01
Ni	(mg kg^{-1})	20.7 ± 19.1^{ab}	26.6 ± 24.1^{a}	22.2 ± 19.3^{ab}	28 ± 5.8^{a}	11.6 ± 10.8^{b}	11.7 ± 3.2^{b}	3.3	0.05
Pb	(mg kg^{-1})	44.5 ± 3.5^{ab}	42.1 ± 8.2^{abc}	39.9 ± 5.3^{abc}	70.8 ± 12.8^{a}	13.2 ± 0.2^{c}	21.5 ± 3.6^{bc}	4.9	0.01
Cd	(mg kg^{-1})	1.32 ± 0.17^{a}	1.18 ± 0.22^{a}	0.89 ± 0.14^{ab}	1.35 ± 0.05^{a}	0.43 ± 0.01^{b}	0.46 ± 0.12^{b}	5.3	0.01

Abbreviations: *LB* Libuše hut, *VT* Vítkovice v Krkonoších, *PC* Pec pod Sněžkou, *HM* Horní Mísečky, *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 (± SE) plant-available content of elements in the upper 10 cm soil layers of the studied localities

Locality		LB	Vítkovice	PC	HM	DCH	Madesimo	*p* value
Fe	(mg kg^{-1})	404 ± 9.5^{d}	527 ± 5.1^{c}	260 ± 6.9^{e}	657 ± 21.2^{a}	204 ± 13^{f}	554 ± 14.3^{b}	< 0.001
Zn	(mg kg^{-1})	39.5 ± 4.7^{b}	10.6 ± 0.3^{d}	62.1 ± 0.7^{a}	25.2 ± 0.2^{c}	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^{b}	99 ± 1.8^{d}	189 ± 3.8^{a}	59 ± 0.8^{e}	131 ± 1.1^{c}	43 ± 2.4^{f}	< 0.001
Al	(mg kg^{-1})	931 ± 24^{d}	1474 ± 72^{b}	648 ± 16.2^{f}	1720 ± 77^{a}	697 ± 16.5^{e}	1256 ± 68^{c}	< 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 ± 0.02bc	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

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^{-1} in HM. The total Pb content ranged from 13.2 in DCH to 70.8 mg kg^{-1} in HM. The total Cd content ranged from 0.43 in DCH to 1.35 mg kg^{-1} 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^{-1} in DCH and HM, respectively. The plant-available content 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 content ranged from 43 in MD to 189 mg kg^{-1} in PC. The plant-available content of Al ranged from 648 to 1720 mg kg^{-1} in PC and HM, respectively. The available As content ranged 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 content of available Ni ranged from 0.34 in DCH to 1.85 mg kg^{-1} in LB. The plant-available content of Pb ranged from 2.15 in HM to 21 mg kg^{-1} in PC. Finally, the available content of Cd ranged from 0.06 in MD to 0.51 mg kg^{-1} in LB. The 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^{-1} 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^{-1} 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^{-1} 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^{-1} 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^{-1} 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^{-1} in senescent leaves in PC_A. The mean As

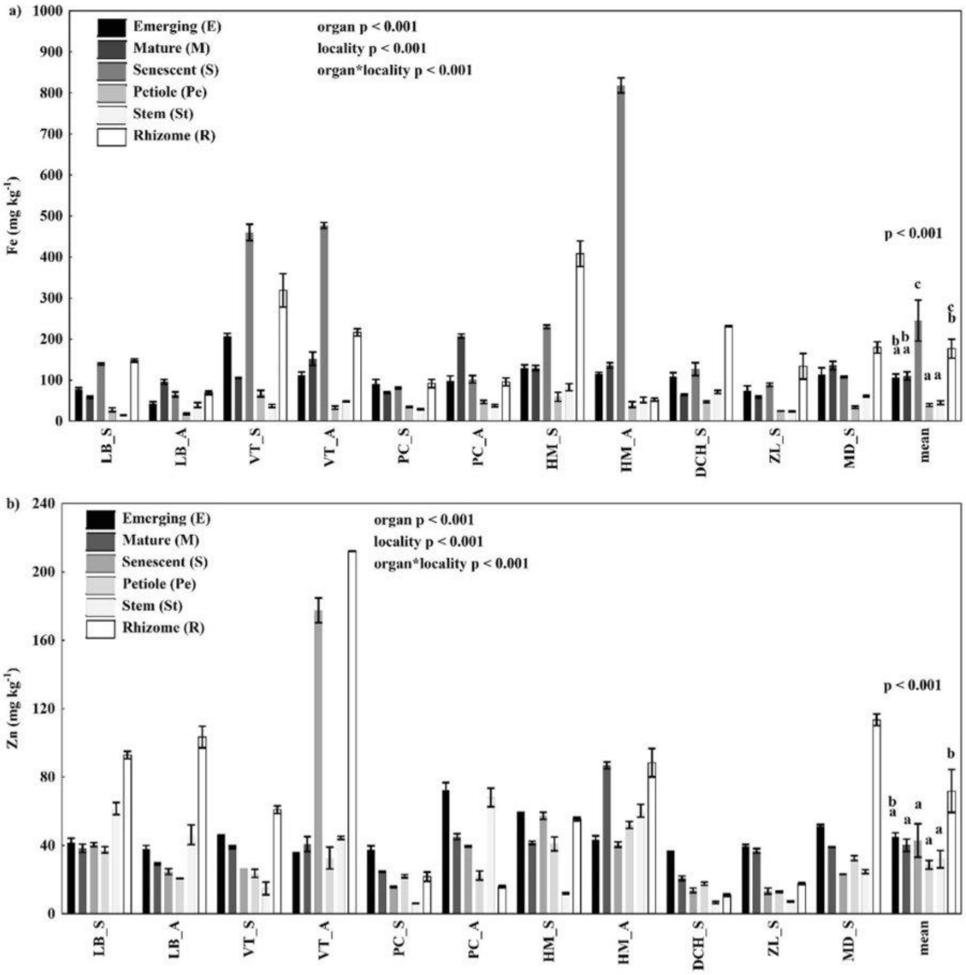


Fig. 3 Effect of locality on the content (mean ± SE) of (**a**) Fe and (**b**) Zn in different organs of *R alpinus*. The *p* value was obtained by factorial ANOVA. The content of elements in individual organs' overall localities was evaluated by One-way ANOVA. Using the Tukey post hoc test, mean contents 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^{-1} 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^{-1} in the rhizomes of VT_A. The mean

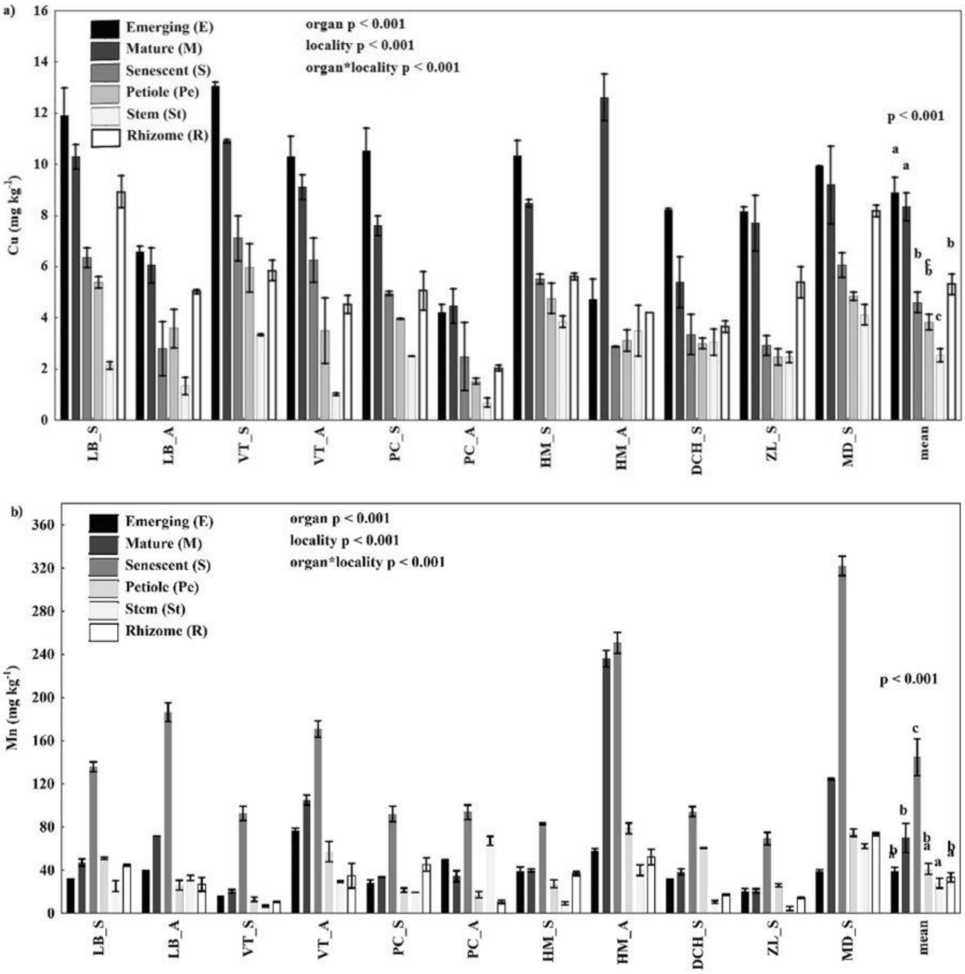
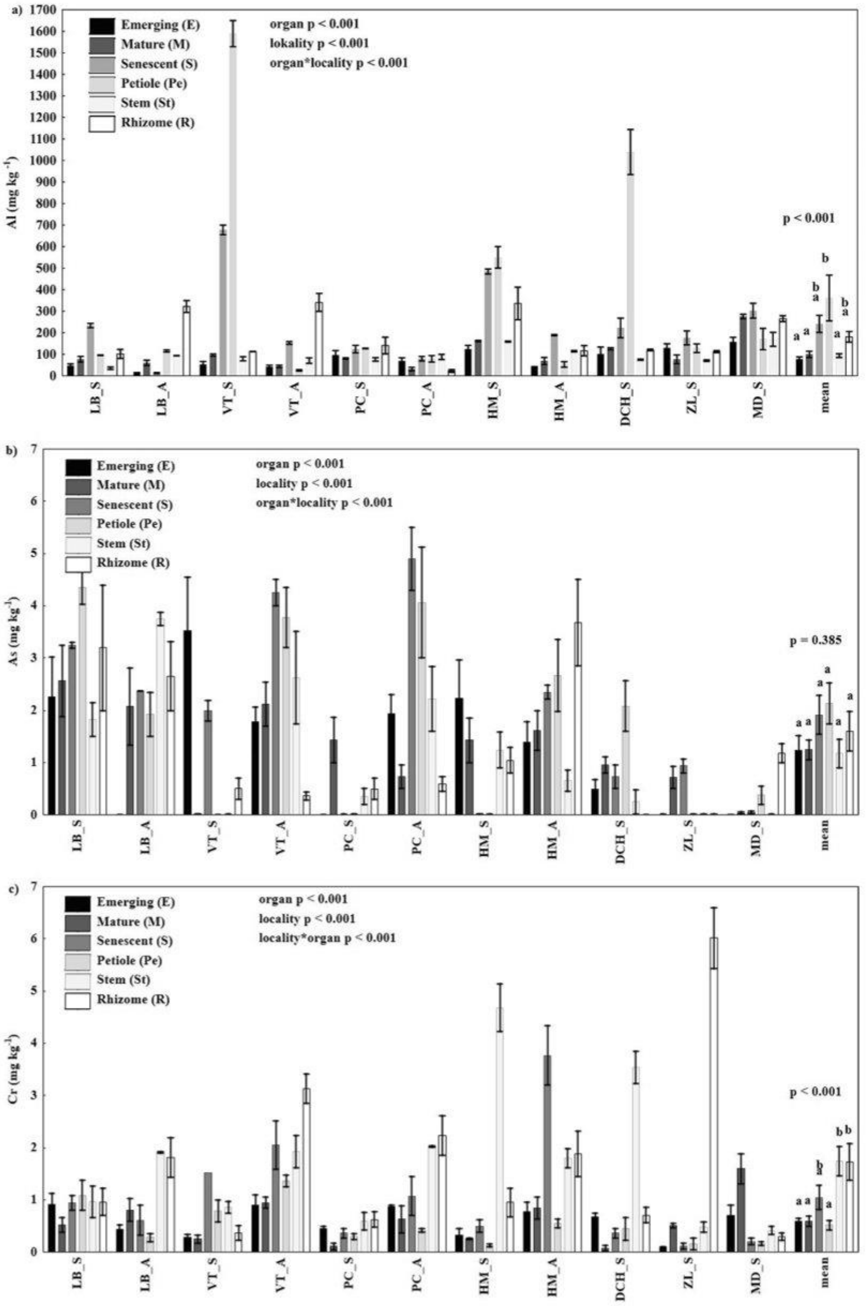


Fig. 4 Effect of locality on the content (mean ± SE) of (**a**) Cu and (**b**) Mn in different organs of *R alpinus*. The *p* value was obtained by factorial ANOVA. The concentration of elements in individual organs' overall localities was evaluated by One-way ANOVA. - Using the Tukey post hoc test, 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^{-1} 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


a)
Emerging (E)
Mature (M)
Senescent (S)
Petiole (Pe)
Stem (St)
Rhizome (R)
organ p < 0.001
lokality p < 0.001
organ*locality p < 0.001
Al (mg kg-1)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
p < 0.001
a a b a b a a b a b
LB_S
LB_A
VT_S
VT_A
PC_S
PC_A
HM_S
HM_A
DCH_S
ZL_S
MD_S
mean
b)
Emerging (E)
Mature (M)
Senescent (S)
Petiole (Pe)
Stem (St)
Rhizome (R)
organ p < 0.001
locality p < 0.001
organ*locality p < 0.001
As (mg kg-1)
0
1
2
3
4
5
6
7
p = 0.385
a a a a a a
LB_S
LB_A
VT_S
VT_A
PC_S
PC_A
HM_S
HM_A
DCH_S
ZL_S
MD_S
mean
c)
Emerging (E)
Mature (M)
Senescent (S)
Petiole (Pe)
Stem (St)
Rhizome (R)
organ p < 0.001
locality p < 0.001
locality*organ p < 0.001
Cr (mg kg-1)
0
1
2
3
4
5
6
7
p < 0.001
a a b a a b b
LB_S
LB_A
VT_S
VT_A
PC_S
PC_A
HM_S
HM_A
DCH_S
ZL_S
MD_S
mean

◂**Fig. 5** Effect of locality on the content (mean ± 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^{-1} 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^{-1} 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 ÷ soil, petiole ÷ soil, rhizome ÷ 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 ÷ rhizome)

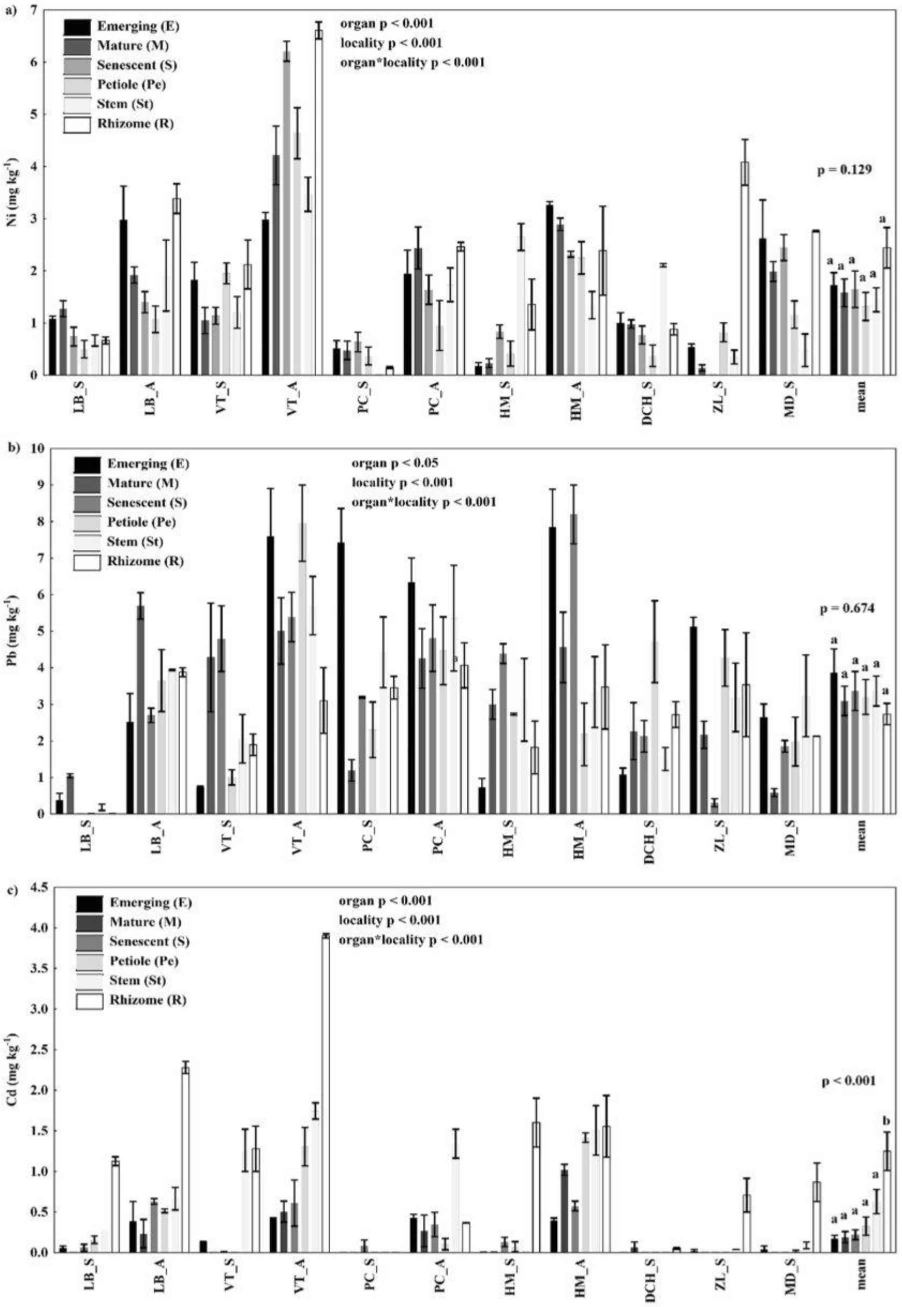
The mean TF of Al, Cr, and Fe was <1 (Table 6). Only in PC_A was the TF of Al above 1, and in MD, Cr was above 1. TF of Cd was <1 in all localities. The TF for Fe was above 1 in autumn in localities LB, PC, and HM. The TF of Cu, Mn, Ni, and Pb was mainly above 1.

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_2O) 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^{-1} 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^{-1} (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^{-1} 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^{-1}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^{3+} and Mn^{4+} predominate, and insoluble Mn oxides will form (Rengel 2000; Marschner 2012). Thus, Mn shows some translocation


a)
Emerging (E)
Mature (M)
Senescent (S)
Petiole (Pe)
Stem (St)
Rhizome (R)
organ p < 0.001
locality p < 0.001
organ*locality p < 0.001
p = 0.129
Ni (mg kg-1)
b)
organ p < 0.05
locality p < 0.001
organ*locality p < 0.001
p = 0.674
Pb (mg kg-1)
c)
organ p < 0.001
locality p < 0.001
organ*locality p < 0.001
p < 0.001
Cd (mg kg-1)
LB_S
LB_A
VT_S
VT_A
PC_S
PC_A
HM_S
HM_A
DCH_S
ZL_S
MD_S
mean

◂**Fig. 6** Effect of locality on the content (mean ± 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^{-1} (Adriano 2001). The average total content 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 anthropogenic activities. The average Ni content in the soil generally is considered to be approximately 20 mg kg^{-1} (Adriano 2001), and the results show t again exceeded in the Krkonoše localities. Notably, contaminated agricultural areas in the Czech Republic have Ni values >200 mg kg^{-1} (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^{-1} (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 ≤ of 6.5 is 300 mg kg^{-1} (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^{-1} 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 ± 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	*F*-value	*P* value	Normal value *
Fe	(mg kg^{-1})	106 ± 8.8^{bc}	110 ± 10^{bc}	245 ± 50^{a}	39.2 ± 3.3^{c}	45 ± 4.4^{c}	177 ± 23^{ab}	11.5	< 0.001	30 – 300 $^{(3)}$
Zn	(mg kg^{-1})	45.3 ± 2.4^{ab}	40.1 ± 3.6^{b}	42.9 ± 9.1^{b}	28.6 ± 2.5^{b}	32 ± 5.1^{b}	75 ± 12.5^{a}	4.6	< 0.001	10 – 150 $^{(2)}$
Cu	(mg kg^{-1})	8.9 ± 0.6^{a}	8.3 ± 0.5^{a}	4.6 ± 0.4^{b}	3.8 ± 0.3^{bc}	2.5 ± 0.3^{c}	5.3 ± 0.4^{b}	33.8	< 0.001	4 – 15 $^{(6)}$
Mn	(mg kg^{-1})	38.8 ± 3.6^{cd}	17.1 ± 13.5^{b}	145 ± 17^{a}	41.3 ± 5.0^{cd}	27.9 ± 4.5^{c}	33.3 ± 4.3^{cd}	21.7	< 0.001	40 – 200 $^{(4)}$
Al	(mg kg^{-1})	79 ± 10^{b}	99.8 ± 14.5^{b}	214 ± 40^{ab}	361 ± 106^{a}	94 ± 8.7^{b}	181 ± 24^{ab}	5.3	< 0.001	_
As	(mg kg^{-1})	1.2 ± 0.3^{a}	1.2 ± 0.2^{a}	1.9 ± 0.4^{a}	1.8 ± 0.4^{a}	1.2 ± 0.3^{a}	1.2 ± 0.3^{a}	1.6	0.173	1 - 1.7 $^{(1)}$
Cr	(mg kg^{-1})	0.6 ± 0.1^{b}	0.6 ± 0.1^{b}	1.0 ± 0.2^{ab}	0.5 ± 0.1^{b}	1.7 ± 0.3^{a}	1.7 ± 0.4^{a}	7.0	< 0.001	0.1 - 0.5 $^{(1)}$
Ni	(mg kg^{-1})	1.7 ± 0.2^{a}	1.6 ± 0.3^{a}	1.6 ± 0.4^{a}	1.3 ± 0.3^{a}	1.4 ± 0.2^{a}	2.4 ± 0.4^{a}	1.8	0.128	0.5 – 5 $^{(5)}$
Pb	(mg kg^{-1})	3.9 ± 0.7^{a}	3.1 ± 0.4^{a}	3.4 ± 0.5^{a}	3.2 ± 0.5^{a}	3.3 ± 0.4^{a}	2.7 ± 0.3^{a}	0.6	0.674	0.5 – 10 $^{(2)}$
Cd	(mg kg^{-1})	0.17 ± 0.04^{b}	0.19 ± 0.07^{b}	0.22 ± 0.06^{b}	0.33 ± 0.11^{b}	0.63 ± 0.15^{b}	1.25 ± 0.24^{a}	10.5	< 0.001	0.05 – 2 $^{(2)}$

*– 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 Güleryüz 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), Güleryüz 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^{-1}, 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^{-1}. For comparison, the Environmental Protection Agency (EPA, 2021)

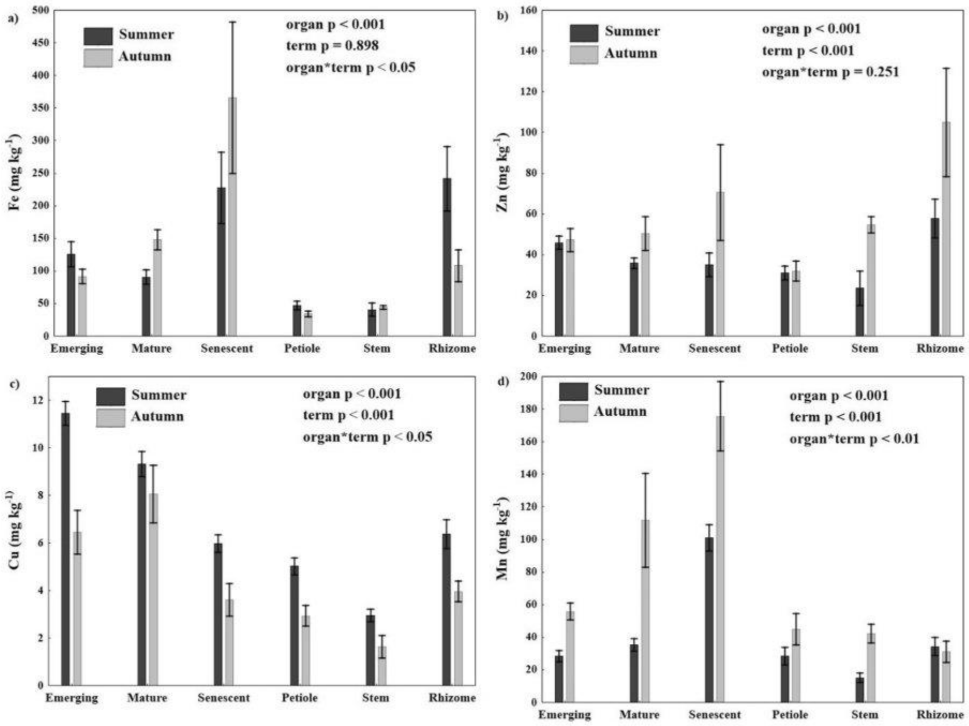


Fig. 7 Effect of term on the content (mean ± SE) of (**a**) Fe, (**b**) Zn, (**c**) Cu, and (**d**) Mn in different organs of *Rumex alpinus*, collected in the Krkonoše Mountains. The *p* value was obtained by factorial ANOVA

allows maximum level limits of arsenic in US drinking water to 10 micrograms per liter (μg 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 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 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 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

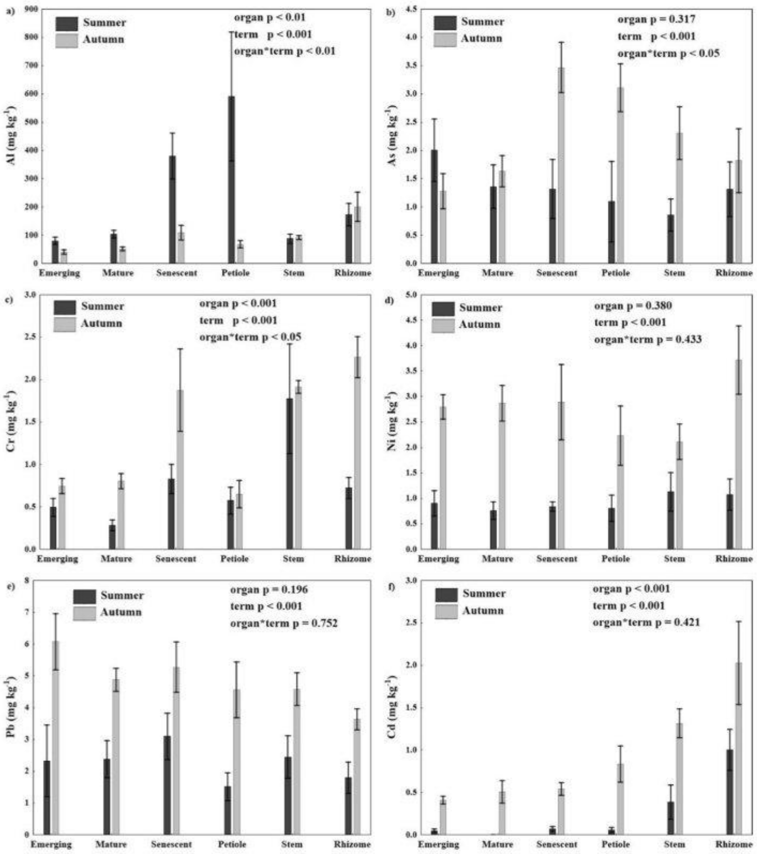


Fig. 8 Effect of term on the content (mean ± SE) of (**a**) Al, (**b**) As, (**c**) Cr (**d**), Ni, (**e**) Pb, and (**f**) Cd in different organs of *R. alpinus*, collected in the Krkonoše Mountains. The *p* value was obtained by factorial ANOVA

public health. For comparison, according to the World Health Organization, the highest dose is equivalent to 1210 mg kg^{-1} 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 belowground organs, we found Pb to be higher in aboveground 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^{-1} 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 ± 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 ± 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}	$1.01 \pm 0.4b^{cd}$	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}	$4.8 \pm 1.1b$	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

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 aboveground 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^{-1} according to Commission Regulation (EU) No. 1275/ 2013. The permitted weekly limit for a 60 kg human is to consume 0.43 mg kg^{-1} 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 ± 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 ± 0.01^{a}	0.43 ± 0.01^{ab}	1.2 ± 0.05^{a}	0.88 ± 0.05^{a}	0.61 ± 0.02^{abc}	0.88 ± 0.34^{a}	0.8 ± 0.18^{a}	1.8 ± 0.3^{ab}	0 ± 0^{a}	0.03 ± 0.01^{a}
LB_A	1 ± 0.14^{ab}	0.32 ± 0.03^{ab}	1.3 ± 0.11^{a}	2.2 ± 0.51^{ab}	0.12 ± 0.03^{b}	0.38 ± 0.05^{a}	0.38 ± 0.16^{a}	0.72 ± 0.01^{ab}	1.1 ± 0.2^{ab}	0.14 ± 0.1^{a}
VT_S	0.5 ± 0.08^{a}	0.7 ± 0.04^{ab}	2.1 ± 0.12^{a}	0.68 ± 0.02^{a}	0.66 ± 0.03^{ac}	4.7 ± 2.9^{a}	0.83 ± 0.29^{a}	0.72 ± 0.2^{ab}	1.3 ± 0.2^{ab}	0.05 ± 0.01^{a}
VT_A	0.61 ± 0.08^{a}	0.18 ± 0.01^{a}	2.2 ± 0.31^{a}	3 ± 0.92^{ab}	0.13 ± 0.03^{bc}	5.9 ± 2.2^{a}	0.3 ± 0.08^{a}	0.55 ± 0.1^{ab}	2.2 ± 0.7^{b}	0.12 ± 0.02^{a}
PC_S	0.89 ± 0.18^{ab}	1.4 ± 0.13^{c}	1.8 ± 0.14^{a}	0.69 ± 0.6^{a}	0.65 ± 0.09^{ac}	1.9 ± 1.2^{a}	0.47 ± 0.09^{a}	3.5 ± 0.7^{c}	1.24 ± 0.1^{ab}	0.69 ± 0.04^{a}
PC_A	1.6 ± 0.3^{bc}	3.7 ± 0.4^{d}	2.2 ± 0.36^{a}	4 ± 0.84^{b}	2.2 ± 0.09^{d}	2.5 ± 1.1^{a}	0.34 ± 0.01^{a}	0.88 ± 0.14^{ab}	1.3 ± 0.01^{ab}	0.94 ± 0.21^{a}
HM_S	0.32 ± 0.02^{a}	0.9 ± 0.03^{bc}	1.7 ± 0.03^{a}	1.1 ± 0.01^{a}	0.44 ± 0.08^{abc}	1.8 ± 0.3^{a}	0.35 ± 0.16^{a}	0.20 ± 0.13^{ab}	1.13 ± 0.3^{ab}	0 ± 0^{a}
HM_A	2.4 ± 0.3^{c}	0.74 ± 0.1^{abc}	2.1 ± 0.20^{a}	2.9 ± 0.4^{ab}	0.5 ± 0.16^{abc}	0.4 ± 0.01^{a}	0.48 ± 0.22^{a}	1.5 ± 0.5ab	2 ± 0.7^{b}	0.48 ± 0.1^{a}
DCH_S	0.37 ± 0.03^{a}	3 ± 0.16^{e}	1.9 ± 0.03^{a}	2 ± 0.01^{ab}	0.94 ± 0.09^{a}	0 ± 0^{a}	0.55 ± 0.13^{a}	1.15 ± 0.2^{ab}	0.6 ± 0.1^{ab}	0.55 ± 0.5^{a}
ZL_S	0.51 ± 0.1^{a}	2 ± 0.0^{e}	1.5 ± 0.29^{a}	1.4 ± 0.1^{a}	0.92 ± 0.21^{a}	0 ± 0^{a}	0.05 ± 0.01^{a}	0.08 ± 0.01^{b}	1.2 ± 0.5^{ab}	0.02 ± 0.02^{a}
MD_S	0.7 ± 0.13^{a}	0.4 ± 0.02^{ab}	1.2 ± 0.06^{a}	1.1 ± 0.02^{a}	0.81 ± 0.02^{a}	0 ± 0^{a}	4.4 ± 1.94^{b}	0.83 ± 0.2^{ab}	0.8 ± 0.11^{ab}	0.03 ± 0.03^{a}
Mean	0.86 ± 0.14	1.3 ± 0.24	1.7 ± 0.09	1.9 ± 0.24	0.73 ± 0.12	1.7 ± 0.5	0.81 ± 0.28	1.1 ± 0.2	1.17 ± 0.15	0.28 ± 0.1
P 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 exudate 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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4.3 CHAPTER 3 (PAPER 3)

Origin and Genetic Variability of Populations of the Invasive Plant *Rumex alpinus* L. in the Giant (Krkonoše) Mountains

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RESEARCH ARTICLE

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Origin and genetic variability of populations of the invasive plant *Rumex alpinus* L. in the Giant (Krkonoše) Mountains

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Abstract

Monk's rhubarb, *Rumex alpinus* L. (*R. alpinus*), is a perennial plant native to the mountains of Central and Southern Europe. Currently, the distribution of *R. alpinus* has been partly affected by its utilization as a vegetable and a medicinal herb. In the mountains of the Czech Republic, it is considered an invasive plant, probably introduced into the Krkonoše Mountains by colonists from the Alps. This study's main aim was to verify whether *R. alpinus* was introduced into the Krkonoše Mountains by alpine colonists or whether it was anthropogenically introduced from the Carpathians. Furthermore, the genetic structure of native and introduced populations of *R. alpinus* was determined. For the evaluation of genetic structure, 417 samples of *R. alpinus* were collected from the Alps, Carpathians, Balkan, Pyrenees, and Czech Mountains. In total, 12 simple sequence repeat (SSR) markers were applied. The results of AMOVA showed a high 60% variation within populations, 27% variation among groups, and 13% among the population within groups. The overall unbiased gene diversity was high ($\check{h}=0.55$). The higher level of genetic differentiation among populations (FST = 0.35; $p<.01$) indicated restricted gene flow between populations. Compared to native populations, limited genetic variability was observed in the nonnative populations. It was concluded that local adaptation, low gene exchange, and genetic drift affected the genetic diversity of nonnative *R. alpinus*. The results support a genetic link between Alpine and Czech genotypes of *R. alpinus*, while the Carpathians genotypes corresponded to the Balkan genotype.

KEYWORDS
alpine dock, genetic variability, invasive plant, microsatellite, weed species

TAXONOMY CLASSIFICATION
Population genetics

1 | INTRODUCTION

Monk's rhubarb (*Rumex alpinus L.*) is native to the high mountains of Western, Central, and Eastern Europe, including the Iberian and Balkan Peninsulas and the East and Western Carpathians (Šťastná et al., 2010). In the Krkonoše (Giant) Mountains, *R. alpinus* was probably introduced by German-speaking colonists from the Alps in the 16th century AD and used for the treatment of different diseases. Boiled plants were used as a fodder crop for pigs and goats (Brockmann-Jerosch, 1921; Kopecký, 1973; Kubát, 1990; Lokvenc, 1978; Šťastná et al., 2010). The leaves were used for packing butter in Tyrol and in some parts of the Carpathians (Kopecký, 1973). Seeds, roots, and rhizomes of *R. alpinus* have also been used for the treatment of several health disorders, such as diarrhea, dysentery, stomach problems, and kidney disorders. Powder, decoction, infusion, poultice, and ointments prepared from the roots, seeds, leaves, or whole plants have also been used for the treatment of different types of tumors (Bogl et al., 2013; Hartwell, 1970; Jang et al., 2012; Vasas et al., 2015).

Despite its medicinal properties and uses, *R. alpinus* is a troublesome weed and invasive plant in some mountain areas of Europe, growing in permanent monodominant stands characterized by low natural conservation and agricultural value (Delimat & Kiełtyk, 2019). Due to its successful dissemination, other plant species are suppressed by shading and "fast casting" (the ability of these invasive plants to grow and spread quickly) of above ground and underground parts (Bohner, 2005; Raycheva & Dimitrova, 2007). Some invasive plant species have adaptations that allow them to outcompete native plants, such as rapid growth, early leaf-out, efficient nutrient uptake, or release of chemicals that inhibit the growth of other plants. This can lead to the displacement of native plant species and reduced biodiversity in the affected area (Šťastná et al., 2010). The overshadowing effect of large leaves and horizontal development of *R. alpinus* rhizomes create such difficult conditions that only a few species (*Urtica dioica* L., *Deschampsia caespitosa* L., *Chaerophyllum hirsutum* L., and *Stellaria nemorum* L.) are capable of surviving on these sites (Stachurska-Swakoń, 2009).

Rumex alpinus was found to be an invasive neophyte in the Czech Republic in recent decades (Pyšek et al., 2012), invading many localities in the Krkonoše (Giant) Mountains (Náglová et al., 2014). Its ability to supplant original species leads to ecosystem disbalance (Bohner, 2005; Delimat & Kiełtyk, 2019; Pyšek et al., 2012). Plants of *R. alpinus* occur in nutrient-rich habitats such as mountain pastures, particularly in overfertilized locations, around mountain huts and roads, and along riverbanks (Bohner, 2005; Delimat & Kiełtyk, 2019; Klimeš, 1992; Šťastná et al., 2010). It is an anemophilous plant (Kubát, 1990); however, the pollen produced by flowering plants attracts numerous pollen-feeding insects, thereby taking part in the gene flow of *R. alpinus* populations (Klimeš, 1994; Šťastná et al., 2010). The production of seeds is very high (Šťastná et al., 2010); a flowering plant can produce approximately 11,500 seeds m−2 (Klimeš, 1992). The seeds can remain dormant for many years (Bucharová, 2003), and below the stand is a wealthy seed bank (Šťastná et al., 2010). The seeds are spread over long distances (100 m), mainly downstream, allowing the colonization of new habitats (Červenková & Münzbergová, 2009). Moreover, *R. alpinus* is also a clonal plant that reproduces through rhizomes (Klimeš, 1992), and the growth rate of populations is high and fast (Klimeš et al., 1993).

Whether a plant is native or nonnative in a given area is often difficult to determine. The allochthonous origin of many archaeophytes, epoecophytes, and ephemerophytes, e.g., *Ballota nigra* L. Subsp. Nigra and *Verbena officinalis* L., is associated only with anthropogenically influenced communities (Kopecký, 1973; Pyšek et al., 2012). The distribution of some of them, such as *Lamium album* L. and *Chenopodium bonus-henricus* L., precisely defined the area of the original Czech agricultural settlement (Kopecký, 1973). However, the connection of a species to communities of anthropogenic origin may not provide adequate evidence for its allochthonous origin (Chytrý et al., 2005; Kopecký, 1973; Pyšek et al., 2012). According to Lokvenc (1978), this is the case for the species that have been previously collected as medicinal herbs, and they were also grown in gardens (*Angelica archangelica L.*). Therefore, it is difficult to determine the origin of some species that may have been grown as medicinal or valuable plants in the past.

The literature on the historical colonization of the Úpa and Elbe valleys in the Krkonoše Mountains by settlers from the Alps and their introduction of *R. alpinus* is well-established (Hendrych, 2001; Kopecký, 1973; Kubát, 1990; Lokvenc, 1978). Moreover, in the past decade, Professor Klimeš has been investigating the genetic origins of the Krkonoše settlers. His research has revealed that these ancestors hailed from sites in Styria's Salzkammergut region and South Tyrol in Austria and Italy (Klimeš, 2011). Whether *R. alpinus* is truly nonnative in the Krkonoše Mountains may not be certain. In Poland, among others, besides the Carpathian Mountains, where *R. alpinus* is a native plant (Klimeš, 1992; Stachurska-Swakoń, 2009), there is also a part of the Giant Mountains (Karkonosze), and according to Kwiatkowski (2003), *R. alpinus* is a native plant in Poland. Based on this statement and based on the available information, we decided to verify the origin of *R. alpinus* using SSR markers, because the possibility of a different origin of *R. alpinus* populations found in the Giant Mountains is considered. And we pose the following hypotheses:

(i) *Rumex alpinus*, whose European distribution is determined by human activity, was introduced into the Czech part of the Krkonoše Mountains via the Polish part of the Krkonoše Mountains (Karkonosze Mountains) from the Carpathians or from Austrian parts of Alp? (ii) Do differences in genetic diversity exist within native and nonnative habitats? (iii) Does population structure reflect geographic distances?

This is the first study focusing on the genetic variability and population structure of the problematic weedy plant *R. alpinus*, which could provide new assessments of this species under a genetic context and produce valuable data for further control and management of plant invasions.

2 | MATERIALS AND METHODS

2.1 | Description of localities

Between 2017 and 2020, plant samples of *R. alpinus* were collected from different locations in Europe (Table 1, Figure 1). According to Professor Klimeš research's focused on ancestors of the original alpine colonists and individual families, *R. alpinus* was collected in the exact places (Krkonoše) where the colonist's lived and *R. alpinus* probably occurred. Equally, individual plants of *R. alpinus* were collected in Tyrol and Styria in Austria. As control samples *R. alpinus* samples were collected from other mountain localities in the Alps (Lombardy and Graubünden) and the Pyrenees. Finally, to determine the true origin of *R. alpinus* were collected samples representing plant populations in the East and West Carpathians, and then in the mountainous regions of the Balkans. Populations from other mountainous regions of the Czech Republic (Jizera and Eagle Mountains) were collected for comparison and possible exclusion of other origins of *R. alpinus*.

TABLE 1 Rumex alpinus populations examined in this study.

No	Name of population	Latitude	Longitude	Mountains_Region_Country	Type of locality	Year
1	Garmisch Partenkirchen	47°28′16″ N	11°7′56″ E	Alps_Bavaria_Germany	Pasture	2019
2	Filtzsteiner	47°14′2″ N	12°7′39″ E	Alps_Tyrol_Austria	Next the road, pasture	2017
3	Gerlos	47°13′39″ N	12°3′24″ E	Alps_Tyrol_Austria	Pasture	2017
4	Almdorf Königsleiten	47°14′59″ N	12°7′8″ E	Alps_Tyrol_Austria	Next the road, pasture	2017
5	Seebachbrücke	47°0′28″ N	13°10′35″ E	Alps_Tyrol_Austria	Next the road, pasture	2017
6	Obervellach	46°56′9″ N	13°12′12″ E	Alps_Tyrol_Austria	Next the road, pasture	2017
7	Mallnitz	47°6′52″ N	12°30′14″ E	Alps_Tyrol_Austria	Next the road, pasture, banks	2017
8	Schildalm	47°6′30″ N	12°30′17″ E	Alps_Tyrol_Austria	Next the road, pasture	2017
9	Ahrntal_Anholz	46°53′1″ N	12°9′42″ E	Alps_Tyrol_Italy	Next the road, pasture, banks	2017
10	Umbaltal_Prägraten	47°0′59″ N	12°19′15″ E	Alps_Tyrol_Austria	Next the road, pasture	2017
11	Dachstein	47°27′1″ N	13°37′1″ E	Alps_Styria_Austria	Next the road, pasture	2017
12	Schladming	47°23′33″ N	13°45′30″ E	Alps_Styria_Austria	Pasture	2020
13	Madesimo	46°26′13″ N	9°21′27″ E	Alps_Lombardy_Italy	Road, chalets, pasture, ski slope	2017
14	Valle Spluga	46°28′7″ N	9°20′55″ E	Alps_Lombardy_Italy	Next the road, pasture	2017
15	Splügen	46°31′13″ N	9°19′50″ E	Alps_Lombardy_Switzerland	Next the road, pasture	2017
16	St. Moritz	46°28′47″ N	9°50′45″ E	Alps_Graubünden_Switzerland	Next the road	2017
17	Schuders	46°59′48″ N	9°43′31″ E	Alps_Graubünden_Switzerland	Around the village	2017
18	Davos	46°48′18″ N	9°51′53″ E	Alps_Graubünden_Switzerland	Pasture	2017
19	Horní Mísečky ●	50°43′4″ N	15°32′49″ E	Krkonoše Czech Republic	Road, chalets, ski slope	2017
20	Špindlerův Mlýn ●	50°44′39″ N	15°36′46″ E	Krkonoše Czech Republic	Grassland	2017
21	Velká Úpa ●	50°41′7″ N	15°46′49″ E	Krkonoše Czech Republic	Next the road, chalets, banks	2017
22	Jizera Mountains ●	50°48′42″ N	15°21′8″ E	Jizera Mts. Czech Republic	Next the road, chalets	2018
23	Eagle Mountains ●	50°19′34″ N	16°23′10″ E	Eagle Mts. Czech Republic	Next the road, chalets, ski slope	2018
24	Romania_Muntele Mic	45°22′25″ N	22°28′26″ E	Carpathians_Bihor Mountains_Romania	Next the road, chalets, ski slope	2018
25	Zakopane_Lejowa glade	49°15′47″ N	19°50′44″ E	Carpathians_Western Tatras_Poland	Pasture	2018
26	Kom_Stara Planina	43°11′15″ N	23°44′1″ E	Balkan Mts._Stara Planina_Bulgaria	Chalets, pasture	2018
27	Vitosha_Sofia	42°34′55″ N	23°14′31″ E	Balkan Mts._Vitosha_Bulgaria	Next the road, chalets, ski slope	2018
28	Rila_Borovets_ Yastrebets	42°13′14″ N	23°34′33″ E	Balkan Mts._Rila_Bulgaria	Next the road, chalets, ski slope	2018
29	Pirin_Bansko SKI	41°46′54″ N	23°26′26″ E	Balkan Mts._Pirin_Bulgaria	Next the road, chalets, ski slope	2018
30	Prats d'Aiguadassi	42°34′6.8″ N	0°55′57″ E	Pyrennes_Catalan_Spain	Pasture	2017
31	Aigüestortes	42°34′8.4″ N	0°56′17″ E	Pyrennes_Catalan_Spain	Pasture	2017

Note: Populations of *Rumex alpinus* that are marked with a ● are nonnative, while the remaining populations are native plants.
Abbreviation: Mts, Stands for "Mountains".

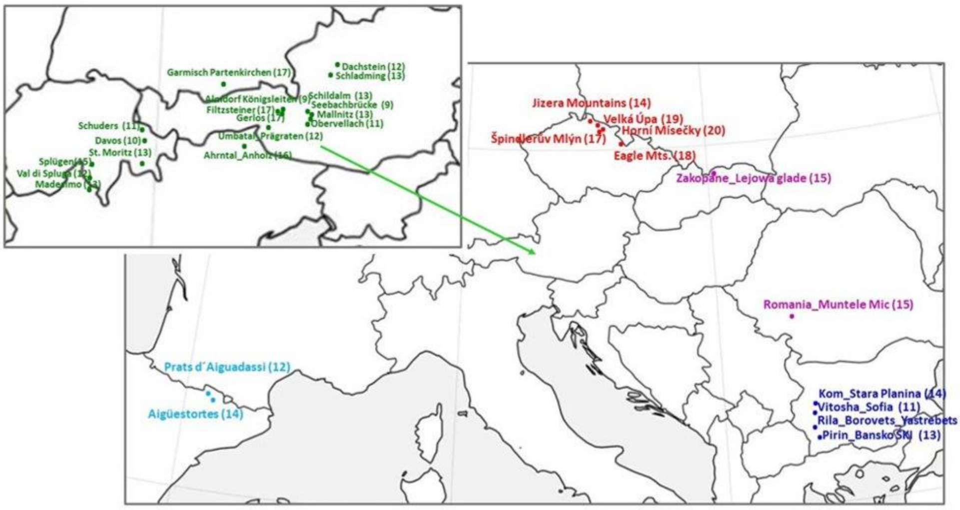


FIGURE 1 Geographical distribution of studied *Rumex alpinus* populations. The names of the populations correspond to Table 1, the numbers in brackets indicating the number of plants tested.

In total, 417 individual leaf samples were collected, representing 31 populations with 9–20 individual plants per population. The 329 plant samples were collected in the European Mountains, Alps, West and East Carpathians, Pyrenees, and Balkan Peninsula, which represent areas where *R. alpinus* is a native species.

In the Czech Mountains, represented by Eagle Mountains, Jizera Mountains, and Krkonoše (Giant) Mountains where *R. alpinus* is considered a nonnative/invasive plant (Kopecký, 1973; Kubát, 1990; Lokvenc, 1978; Pyšek et al., 2012; Šťastná et al., 2010), overall 88 plant samples were collected (Table 1 and Figure 1).

2.2 | Population sampling and DNA extraction

At each locality, leave samples were collected in plants separated approximately 200 m apart to avoid collecting the same plant because *R. alpinus* also reproduces vegetatively and to ensure a representative sample of the population. Samples were dried on silica gel and stored in a collection at the Faculty of Environmental Sciences Czech University of Life Sciences Prague.

Total genomic DNA was extracted from silica gel-dried leaves of *R. alpinus* in two repetitions using the protocol of Doyle and Doyle (1987) with a minor modification that involved the addition of 10 mg of polyvinylpyrrolidone (PVPP) (Carl Roth) and 5 μL of 10 mg/μL RNase A (Thermo Scientific) during the initial phase prior to incubation. The quality and yield of isolated DNA were assessed on a 0.8 % agarose gel in 1× TAE buffer. The concentration and quality of DNA were measured using a spectrophotometer UVS-99/UVIS Drop (Avans Biotech). The extracted DNA samples diluted to the concentration of 20 ng/μL for subsequent analysis and stored at −20°C.

2.3 | Microsatellite (SSR) analysis

Based on the test, 12 polymorphic primer pairs were selected from the 15 primer pairs according to Šurinová et al. (2018) and used (Table S1). DNA amplification was performed in 5 μL reactions consisting of 2.5 μL QIAGEN Multiplex PCR Master Mix; 0.125 μL of each M13-labeled forward, reverse, and fluorolabelled (NED™, PET®, 6-FAM™, VIC® – Table S1) M13 primers (10 μM each in initial volume); 20 ng of DNA dissolved in 0.5 μL TE buffer; and 1.625 μL dH_2O. The PCR protocol was performed according to Schuelke (2000). PCR was performed using an Applied Biosystem Thermal Cycler (Applied Biosystems) as follows: an initial denaturation step at 95°C for 15 min followed by 25 cycles of denaturation (95°C for 20 s), annealing (59°C for 30 s), and extension (72°C for 20 s), followed by 10 cycles of denaturation (95°C for 30 s), annealing (53°C for 45 s), and extension (72°C for 45 s) and a final extension at 72°C for 15 min. During the first 25 cycles, specific PCR products are produced, and in the following 10 cycles, the fluorescent M13 tag is ligated to the M13 forward primer. The quality of PCR products has been verified on 2% agarose gels. Three multiplexes were built (Table S1).

Fragment analyses were performed using capillary electrophoresis in an ABI PRISM 3500 Genetic Analyzer automated sequencer (Applied Biosystems). Electropherograms were analyzed and scored using GeneMarker ver. 1.8 (SoftGenetics).

2.4 | Statistical analysis

Analysis of the molecular variance test (AMOVA) with 1000 permutations was calculated in ARLEQUIN software ver. 3.5.2 (Excoffier & Lischer, 2010). The degree of genetic differentiation among populations was also evaluated using ARLEQUIN software using the distance matrix based on the fixation index (FST) generated by the program. Further, distance matrix based on geographical distances was calculated for *R. alpinus* populations within R program (R Core Team, 2020) version 4.0.3 using the routines in geosphere (Hijmans, 2021) library. These were subsequently logarithmically transformed and correlated with FST distance matrix using the Mantel test and 9999 permutations.

Nei's genetic distance was employed to obtain a UPGMA dendrogram after 1000 bootstrap samplings in TFPGA software (Miller, 1997).

The diversity indices for each population included the percentage of polymorphic loci, the average diversity of the loci using Nei's unbiased gene diversity $\hat{h}$ (Nei, 1973), and the Shannon information index (Lewontin, 1972; Shannon & Weaver, 1949) were calculated using the POPGENE, version 1.32 (Yeh et al., 1999).

To assess the Hardy–Weinberg equilibrium, we used the ARLEQUIN software ver. 3.5.2 (Excoffier & Lischer, 2010). Was conducted an exact test using a Markov chain with a forecasted chain length of 1,000,000 and 100,000 dememorization steps (Guo & Thompson, 1992). Deviation from HWE was assessed at a significance level of $p < .05$. The results were interpreted according to established guidelines (Levene, 1949).

Another approach to studying population structure analysis is based on Bayesian statistics STRUCTURE, version 2.3.4 (Pritchard et al., 2000) was used to determine the genetic architecture of the *R. alpinus* populations. Ten independent runs of 1–20 groups ($K = 1$–20) were performed using the locprior model with admixture and correlated allele frequency (Falush et al., 2003; Hubisz et al., 2009) with the recommended 2,00,000 Markov chain iterations after a burn-in period of 1,00,000 iterations. The optimal value of K was estimated based on ln (K) and on the ΔK calculation, which considers the rate of change in the ln P (D) values among successive K runs to account for patterns of dispersal that are not homogeneous among populations (Evanno et al., 2005). The number (K) of clusters into which the sample data (X) were fitted with posterior probability Pr (X|K) was estimated using the same model with 1,000,000 Markov chain iterations after a burn-in period of 1,00,000 iterations (Evanno et al., 2005).

An exact test for population differentiation was calculated using the Tools for Population Genetic Analyses (TFPGA; version 1.3; Miller, 1997) with 1,00,000 recommended permutation steps.

To identify potential bottleneck events in the populations under investigation, we employed BOTTLENECK 1.2.02 software (Cornuet & Luikart, 1996; Piry et al., 1999) and heterozygosity excess resulting from population reduction was examined. We utilized three models of mutational equilibrium: the infinite allele model (IAM), the stepwise mutation model (SMM), and the two-phase mutation model (TPM), with the latter being the most appropriate for microsatellites. For the TPM, we employed the default settings, which assumed that 70% of mutations occur in a single step, with a variance of 30 among multiple steps. The significance of these models was assessed using a one-tailed Wilcoxon rank test, which is suitable for datasets analysis with less than 20 microsatellite loci (Piry et al., 1999). A population was deemed to have experienced a bottleneck event only if all three models produced significant results (p-value ≤.05).

3 | RESULTS

The number of alleles in different loci is presented in Figure S1, and the sum of all alleles for each population is in Table 2. In the 417 analyzed individuals, 146 alleles were identified for the 12 microsatellite loci (Table S3) with an average of 9.93 polymorphic loci (Table 2). The mean of alleles per locus for all populations was 3 and ranged from the population of Eagle Mountains (1.5) to Carpathian Bihor Mountains population (5.2), where the highest number of nine alleles per locus was identified (Figure S1). While the percentage of polymorphic loci was the highest in the Zakopane Lejowa glade population (100%) the lowest number was found in the Eagle Mountains (42%) (Table 2). A total of 340 multilocus genotypes from 417 individuals of *R. alpinus* were identified. Some populations consisted partly or totally from identical clones, especially the Eagle Mountains population (Table S3).

Nei's average gene diversity values ranged from 0.23 in the population of the Eagle Mountains to 0.52 in the population of Splügen and Schuders (Table 2). The overall gene diversity for all populations was 0.43. The Shannon diversity index (I) was the lowest in the population from the Eagle Mountains ($I = .32$), and the highest ($I = 1.11$) was in the population from Romania Muntele Mic (Table 2). The overall mean value of I was 0.78 when all populations were included (Table 2). The level of population genetic differentiation (FST) ranged from 0.05 to 0.22, with an average of 0.12. While, the gene flow (Nm) level was found in the Czech Mountains (0.88), populations from Pyrenees demonstrated the highest level (5.29), with an average of 2.80 (Table 2).

The level of genic diversity of 5 mountains was lowest in *R. alpinus* populations from Czech Mountains ($\hat{h} = 0.39$; $I = .65$), followed Pyrenees Mountains ($\hat{h} = 0.39$; $I = .79$); Balkan Mountains ($\hat{h} = 0.52$; $I = 1.17$), Carpathian Mountains ($\hat{h} = 0.54$; $I = 1.17$), and the highest level of genic diversity was found in populations from the Alps ($\hat{h} = 0.56$; $I = 1.16$) (Table 3).

Genetic variability was measured as the amount of observed or expected heterozygosity, presented in Table S2. The mean level of Hardy–Weinberg equilibrium for Alpine, Carpathians, and Balkan populations showed that the observed heterozygosity values were not significant, indicating that the populations were in Hardy–Weinberg equilibrium. However, the level of Hardy–Weinberg equilibrium for Czech Mountain populations showed that the observed heterozygosity values were significantly lower than the expected ones for more than seven loci (p-value <.01), indicating that the population was not in Hardy–Weinberg equilibrium.

TABLE 2 Characteristics of 31 local populations of *Rumex alpinus* and their diversity evaluation based on 12 SSR loci analyses.

No	Name of population	*n*	Mountains	Region_Country	I	St. dev.	$\bar{h}$	St. dev.	*P* (%)	Σ_poly-morphic loci	Σ-alleles	FST / Mts.	Nm*/Mts.
1	Garmisch Partenkirchen	17	Alps	Bavaria_Germany	0.69	0.48	0.38	0.26	83	10	37	0.2	1.03
2	Filtzsteiner	12	Alps	Tyrol_Austria	0.79	0.54	0.43	0.27	83	10	37		
3	Gerlos	9	Alps	Tyrol_Austria	0.79	0.49	0.45	0.24	92	11	37		
4	Almdorf Königsleiten	9	Alps	Tyrol_Austria	0.79	0.49	0.44	0.24	92	11	38		
5	Seebachbrücke	9	Alps	Tyrol_Austria	0.77	0.52	0.43	0.27	83	10	35		
6	Obervellach	11	Alps	Tyrol_Austria	0.84	0.49	0.47	0.26	92	11	40		
7	Mallnitz	13	Alps	Tyrol_Austria	0.84	0.56	0.46	0.29	92	11	40		
8	Schildalm	13	Alps	Tyrol_Austria	0.76	0.43	0.44	0.24	92	11	34		
9	Ahrntal_Anholz	16	Alps	Tyrol_Italy	0.68	0.63	0.35	0.30	92	11	38		
10	Umbaltal_Prägraten	12	Alps	Tyrol_Austria	0.81	0.61	0.42	0.28	92	11	43		
11	Dachstein	12	Alps	Styria_Austria	0.74	0.44	0.43	0.25	92	11	36		
12	Schladming	13	Alps	Styria_Austria	0.75	0.36	0.44	0.20	92	11	35		
13	Madesimo	13	Alps	Lombardy_Italy	0.83	0.53	0.46	0.28	83	10	39		
14	Valle Spluga	12	Alps	Lombardy_Italy	0.92	0.61	0.49	0.28	83	10	43		
15	Splügen	15	Alps	Lombardy_Switzerland	0.97	0.57	0.52	0.25	92	11	47		
16	Schuders	11	Alps	Graubünden_ Switzerland	0.97	0.52	0.52	0.25	92	11	45		
17	St. Moritz	13	Alps	Graubünden_ Switzerland	0.80	0.52	0.44	0.28	92	11	37		
18	Davos	10	Alps	Graubünden_ Switzerland	0.87	0.58	0.46	0.30	83	10	43		
19	Horní Mísečky ●	20	Krkonoše	Krkonoše Czech Republic	0.58	0.38	0.38	0.24	75	9	24	0.22	0.88
20	Špindlerův Mýn ●	17	Krkonoše	Krkonoše Czech Republic	0.47	0.37	0.30	0.25	75	9	24		
21	Velká Úpa ●	19	Krkonoše	Krkonoše Czech Republic	0.59	0.40	0.39	0.25	75	9	24		
22	Jizera Mountains ●	14	Jizera Mts.	Jizera Mts. Czech Republic	0.44	0.44	0.28	0.28	58	7	22		
23	Eagle Mountains ●	18	Eagle Mts.	Eagle Mts. Czech Republic	0.32	0.36	0.23	0.26	42	5	16		

TABLE 2 (Continued)

No	Name of population	n	Mountains	Region_Country	I	St. dev.	ĥ	St. dev.	P (%)	Σ_poly-morphic loci	Σ-alleles	FST / Mts.	Nm*/Mts.
24	Romania_Muntele Mic	16	Carpathians	Bihor Mountains_ Romania	1.11	0.73	0.52	0.32	92	11	62	0.05	5.05
25	Zakopane_Lejowa glade	15	Carpathians	Western Tatras_Poland	0.99	0.58	0.50	0.30	100	12	50		
26	Kom_Stara Planina	14	Balkan	Stara Planina_Bulgaria	0.99	0.70	0.49	0.32	75	9	51	0.12	1.75
27	Vitosha_Sofia	11	Balkan	Vitosha_Bulgaria	0.92	0.76	0.46	0.35	67	8	45		
28	Rila_BorovetsYastrebets	14	Balkan	Pirin_Bulgaria	1.05	0.81	0.50	0.36	75	9	55		
29	Pirin_Bansko SKI	13	Balkan	Rila_Bulgaria	0.73	0.71	0.37	0.32	75	9	42		
31	Prats d'Aiguadassi	14	Pyrennes	Catalan_Spain	0.65	0.59	0.35	0.30	75	9	35	0.05	5.29
30	Aigüestortes	12	Pyrennes	Catalan_Spain	0.82	0.63	0.42	0.31	92	11	42		
Mean					0.78	0.54	0.43	0.28	82.8	9.93	38.5	0.13	2.80

Note: Nm* = Gene flow estimated from FST = 0.25 (1 – FST)/FST. Populations of *Rumex alpinus* that are marked with a ● are nonnative, while the remaining populations are native plants.

Abbreviations: FST/Mts. the fixation index per the Mountains; ĥ, observed heterozygosity; I, Shannon-Wiener Diversity Index; Nm*/Mts, the effective number of migrants per the Mountains (A higher Nm* indicates higher gene flow between populations); St. Dev. P (%), the percentage of polymorphic loci; St. Dev, standard deviation; Σ_polymorphic loci, the total number of loci in a population that has more than one allele; Σ-alleles, the total number of different alleles observed across all loci in a population.

Based on the results, all Alpine, Balkan, and Czech Mountain populations had at least one monomorphic locus, meaning that every individual in the population had the same homozygous genotype at that locus. Specifically, in the Eagle Mountains population, loci 1, 2, 3, 4, and 10 were monomorphic. In contrast, loci 5, 7, 8, 9, and 12 exhibited an observed heterozygosity (HO) of 1000 and an expected heterozygosity (HE) of 0.514, suggesting that the population is highly inbred or clonal, as all individuals have the same genotype at the locus. On the other hand, the results for the Carpathian population Zakopane_Lejowa glade did not record any monomorphic locus. However, significant results on some loci suggest that the population might be experiencing some form of selection (Table S2).

The results of the AMOVA for all native and non-native populations (Table 4) indicated that a significant proportion of the genetic variation (26.8%) occurs both among groups (mountains) of populations and mainly within populations (60.2%). A smaller proportion (13.0%) was found among populations within groups. Like to the previous results, AMOVA results for the native populations (Table 5) suggested that a significant proportion of the genetic variation is found among groups (mountains) of populations (33.6%) and primarily within populations (54.2%). In contrast, a smaller proportion (12.2%) was found among populations within groups. Similar to the previous two AMOVA results for nonnative populations (Table 6) suggested that a significant proportion (71.7%) of the genetic variation can be explained by differences within populations, while a smaller 28.1% of differences can be explained by the variability among groups (mountains). As only 0.2% of the genetic variation was found among populations within groups, indicating only minor genetic differences among the populations within each group (Table 6).

The two first axes of the PCoA analysis (Figure 2) of SSR data explained 15.76% and 11.74% of the total variance, respectively, and separated individuals into three main groups (Figure 2). The largest group in the lower quadrant of the plot comprised the majority of all sampled individuals from the Alps (Bavaria, Tyrol, Styria, Lombardy) and Pyrenees. All individuals (except the one) of Carpathians and Balkans populations formed the second group, separated from the main group along the first axis, whereas the third group containing all the populations of the Czech Mountains was separated along the first and second axes.

Pair differences and the degree of variability between *R. alpinus* populations were quite high. The total value for the (FST) between populations was 0.40. The differences and degree of variability between the populations were clear, as shown in Figure 3.

The Mantel test revealed a significant positive correlation between geographical and genetic distances ($r = .65$; $p < .01$) across all the localities. The linear regression model was identified as a representation of the relationship between geographical and genetic distances (Figure 4).

The UPGMA dendrogram based on Nei's genetic distance matrix (Figure 5) represented two main groups. The first group was composed of two clusters containing populations from the Balkan and Carpathian Mountains. The second group was further divided into three clusters, one of which contained populations from the Czech

TABLE 3 Analysis of genic variation statistics for all loci according to Nei (1987) developed for all five mountain populations.

Name of mountains	I	St. dev.	$\hat{h}$	St. dev.	P (%)	Σ_polym.Loci	Σ-alleles
Alps	1.16	0.70	0.55	0.29	100	12	6
Czech Mountains	0.65	0.47	0.39	0.26	75	9	3
Carpathians	1.17	0.69	0.54	0.30	100	12	6
Balkans	1.17	0.88	0.52	0.36	75	9	6
Pyrenees	0.79	0.62	0.39	0.31	100	12	4

Abbreviations: $\hat{h}$, observed heterozygosity; I, Shannon-Wiener Diversity Index; P (%), the percentage of polymorphic loci; St. Dev, standard deviation; Σ_polymorphic loci, the total number of loci in a population that has more than one allele; Σ-alleles, the total number of different alleles observed across all loci in a population.

TABLE 4 Analysis of molecular variance (AMOVA) results with the 31 native and nonnative populations of *Rumex alpinus*.

Source of variation	d.f.	Sum of squares	Variance of components	Percentage of variation	Fixation indices	*p*-Value
Among groups	4	553	0.907	26.8	0.398	.001
Among populations within groups	26	355	0.439	13.0	0.177	.001
Within populations	803	1635	2.036	60.2	0.268	.001
Total	833	2543	3.382			

Note: The populations were divided into groups according to mountain communities (the Alps, Czech Mountains, Carpathians, Balkans, and Pyrenees).

TABLE 5 Analysis of molecular variance (AMOVA) results with the 26 native populations of *Rumex alpinus*.

Source of variation	d.f.	Sum of squares	Variance of components	Percentage of variation	Fixation indices	*p*-Value
Among groups	3	389	1.045	33.6	0.458	.001
Among populations within groups	22	246	0.381	12.2	0.184	.001
Within populations	632	1067	1.689	54.2	0.336	.001
Total	657	1703	3.115			

Note: The populations were divided into groups according to mountain communities (the Alps, Carpathians, Balkans, and Pyrenees).

TABLE 6 Analysis of molecular variance (AMOVA) results with the 5 nonnative populations of *Rumex alpinus*.

Source of variation	d.f.	Sum of squares	Variance of components	Percentage of variation	Fixation indices	*p*-Value
Among groups	2	71	0.717	28.1	0.283	.001
Among populations within groups	2	4	0.005	0.2	0.003	.148 ± .010
Within populations	171	313	1.831	71.7	0.281	.106 ± .008
Total	175	388	2.553			

Note: The populations were divided into three groups according to mountain communities (the Krkonoše Mountains, Jizera Mountains; and Eagle Mountains).

Mountains (Krkonoše and Jizera Mountains), the second was formed by populations from the Pyrenees and a population from the Eagle Mountains. The third most comprehensive alpine cluster was divided into two subgroups, one contained populations from Lombardy and Garmisch Partenkirchen, while the populations from Styria, and the Tyrolean Alps formed the second subgroup Tables 6 and 7.

A noticeable genetic structure of *R. alpinus* populations was found. Although the optimal value of K = 8 was identified, the subsequent analysis results with K from two to eight were displayed (Figure 6b). When K = 2, two genetic populations representing Alpine and Balkans groups of local populations were identified. Whereas, the results for K = 3, the Czech Mountains' local populations (dark blue) were distinguished from the Alpine ones. When K = 4, Tyrolean local population (yellow) was separated from Alpine Mountains local populations, and Eagle Mountains local populations were colored identically to the Alps populations (Figure 6b). While the result for

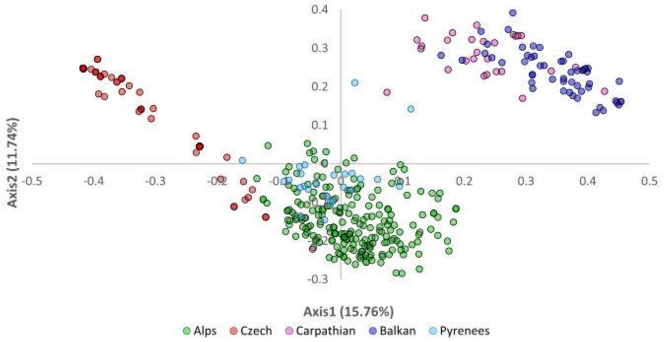


FIGURE 2 The plot of PCoA analyzed populations of *Rumex alpinus* from the Alps, Czech, Carpathians, Balkans, and Pyrenees Mountains.

K = 5, are additionally identically colored, the Lombardy populations with Bavaria local populations (dark blue) and the Pyrenees with the Eagle Mountains local populations (red). When K = 6, the Pyrenees were distinguished as a separate genetic population In the case of K = 7, the alpine regions were divided into more distinguished groups of local populations, where Styria is seen as colored pink (Figure 6b). As in the previous results (K = 4, 5, 6), the local Bavarian populations formed genetically the same population as the Lombard ones (Figure 6b).

Moreover, the Evanno method showed that the best number of populations is K = 8 and is most similar to the result of UPGMA analysis. The *ΔK* value identified eight clusters K1–K8 among *R.* alpinus populations (see Figure 6b—k = 8). Expected heterozygosity between individuals within the same cluster ranged from 0.252 (K4), which consisted of populations Alps_Lombardy, to 0.571 (K3), which presented populations from Alps_Styria, with an average of 0.406. FST values ranged from 0.234 (K3) to 0.792 (K4), with an average of 0.479. The FST value of cluster K5, which consisted of Krkonoše and Jizera Mountains, was 0.303. The mean value of α was 0.03, indicating that most *R.* alpinus genotypes were not genetically admixed (Falush et al., 2003). The representation of individual samples of *R. alpinus* in eight genetic populations for each local population can be seen in the map (Figure 6a) corresponding with K = 8 (Figure 6b). While genetic population K1 is typical for Alps_Bavaria *R. alpinus* local populations (88%), K2 for Alps_Tyrol and Alps_Styria local populations (52%, 31%), K3 for Styria local populations (89%), and K4 consists for Alps_Lombardy local populations (89%), genetic populations K5and K6 are predominant for Czech Mountain local populations (81%, 99%, resp.). Cluster K8 consisted exclusively of Pyrenees populations (93%). The distribution of allele frequency (Figure 7) suggested the occurrence of a bottleneck effect in four Alpine populations (Gerlos, Seebachbürcke, Schildalm, and Davos) and all five populations from the Czech Republic. However, upon further analysis using the one-tailed Wilcoxon rank test, only four populations of Czech origin produced significant results for all three mutation models (Table 7). This confirmed that populations from Horní Mísečky, Velká Úpa, Jizera Mountains, and Eagle Mountains experienced bottleneck events.

4 | DISCUSSION

The possibility of comparing the genetic variation of *R. alpinus* with other Rumex species is limited due to the low number of species examined for SSR marker diversity. Indeed, the physiology and ecology of *R. alpinus* have been studied (Hujerová et al., 2013; Jungová et al., 2022; Řičařová, 2011) much more than genetic variability, with the only published genetic work being on *Rumex bucephalophorus* subsp. canariensis (Viruel et al., 2015). Unfortunately, *Rumex bucephalophorus* is an annual Mediterranean plant.

This is the first study on genetic variability and population structure of the weedy plant *R. alpinus*, which could provide new assessments of this species in a genetic context and produce valuable data for further control management of plant invasions (Le Roux & Wieczorek, 2008). Molecular markers helped elucidate the introduction history and contributed to the explanation of genetic variation in invaders, as in other studies (Bímová et al., 2003; DeWalt et al., 2011; Matesanz et al., 2014; Šurinová et al., 2018). The 12 SSR loci used in this study were significantly polymorphic and useful for differentiation among the *R. alpinus* populations studied (Šurinová et al., 2018).

The population structure of *R. alpinus* in the introduced range is consistent with the random establishment of genotypes in different localities developed by human dispersal. Probably for various socio-economic reasons, *R. alpinus* extended in many Central European mountains (Stachurska-Swakoń, 2008; Delimat & Kiełtyk, 2019) in abandoned or inappropriately managed mountain pastures (Bohner, 2005; Rehder, 1982), including the Krkonoše Mountains (Červenková & Münzbergová, 2009; Pyšek et al., 2012; Šťastná et al., 2010). This is evident from the pairwise differences, which showed that population structure reflected a pattern of isolation by distance associated with human dispersal in the past (Kopecký, 1973; Kubát, 1990; Lokvenc, 1978; Maude & Moe, 2005; Šťastná et al., 2010; Vasas et al., 2015). The correlation analysis relevated a moderate to a strong positive correlation between paired FST values and the differences in geographical distances between *R. alpinus* populations. This suggests no gene flow between populations. Some degrees of gene flow were caused

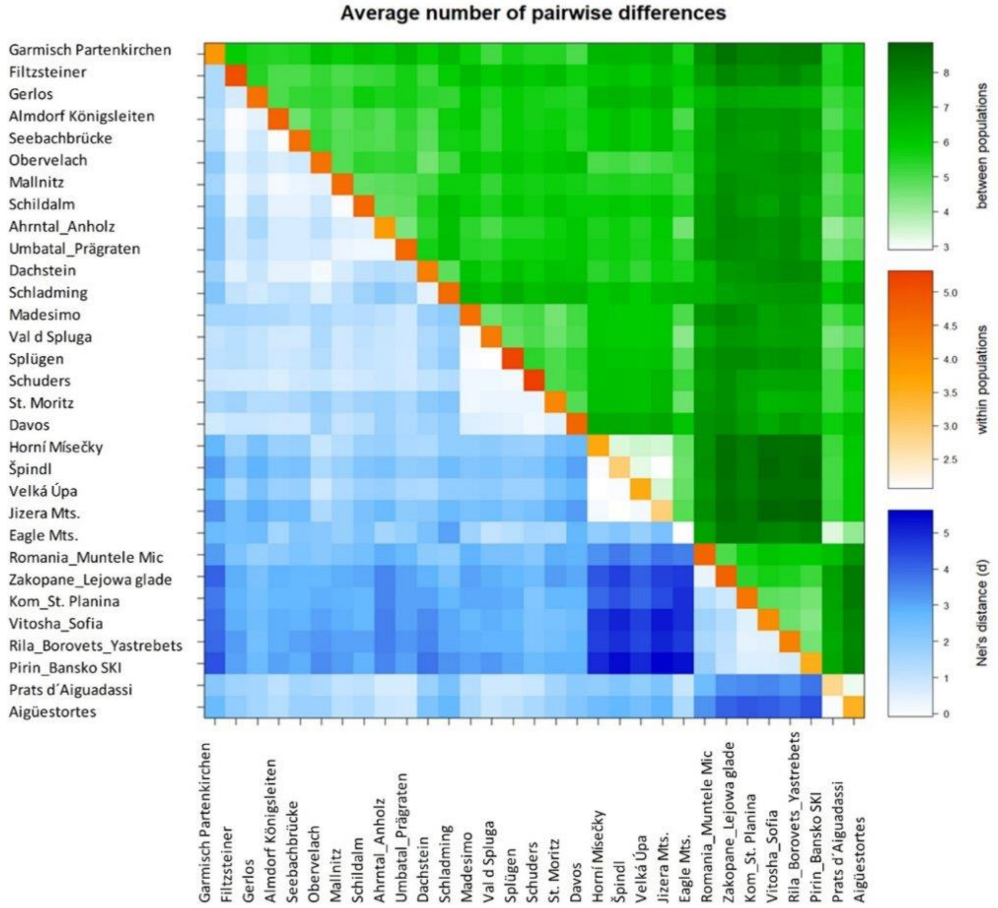


FIGURE 3 The correlation between pairwise FST values and logarithm of pairwise geographical distance between populations of *Rumex alpinus*.

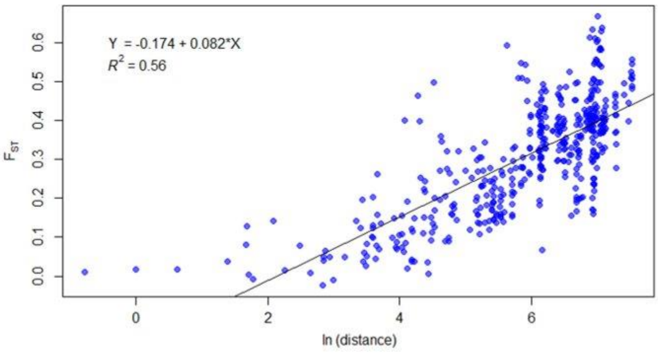


FIGURE 4 Correlation between paired FST values and logos by the rhythm of paired geographical distances between *Rumex alpinus* populations ($r = .65$ $p < .001$) calculated by Mantel test and expressed by the linear regression model, where Y is the fixation index FST and X is the logarithm of distances in expressed in km.

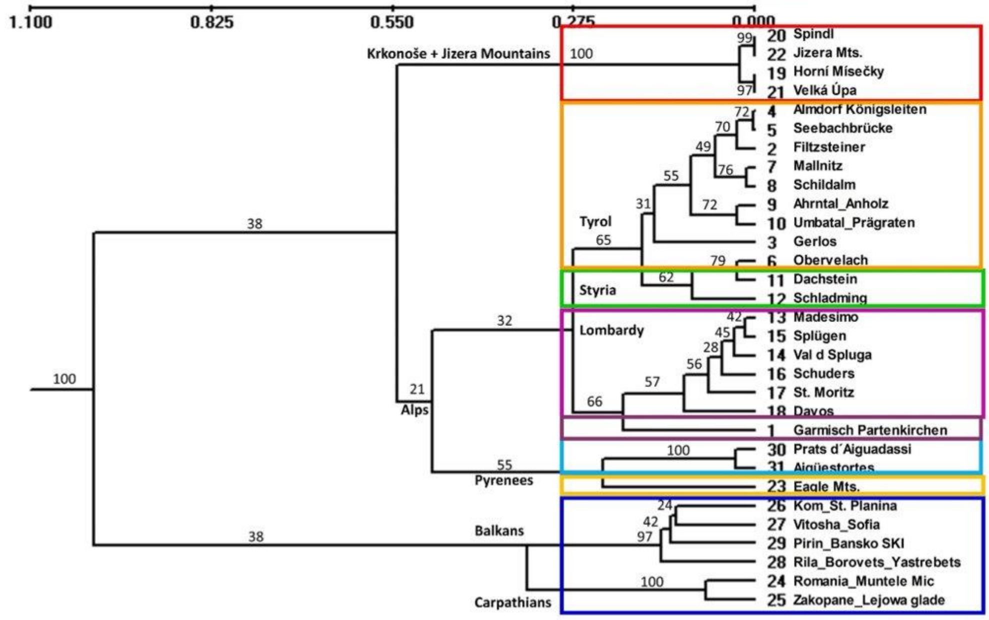


FIGURE 5 UPGMA dendrogram was constructed based on 12 molecular microsatellite markers using Nei's genetic distances (Nei, 1978) for 31 populations of *Rumex alpinus* in the European Mountains. Bootstrap values of the consensus tree are given in branches. The length of the branches is proportional to the genetic distance. Numbers and the name of populations see in Tables 1, 2. The colors correspond to the colors of the mountains in the Figure 6a.

most likely by repeated human impact (Matesanz et al., 2014), which was in accordance with genetic admixture detected in Structure analysis. The anthropogenic situation has probably promoted the movement of propagules across the introduced range (Matesanz et al., 2014), and individual populations have been founded by relatively few individuals (Dlugosch & Parker, 2008; Genton et al., 2005). The results of the Hardy–Weinberg equilibrium analysis showed that most Alpine and Balkan populations are in equilibrium and gene flow is occurring here. However, the situation is different in the Czech populations, especially in the Eagle Mountains population, where only one clone was identified. Nevertheless, the source of those individuals bears a relationship to the geographical or ecological distance from the site where they were established, which is seen from other analyses. The principal coordinate analysis PCoA showed that two groups, one of the Czech Mountains populations and the Carpathians and the Balkans Mountain populations, separated from the parental Alpine population over the centuries. Based on these results, it is unlikely that *R. alpinus* was introduced from the Carpathians to the Krkonoše Mountains. Individuals from the Carpathian Mountains populations were associated with the Balkan populations in one cluster, as the Structure results indicated gene flow between them. On the other hand, from the PCoA and in more detail Structure analyses, it was also evident that the Czech Mountains cluster was more clearly separated from the alpine cluster, suggesting that *R. alpinus* could be a native plant of the Krkonoše Mountains.

However, based on historical sources (Hendrych, 2001; Lokvenc, 2007), it is known that botanist Caspar Schwenckfelt (1607) in his botanic book Scite aus dem botanischen Teil des Buches did not mention the very conspicuous herb *R. alpinus*, and such a large plant cannot be overlooked (Hendrych, 2001). The first historical record that supported the allochthonous origin of *R. alpinus*, but with a different idea of its introduction, is mentioned only by Wimmer in 1844, who described the findings of these plants in mountain huts (Hendrych, 2001). However, the results of Structure analyses showed that the Krkonoše populations corresponded to the average posterior likelihood that the individual was assigned to the cluster with populations from Styria. Based on the results of these analyses, supported by the historical research of Professor Klimeš, it is evident that *R. alpinus* was introduced to the Krkonoše Mountains mainly by colonists from Styria, whether they were lumberjacks (Klimeš, 2011) or raftsmen (Smrčka, 2016).

Nevertheless, the less distinct molecular difference was found between *R. alpinus* from the Czech Mountains and *R. alpinus* in the Alps, which was probably caused due to the bottleneck effect in all of Krkonoše populations except Špindl (DeWalt et al., 2011; Durka

TABLE 7 Bottleneck testing using one tailed Wilcoxon rank test for IAM, TPM, and SMM model. Populations where the probability level for all three models where under significance threshold ($p < .05$) are highlighted by yellow text.

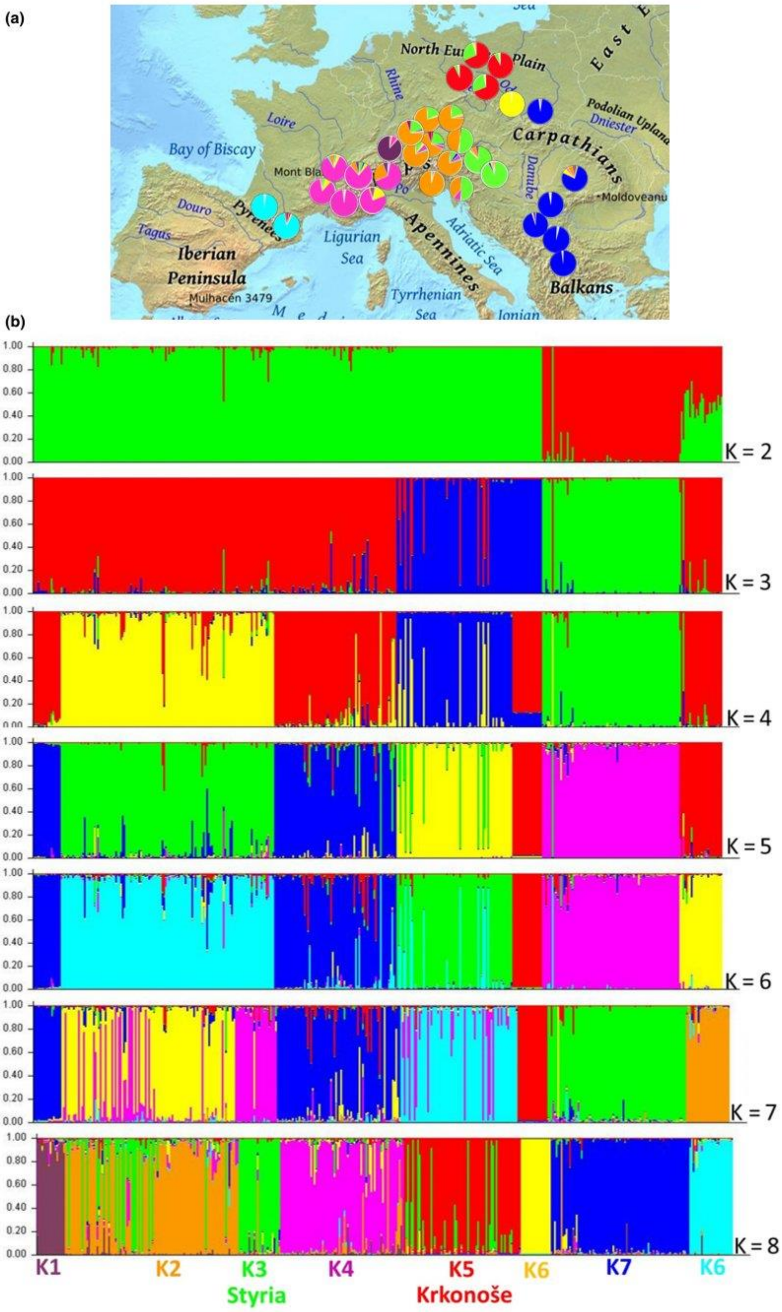
Mountains	Population	Population name	IAM	TPM	SMM
Alps	Pop1_GP17	Garmisch Partenkirchen	0.21582	0.57715	0.75391
Alps	Pop2_Fz18	Filtzsteiner	0.01221	0.04199	0.16113
Alps	Pop3_Gs09	Gerlos	0.07373	0.25977	0.64990
Alps	Pop4_Kg15	Almdorf Königsleiten	0.23242	0.61768	0.91260
Alps	Pop5_Kv22	Seebachbrücke	0.05273	0.11621	0.34766
Alps	Pop6_Mn23	Obervelach	0.12012	0.13916	0.48291
Alps	Pop7_Mt22	Mallnitz	0.13916	0.25977	0.55078
Alps	Pop8_Sch20	Schildalm	0.03369	0.08740	0.31885
Alps	Pop9_An21	Ahrntal_Anholz	0.38232	0.68115	0.86084
Alps	Pop10_Pg22	Umbatal_Prägraten	0.35010	0.64990	0.83984
Alps	Pop11_Ds26	Dachstein	0.28857	0.38232	0.64990
Alps	Pop12_Sg15	Schladming	0.02686	0.07373	0.31885
Alps	Pop13_Md19	Madesimo	0.00488	0.08008	0.61523
Alps	Pop14_Vl12	Val d Spluga	0.00244	0.04199	0.42285
Alps	Pop15_Sp15	Splügen	0.00244	0.03369	0.38232
Alps	Pop16_Ch19	St. Moritz	0.00610	0.07373	0.41553
Alps	Pop17_Mz20	Schuders	0.03369	0.20654	0.48291
Alps	Pop18_Dv16	Davos	0.42285	0.61523	0.81250
Czech Mts.	Pop19_H20	Horní Mísečky	0.00098	0.00098	0.00195
Czech Mts.	Pop20_K20	Špindl	0.01367	0.10156	0.21289
Czech Mts.	Pop21_U20	Velká Úpa	0.00098	0.00098	0.00195
Czech Mts.	Pop22_J18	Jizera Mountains	0.01953	0.03906	0.03906
Czech Mts.	Pop23_S22	Eagle Mountains	0.01563	0.01563	0.01563
Carphatian	Pop24_Ro20	Romania_Muntele Mic	0.44922	0.71143	0.91260
Carphatian	Pop25_PL22	Zakopane_Lejowa glade	0.39551	0.63330	0.88330
Balkan	Pop26_Kc20	Kom_St. Planina	0.21289	0.32617	0.50000
Balkan	Pop27_Vt22	Vitosha_Sofia	0.00195	0.00195	0.09766
Balkan	Pop28_RL23	Pirin_Bansko SKI	0.01367	0.12500	0.63281
Balkan	Pop29_PN14	Rila_Borovets_Yastrebets	0.38477	0.75391	0.88379
Pyrenees	Pop30_IB22	Aigüestortes	0.41553	0.64990	0.92627
Pyrenees	Pop31_Py22	Prats d´Aiguadassi	0.24805	0.58984	0.87500

et al., 2005; Genton et al., 2005; Hardesty et al., 2012). In the localities, there may have been a drastic reduction in the number of individuals, inbreeding, and thus allele loss as the population settled into the new territory (Wright, 1931; Maron et al., 2004; Keshavarzi & Mosaferi, 2019).

On the other hand, a founder effect could have occurred there when a new territory was settled, and an individual with a unique allele was introduced (Bossdorf et al., 2005; Matesanz et al., 2014; Oduor et al., 2016). There could have also been an accumulation of mutations or hybridization with the related species Rumex species (Kubát, 1990; Rechinger, 1957; Šťastná et al., 2010; Stehlik, 2002).

Amova results of *R. alpinus* revealed high variability within populations rather than between populations themselves (Mosaferi et al., 2015; Sheidai et al., 2016). This fact was subsequently confirmed by the results of the Hardy–Weinberg equilibrium, especially in Alpine populations. Similarly, higher genetic polymorphism in native localities was confirmed in other studies (Chen et al., 2009; Keshavarzi & Mosaferi, 2019; Leišová-Svobodová et al., 2018; Rollins et al., 2013). It is consistent with the statement that in natural habitats, plants such as *R. alpinus* reproduced by generative proliferation (Červenková & Münzbergová, 2009; Klimeš et al., 1993, 1997), leading to subsequent higher genetic variability (Briggs & Walters, 2016).

FIGURE 6 (a) Sample localities of *Rumex alpinus* populations with pie charts describing the proportions of individuals classified into one of the eight clusters defined using the Bayesian approach (Pritchard et al., 2000). Color coded pie charts indicate the proportion of individuals within each population that corresponds to a particular STRUCTURE identified genetic deme (coresponding with Figure 6b K = 8 for details). (b) Bayesian model-based clustering of analyzed accessions. Bar plots show the membership coefficient estimate (Q) for each accession for the inferred clusters with maximum log-likelihood probability. Bar colors and lengths represent inferred clusters and Q, respectively, identified by STRUCTURE for K = 2, 3, 4, 5, 6, 7, and 8. As members from PCoA clusters o were rather heterogeneous reasonable division was found by STRUCTURE for K = 8 (detail: K1 Alps Bavaria, K2 Alps Tyrol, K3 Alps Styria, K4 Alps Lombardy, K5 Czech Mountains, K6 Eagle Mountains, K7 Carpathians and Balkans, K8 Pyrenees). Individual colors in do not represent the same clusters for technical reasons given by the software.


(a)
North Eu
Plain
East E
Rhine
Od
Podolian Upland
Dniester
Loire
Carpathians
Bay of Biscay
Mont Bla
Po
Danube
Moldoveanu
Douro
Pyrenees
Tagus
Ligurian Sea
Apennines
Adriatic Sea
Iberian Peninsula
Mulhacén 3479
Tyrrhenian Sea
Ionian
Balkans
(b)
1.00
0.80
0.60
0.40
0.20
0.00
K = 2
K = 3
K = 4
K = 5
K = 6
K = 7
K = 8
K1
K2
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Styria
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K7
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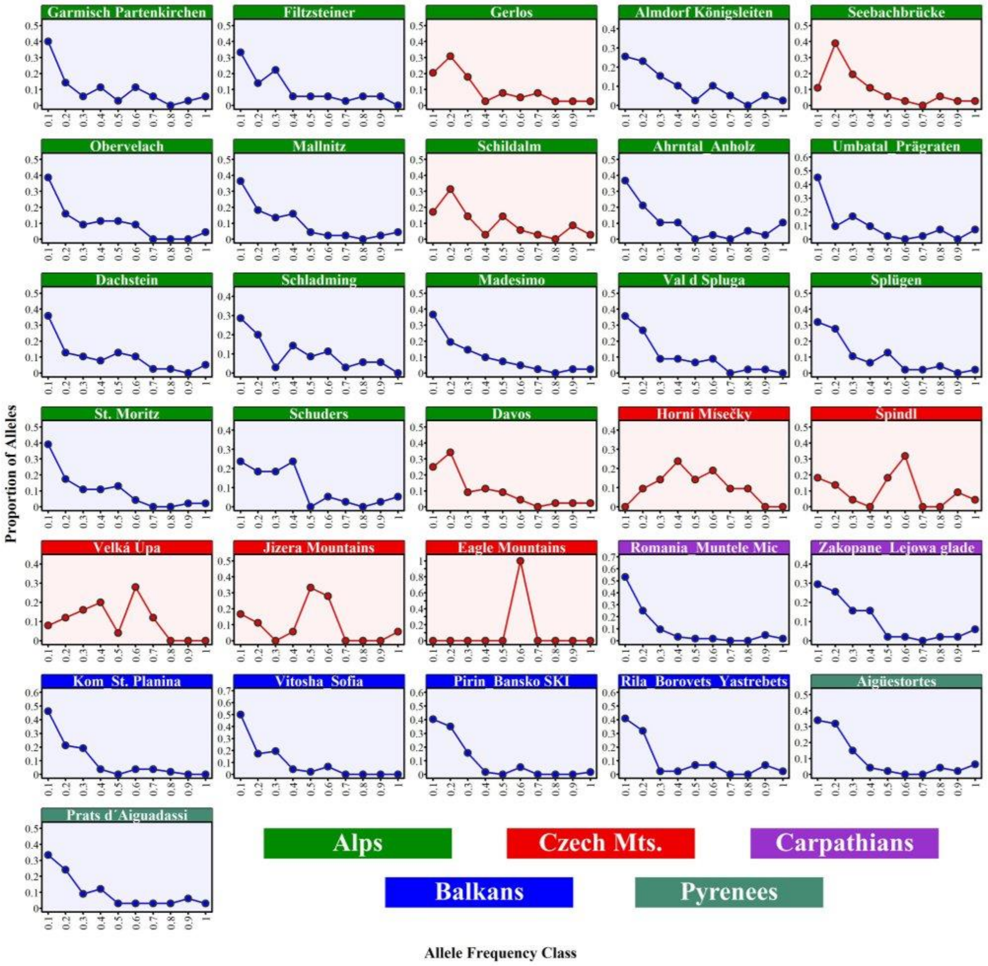

FIGURE 7 Mode-shift plot indicating the occurrence of a recent bottleneck event. The figure displays the distribution of allele frequencies for 12 microsatellite loci in 31 populations of *Rumex alpinus* collected from the Alps (green), Czech Republic (red), Carpathians (purple), Balkans (blue), and Pyrenees (aqua). The x-axis shows the allele frequencies grouped into categories of 0.1, while the y-axis indicates the percentage of alleles in each frequency category. The red lines on the plot denote populations that exhibit a mode shift in the frequency distribution, which is a hallmark of the bottleneck effect.

This is mainly due to their natural enemies, such as insect pests or fungal diseases, and competition (Bímová et al., 2003; Barrett, 2015; Kaljund et al., 2013; Mandák et al., 2005). While in nonnative sites without native enemies, plants prefer clonal reproduction (Bímová et al., 2003; Klimeš et al., 1997), even at the cost of lower genetic variability (Briggs & Walters, 2016; Dlugosch & Parker, 2008).

In addition, the Hardy–Weinberg equilibrium results showed the Czech Mountains, especially in the Eagle Mountains, no genetic variation at several loci. It is assumed that the population in Eagle Mountains is highly inbred (Šurinová et al., 2018), and the population consisted only of clones (reproduced via rhizomes in falanga habit Klimeš et al., 1993), contributing to the opinion that this species is nonoriginal and is, therefore, a secondary occurrence (Hollingsworth & Bailey, 2000). Obtained results confirmed the previously published hypothesis that *R. alpinus* was spread mainly by human distribution from small sources of populations in the Middle Ages (Kopecký, 1973; Lokvenc, 2007).

Additionally, the study found that the genetic variability of *R. alpinus* populations in the Alps, Carpathians, and Pyrenees—where it is considered to be native (Bohner, 2005; Delimat & Kiełtyk, 2019;

Raycheva & Dimitrova, 2007; Stachurska-Swakoń, 2008)—was higher compared to the nonnative populations in the Czech Mountains. These findings, as reported by Amsellem et al. (2000), support the conclusion that *R. alpinus* is indeed a nonnative species in the Czech Mountains.

The variation in the Balkan and Carpathian populations suggests that a single introduction from one native-range population is unlikely. In contrast, the great diversity and the high interpopulation differentiation found in Carpathian populations indicated more native sources.

5 | CONCLUSION

The genetic variability and population structure of the weedy plant *R. alpinus* were studied to provide new insights into this species under a genetic context and to generate valuable data to control plant invasions. The study employed 12 SSR loci that were significantly polymorphic and helpful for distinguishing among the populations of *R. alpinus* studied. *R. alpinus* population structure in the introduced range suggests that the genotypes were randomly established in different localities through human dispersal, with no gene flow between populations. Hardy–Weinberg equilibrium results indicated that most native populations of *R. alpinus* are in equilibrium, and gene flow is occurring. The source of the individuals is related to the geographical or ecological distance from the site where they were settled. It seems that *R. alpinus* was introduced to the Krkonoše Mountains mainly by colonists from Styria. Molecular differences between *R. alpinus* in the Czech Republic and *R. alpinus* in the Alps are probably caused by a founder effect and bottleneck effect. Native *R. alpinus* populations showed higher genetic polymorphism, mainly due to their natural enemies and competition. In nonnative sites without native enemies, plants prefer clonal reproduction, especially the Eagle Mountains, which showed no genetic variation at several loci. Overall, the low genetic variability in the Czech populations indicated that major expansion of this invasive plant species in nonnative habitats is unlikely, and appropriate management can help maintain it in the future.

AUTHOR CONTRIBUTIONS

Michaela Jungová: Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); project administration (equal); resources (equal); writing – original draft (equal). **Vladimíra Müllerová Jurasová:** Conceptualization (equal); formal analysis (equal); methodology (equal); resources (equal); validation (equal); writing – review and editing (equal). **Petra Hlasna Čepková:** Data curation (equal); investigation (equal); methodology (equal); resources (equal); supervision (equal); writing – review and editing (equal). **Leona Leisova Svobodová:** Data curation (equal); formal analysis (equal); software (equal); writing – review and editing (equal). **Pavel Svoboda:** Data curation (equal); software (equal); writing – original draft (equal). **Michal Hejcman:** Conceptualization (equal); investigation (equal); project administration (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

None declared.

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DATA AVAILABILITY STATEMENT

All data are in supporting information, and they will be available at time of publication.

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SUPPORTING INFORMATION

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5. PRINCIPAL CONCLUSIONS OF THE THESIS

SUMMARY IN ENGLISH

The doctoral thesis focuses on the ecology and genetic variability of the invasive plant *R. alpinus* in the Krkonoše Mountains and the problematic weed in mountainous regions of Europe. The results and conclusions of this study are summarized below.

5.1

The study's results revealed that different organs of *R. alpinus* reflected varying levels of macroelement content. The variability in N, P, K, Ca, and Mg content in the organs was directly related to their content in the soil, reflecting pedogenic processes, specific environmental conditions, and anthropogenic influences at the sites. The overall N content in the Krkonoše localities was similar to that in alpine localities and corresponded to long-term fertilized grassland vegetation. An exception was observed at the Ramsau am Dachstein locality, which likely resulted from the northern slope inclination in the Alps, where N losses incur due to leaching and denitrification from low temperatures. The average N content in all leaves was higher (22.6-52.2 g kg^{-1}) compared to plants from different plant communities, such as *Urtica dioica* (23.2 g kg^{-1}), and many other grassland species ranging from 7 to 17 g kg^{-1}. The high N content in emerging leaves was primarily due to its utilization at the cellular level, especially for enzymatic activities and facilitating photosynthesis. The lowest N content was found in the stem and rhizome. The stem serves as a transport conduit from the roots to the photosynthetic tissues, hence having lower nutrient content compared to leaves, for example. Although the overall P content in the soil was relatively lower than most reported values in mountain soils (1.11 - 1.88 g kg^{-1}), the available P fraction for plants was relatively higher than that reported in other mountain soils. However, the total P content (0.8 g kg^{-1}) in fertilized mountain grasslands was similar to the P content (0.72 g kg^{-1}) in this study, indicating significant variability in P content in mountainous sites. The high P content in emerging and mature leaves is likely attributed to their high metabolic activity. The content of individual nutrients, N and P, in mature and senescent leaves varied across different sites, indicating competitive interactions among plant species. Human-influenced sites often lead to higher N and P content, potentially increasing the number of competitive organisms for *R. alpinus*. Nonetheless, *R. alpinus* may acquire N and P from the soil profile through mycorrhizae.

The N:P and N:K ratios in mature leaves of *R. alpinus* ranged within the typical range for plant tissues, indicating a relatively high demand for P. The average N resorption (52%) and P resorption (50%) were lower compared to the average percentage in many other vascular plants (62.1% N and 64.9% P). Compared to senescent leaves, mature leaves have rapid biomass production and, therefore, higher demands for N and P, which could be potential reasons for the higher efficiency of N and P resorption in this study. Furthermore, significant differences in N and P resorption between seasons at the Krkonoše localities suggest that mature leaves remain metabolically active during the summer. The efficiency of N and P resorption in *R. alpinus* leaves followed a similar pattern across all studied sites. The high efficiency of N and P utilization within *R. alpinus* leaves underscores its adeptness at internally cycling these elements. This adaptation highlights the species' capacity to thrive under challenging conditions and in environments with limited N and P availability within the soil.

5.2

The main message of the second study is that the edibility of *R. alpinus* (a plant species) can be questionable due to the accumulation and distribution of risk elements in different plant organs. The levels of risk element accumulation in *R. alpinus* vary depending on site-specific factors such as geological substrate, environmental conditions, and anthropogenic activities. Risk element release is influenced by natural (lithogenic) and human-related (anthropogenic) sources, leading to their subsequent accumulation in different parts of organ *R. alpinus*. The study also found that the accumulation of certain risk elements (such as As, Cr, Ni, Pb, and Cd) in *R. alpinus* varied with seasons, with higher contents observed during autumn than summer in the Krkonoše Mountains.

Regarding the chemical characterization of soil, the study found that the reduced soil acidity in all localities was due to high Ca and Mg contents, predominantly derived from geological substrates. The total Zn and Cu contents in the soils exceeded the permissible limits for agricultural soils, indicating potential contamination from historic mining and smelting activities. The total content of Mn in the soil also exceeded the average value, likely due to acidic soil conditions and limited soil moisture. The total contents of Al, Cr, Ni, Pb, and Cd in the Krkonoše Mountains soil were within the reported range for most agricultural soils but higher than in the Alps, suggesting the influence of past mining activities in the Krkonoše Mountains.

Regarding the distribution of elements in the organs of *R. alpinus*, the study found that the mean contents of Fe, Zn, Cu, and Mn were relatively low and within the ranges of geochemical background values. The variability in the content of microelements in the organs was attributed to compartmentalization and translocation within the plant's vascular system. Different organs exhibited different patterns of element distribution. For example, Zn content was highest in the rhizome, while Fe content was lowest in the stem. The content of Cu varied between emerging and mature leaves. The study also observed low contents of Al, As, Cr, Ni, Pb, and Cd in the organs of *R. alpinus*, indicating limited uptake and accumulation of these elements. However, the study noted that the content of As slightly exceeded the limit set by regulations, suggesting potential risks to food safety and human health.

Overall, the study highlights the site-specific accumulation and distribution of risk elements in *R. alpinus*, raising concerns about its edibility. The results indicate the influence of geological substrate, environmental conditions, and anthropogenic activities on element accumulation in the plant. It also emphasizes the importance of monitoring and assessing the levels of risk elements in edible plants to ensure food safety and human health.

5.3

The genetic variation of *R. alpinus* compared to other *Rumex* species is limited due to the scarcity of studies exploring SSR marker diversity among species. While the physiology and ecology of *R. alpinus* have been extensively studied, the genetic variability has received less attention, with only one published study focusing on the genetic work of *Rumex bucephalophorus* subsp. canariensis. This study aims to fill that gap by investigating the genetic variability and population structure of *R. alpinus*. The results can provide valuable insights into the species' genetic context and contribute to the management of plant invasions.

Molecular markers have proven helpful in understanding invasive species' introduction history and genetic variation. In this study, 12 SSR loci were used, showing significant polymorphism and enabling differentiation among the studied *R. alpinus* populations.

The population structure of *R. alpinus* in the introduced range indicates the random establishment of genotypes in different localities, likely influenced by human dispersal. Gene flow between populations is limited, primarily due to anthropogenic factors. However, some degree of gene flow has occurred, probably due to repeated human impact.

The study results suggest that *R. alpinus* populations in the Czech Mountains are distinct from the Alpine populations, indicating the possibility of the species being native to the Krkonoše Mountains. However, historical records and analyses suggest that *R. alpinus* was likely introduced to the Krkonoše Mountains by colonists from Styria. Our analyses subsequently confirmed this.

Regarding genetic variability, there are notable differences between *R. alpinus* populations in the Czech Mountains and those in the Alps. This could be attributed to a bottleneck and founder effect during the population's colonization of new territories. It is also possible that mutations and hybridization with related *Rumex* species have contributed to genetic variation. The AMOVA analysis revealed higher variability within populations rather than between populations. This pattern is consistent with other studies and can be attributed to generative proliferation in natural habitats, where plants like *R. alpinus* reproduce, as well as the presence of natural enemies and competition. Clonal reproduction is preferred in nonnative sites, leading to lower genetic variability.

The study also found reduced genetic variation and possible inbreeding in the Eagle Mountains population of *R. alpinus* and supports the notion that this population is nonoriginal and a secondary occurrence. The results confirm previous hypotheses suggesting the human-mediated spread of *R. alpinus* from small populations in the Middle Ages.

Furthermore, the study highlights the higher genetic variability of *R. alpinus* populations in the native regions of the Alps, Carpathians, and Pyrenees compared to nonnative populations in the Czech Mountains. These results support the conclusion that *R. alpinus* is indeed a nonnative species in the Czech Mountains. The Balkan and Carpathian populations exhibit substantial variation, indicating multiple native sources rather than a single introduction event.

Overall, this study provides valuable insights into the genetic variability and population structure of *R. alpinus* and explains its origins and spread.

Questions for future research

In the future would be good to focus on a closer examination of the grazing behavior of different animals to the abundance, reproduction, and distribution of life stages of *R. alpinus*. Notably, insights from M. Hejcman's antelope experiments (personal communication) reveal their consumption of sorrel. Given their current breeding experiments in the Alps (personal observation), it's intriguing to explore whether lamas and antelopes might prefer *R. alpinus* biomass on pastures. Furthermore, it is worth exploring the potential for combined grazing of more than one livestock species to be effective against this weedy species.

Considering that few non-chemical control methods have demonstrated effectiveness against *R. alpinus* populations. A pivotal strategy involves preventing seed germination, among others, by introducing competing grasses on degraded sites.

Furthermore, the experiment was carried out in a climatic box with the germination of seeds of three *Rumex* species (*alpinus, obtusifolius,* and *longifolius*) depending on different temperatures (6, 12, 18, 24, 29, and 35 °C) and followed by seed germination experiments from different locations in the Alps and the Giant Mountains. In this context, germination rates and germination of seeds from native and non-native sites were compared. Considering the insights acquired from the aforementioned experiments and the impending climate change along with its accompanying warming trends, a subsequent study was designed to explore the growth of seedlings from three *Rumex* species (*alpinus, obtusifolius,* and *longifolius*) under elevated temperatures (data remains unpublished). The results showed that *Rumex obtusifolius* and *Rumex longifolius* seedlings were more tolerant to high temperatures, whereas *R. alpinus* could not cope with temperatures of 40 °C. Furthermore, the experiment showed that *R. alpinus* was susceptible to lowland diseases (powdery mildew) and pests, which it could not tolerate. Thus, it would be interesting to focus if seedlings surviving in lowland habitats using transplantation experiments to focus on the germination and growth of *R. alpinus* from seed or vegetative propagation.

Finally, I believe that a well-designed study of interviews with farmers and conservationists could be an invaluable contribution to further work that seeks to determine which factors and measures against the expansion of this weed are important in setting up management for the eradication of *R. alpinus* and which are not.

Besides examining the influence of temperature on *Rumex* seeds germination and seedlings' growth in the experiments mentioned above, it is crucial to comprehend the species' response to climate change and consider factors driving its spread. Certain *Rumex* species have already been studied, for example, regarding elevated CO_2 concentrations and heightened solar radiation effects.

SOUHRN V ČEŠTINĚ

Disertační práce je zaměřena na ekologii a genetickou variabilitu invazní rostliny *R. alpinus* v Krkonoších, který je současně obtížným plevelem v horách Evropy. Výsledky a závěry této práce jsou shrnuty níže.

5.1

Z výsledků první studie vyplynulo, že studované orgány *R. alpinus* odrážely různý obsah makroprvků. Variabilita v obsahu N, P, K, Ca a Mg v orgánech nepřímo souvisela s jejich obsahem v půdě a odrážela půdotvorné procesy, specifické podmínky prostředí a antropogenní vlivy na lokalitách. Celkový obsah N na krkonošských lokalitách byl podobný jako na alpských lokalitách a odpovídal dlouhodobě hnojeným travním porostům. S výjimkou lokality Ramsau am Dachstein, což bylo pravděpodobně dáno sklonem na severním svahu Alp, kde ztráty N vznikají v důsledku vyluhování a denitrifikaci při nízkých teplotách. Průměrný obsah N ve všech listech byl vyšší (22.6-52.2 g kg^{-1}) než u rostlin z různých rostlinných společenstev, např.: *Urtica dioica* (23.2 g kg^{-1}) a mnoho dalších lučních druhů - 7 až 17 g kg^{-1}. Vysoký obsah N ve vzrůstajících (emerging) listech byl dán tím, že je N využíván především na buněčné úrovni, a to především pro enzymatické aktivity a k umožnění fotosyntézy. Nejnižší obsah N se vyskytoval ve stonku a oddenku. Pravděpodobně proto, že stonek slouží jako transportní kanál od kořenů k fotosyntetickým pletivům, a proto má nižší obsah živin, ve srovnání např. s listy. Přestože celkový obsah P v půdě byl relativně nižší než byl zaznamenán na horských půdách (1.11 - 1.88 g kg^{-1}); tak obsah rostlinám dostupného P byl relativně vyšší než bylo zaznamenáno na jiných horských půdách. Celkový obsah P (0.8 $g.kg^{-1}$) ve hnojených horských travních porostech byl však podobný obsahu P (0.72 $g.kg^{-1}$) zaznamenaném v této studii, což ukazuje na velkou variabilitu obsahu P na horských lokalitách. Vysoký obsah P v nově vznikajících (emerging) a zralých (mature) listech je pravděpodobně dán jejich vysokou metabolickou aktivitou. Obsahy jednotlivých živin N a P ve zralých (mature) a senescentních (senescent) listech se na různých lokalitách lišily, což svědčí o konkurenčních interakcích mezi rostlinnými druhy. Lokality ovlivněné lidskou činností často vedou k vyššímu obsahu N a P, což může zvýšit počet konkurenčních organismů pro *R. alpinus*. Přesto, může *R. alpinus* získávat N a P z půdního profilu prostřednictvím mykorhizy.

Poměry N:P a N:K ve zralých listech *R. alpinus* se pohybovaly v rozmezí pro rostlinné tkáně a ukazují na poměrně vysokou potřebu P. Průměrná resorpce N (52 %) a P (50 %) byla menší v porovnání s průměrným procentním podílem u mnoha jiných cévnatých rostlin (62,1 % N a 64,9 % P). Ve srovnání se senescentními listy mají zralé listy rychlou produkci biomasy, a tím i vyšší nároky na N a P, což by mohlo být možnými příčinami vyšší účinnosti resorpce N a P v této studii. Navíc významné rozdíly v resorpci N a P mezi jednotlivými sezónami na krkonošských lokalitách naznačují, že zralé listy jsou metabolicky aktivní během léta. Efektivita resorpce N a P v listech *R. alpinus* měla podobný průběh na všech studovaných lokalitách. Vysoce efektivní N a P v listech *R. alpinus* ukazují na lepší vnitřní koloběh těchto prvků a schopnost druhu vyrovnávat se s nepříznivými vlivy a s nízkou dostupností N a P v půdě.

5.2

Hlavním výsledkem druhé studie je zjištění, zda je možná poživatelnost *R. alpinus* vzhledem ke kumulaci a distribuci rizikových prvků v jeho rostlinných orgánech. Úroveň akumulace prvků v *R. alpinus* se lišila v závislosti na faktorech specifických pro danou lokalitu, jako je geologický substrát, podmínky prostředí a antropogenní činnost Uvolňování stopových/rizikových prvků bylo ovlivněno jak přírodními (litogenními), tak lidskými (antropogenními) zdroji, což vedlo k jejich různé akumulaci v různých orgánech *R. alpinus*. Studie rovněž zjistila, že akumulace některých rizikových prvků (jako As, Cr, Ni, Pb a Cd) v *R. alpinus* se lišila v závislosti na ročním období, přičemž vyšší obsahy byly v Krkonoších pozorovány na podzim než v létě.

Studie dále odhalila, že *R. alpinus* má značné akumulační strategie pro Cr, Zn, Cu a Ni ve srovnání s Fe, Mn, Al a Pb. Kromě toho je studovaný druh tolerantní k As, což naznačuje vysoký bioakumulační faktor (BF) oddenku (podzemní biomasa) a totální obsah As v půdách, který byl nadlimitní převážně na Krkonošských lokalitách. Nicméně hodnoty těchto prvků v konzumovaných rostlinných pletivech (listy a řapík) nepředstavují riziko pro lidské zdraví, jak uvádí Světová zdravotnická organizace (WHO).

Pokud jde o chemickou charakteristiku půdy, studie zjistila, že snížená kyselost půdy na všech lokalitách byla způsobena vysokými obsahy Ca a Mg, které pocházejí převážně z geologických substrátů. Obsahy Al, Cr, Ni, Pb a Cd v půdě se pohybovaly v rámci udávaných hodnot pro většinu zemědělských půd, ale byly vyšší v Krkonoších než v Alpách, což naznačuje vliv minulé důlní činnosti v Krkonoších. Studie však zaznamenala, že celkový obsah As v půdě na několika lokalitách mírně překračuje limit stanovený legislativou, což naznačuje potenciální rizika pro bezpečnost potravin a lidské zdraví.

Z hlediska rozložení prvků v orgánech *R. alpinus* studie zjistila, že průměrné obsahy Fe, Zn, Cu a Mn byly relativně nízké a v rozmezí hodnot geochemického pozadí. Variabilita v obsahu mikroprvků v orgánech byla přisuzována oddělení a translokaci v rámci cévního systému rostliny. Různé orgány vykazovaly různé vzorce rozložení mikroprvků. Například obsah Zn byl nejvyšší v oddenku, zatímco obsah Fe byl nejnižší ve stonku. Obsah Cu se lišil mezi vzcházejícími (emerging) a zralými (mature) listy. Ve studii byly rovněž zjištěny nízké obsahy Al, As, Cr, Ni, Pb a Cd v orgánech *R. alpinus*, což svědčí o omezeném příjmu a akumulaci těchto prvků.

Kromě toho byla akumulace As, Cr, Ni, Pb a Cd u *R. alpinus* ovlivněna sezónními změnami. Například srážky během podzimu mohly přispívat k uvolňování prvků do půdy, což se projevilo vyššími obsahy v orgánech ve srovnání s obsahy v létě na lokalitách v Krkonoších. V nadzemní biomase (rašící a zralé listy a řapíky) sice docházelo k určité míře akumulace dalších prvků, např. Cu, avšak Al a Cd se akumululovaly v podzemní biomase (oddenek) a v případě Cd pouze ve stonku. Obsah As, Cr, Ni, Pb a Cd ve vzcházejících, zralých a senescentních listech a řapíku se však pohyboval v rozmezí koncentrací typických pro cévnaté rostliny. Tato studie rovněž prokázala translokaci Zn, Cu, Mn, As, Ni a Pb do nadzemní biomasy *R. alpinus*.

Zároveň doporučujeme velkou opatrnost při konzumaci této zeleniny z kontaminovaných půd, zejména v případě Pb, kde hodnoty na některých lokalitách překračovaly přípustné limity a mohou představovat riziko pro lidské zdraví, jak uvádí Světová zdravotnická organizace. Doporučujeme proto provést podrobnou prvkovou analýzu orgánů tohoto druhu před jeho použitím jako léčivé byliny a potraviny.

5.3

Genetická variabilita *R. alpinus* nebyla doposud zkoumána, přestože fyziologii a ekologii *R. alpinus* byla věnována větší pozornost. Byla zaznamenána pouze jedna publikovaná studie, která se zaměřila na genetickou práci s *Rumex bucephalophorus* subsp. canariensis. Právě proto byl cílem třetí studie zkoumat genetickou variabilitu a populační strukturu invazní rostliny *R. alpinus* v Krkonoších, stejně jako na původních lokalitách. Výsledky mohou poskytnout cenné poznatky o genetických souvislostech tohoto druhu a přispět tak k efektivnějšímu k managementu rostlinných invazí. Molekulární markery se ukazují jako užitečné pro pochopení historie introdukce a genetické variability invazních druhů. Proto bylo v této studii použito 12 SSR markerů, které vykazovaly významný polymorfismus a umožňovaly diferenciaci mezi studovanými populacemi *R. alpinus*.

Populační struktura *R. alpinus* v introdukovaném areálu naznačuje náhodné usazování genotypů na různých lokalitách, pravděpodobně byla nejvíce ovlivněna vlivem člověka. Tok genů mezi populacemi byl omezený, přesto k určitému toku genů docházelo, pravděpodobně důsledkem opakovaného vlivu člověka.

Výsledky studie naznačily, že populace *R. alpinus* v českých horách jsou odlišné od alpských populací, což poukazovalo na možnost toho, že druh je v Krkonoších původní. Z hlediska genetické variability byly mezi populacemi *R. alpinus* v českých horách a v Alpách nápadné rozdíly. To však lze přičíst efektu úzkého hrdla a efektu zakladatele při kolonizaci nových území populací. Navíc je možné, že ke genetické variabilitě přispěly mutace a hybridizace s příbuznými druhy rodu Rumex. Historické záznamy a další genetické analýzy však potvrdily, že *R. alpinus* byl do Krkonoš zavlečen kolonisty, a podle analýzy Structure, to bylo ze Štýrska. Následně analýza AMOVA odhalila vyšší variabilitu spíše uvnitř populací než mezi populacemi *R. aplinus*. Studie navíc upozorňuje na vyšší genetickou variabilitu populací *R. alpinus* v původních oblastech Alp, Karpat a Pyrenejí ve srovnání s nepůvodními populacemi v českých horách. Tento vzorec byl v souladu s jinými studiemi a lze jej přičíst právě generativnímu šíření na přirozených lokalitách. Což je v důsledku přítomnosti přirozených nepřátel a v rámci mezidruhové konkurence. Zatímco, na nepůvodních lokalitách je preferováno klonální rozmnožování, což vede pak k nižší genetické variabilitě.

Studie rovněž zjistila sníženou genetickou variabilitu a možné příbuzenské křížení v populaci *R. alpinus* v Orlických horách.

Tato skutečnost posiluje předpoklad, že populace *R. alpinus* v Krkonoších není původní a představuje sekundární výskyt. Zároveň naznačuje, že správně nastavený manažerský přístup, stejně jako v případě Orlických hor, může výrazně omezit růst tohoto druhu v dalších horských oblastech. Výsledky této studie rovněž potvrzují předchozí hypotézy, které předpokládaly šíření *R. alpinus* prostřednictvím člověka z malých populací v období středověku.

Otázky pro budoucí výzkum

Do budoucna by bylo dobré se zaměřit na bližší zkoumání pastevního chování různých zvířat v souvislosti s abundancí, rozmnožováním a šířením rostlinných částí *R. alpinus* na pastvinách. Zajímavými poznatky, které přinesly experimenty Michala Hejcmana prováděné s antilopami, je objev, že tyto zvířata šťovík konzumují. Vzhledem k současným chovným experimentům s lamami v Alpách (osobní pozorování) je zajímavé prozkoumat, zda by lamy a antilopy mohly preferovat biomasu *R. alpinus* na pastvinách. Kromě toho stojí za to prozkoumat účinnou kombinovanou pastvu více než jednoho druhu hospodářských zvířat proti tomuto plevelnému druhu.

Vzhledem k tomu, že jen málo nechemických likvidačních metod prokázalo účinnost proti rostlinám *R. alpinus*, tak stěžejní strategie zahrnuje zabránění klíčení semen, mimo jiné, vysazováním konkurenčních trav na degradovaná místa.

Z tohoto důvodu byl proveden experiment v klimaboxech, který se týkal klíčení semen tří druhů *Rumex* (*alpinus, obtusifolius* a *longifolius*) v závislosti na různých teplotách (6, 12, 18, 24, 29 a 35 °C). Následovaly pokusy s klíčením semen *R. alpinus* z různých lokalit v Alpách a Krkonoších. V této souvislosti byly porovnány rychlosti klíčení a klíčivost semen z původních a nepůvodních lokalit. S přihlédnutím k poznatkům získaným z výše uvedených experimentů a k hrozící změně klimatu a souvisejícímu oteplování byl dále navržen experiment, který měl prozkoumat růst semenáčků výše uvedených tří druhů *Rumex* (*alpinus, obtusifolius* a *longifolius*) při vyšších teplotách (data nebyla dosud publikována). Předběžné výsledky ukázaly, že sazenice *Rumex obtusifolius* a *Rumex longifolius* byly tolerantnější k vysokým teplotám, zatímco *R. alpinus* nezvládl teploty 40 °C. Dále bylo z experimentu zjištěno, že *R. alpinus* je náchylný k nížinným chorobám (padlí) a také ke škůdcům (mšice), které netoleroval. Bylo by tedy zajímavé zaměřit se na klíčivost a růst rostlin *R. alpinus* ze semene nebo vegetativního množení na venkovních stanovištích (transplantační pokusy).

Domnívám se, že dobře navržená studie rozhovorů s farmáři a ochránci přírody by mohla být neocenitelným příspěvkem k další práci, která se snaží zjistit, které faktory a opatření proti šíření tohoto plevele jsou důležité při nastavení managementu pro eradikaci *R. alpinus* a které nejsou.

Kromě zkoumání vlivu teploty na klíčení semen *R. alpinus* a růst sazenic ve výše uvedených experimentech je zásadní pochopit reakci druhu na změnu klimatu a zvážit i další faktory, které řídí jeho šíření. Některé druhy rodu *Rumex* již byly takto v minulosti studovány, a to například v souvislosti se zvýšenou koncentrací CO_2 a dále například se zvýšenými účinky slunečního záření.

6. LIST OF PUBLICATIONS

6.1 Papers in scientific journals with impact factor:

Jungová M, Asare MO, Jurasová Müllerová V, Hejcman M (2022) 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. Plant and Soil 477, 553–575. https://doi.org/10.1007/s11104-022-05440-2

The percentage of Jungové work done on the article: **90%**

Jungová M, Asare MO, Hejcman M, Hakl J, Pavlů V (2023) Distribution and Resorption Efficiency of Macroelements (N, P, K, Ca, and Mg) in Organs of *Rumex alpinus* L. in the Alps and the Giant (Krkonoše) Mountains. Journal of Soil Science and Plant Nutrition 23, 469–484. https://doi.org/10.1007/s42729-022-01059-5

The percentage of Jungové work done on the article: **85%**

Jungová M, Jurasová Müllerová V, Čepková Hlásná P, Svobodová Leišová L, Svoboda P, Hejcman M (2023) Origin and genetic variability of populations of the invasive plant *Rumex alpinus* L. in the Giant (Krkonoše) Mountains. Ecology and Evolution, 13, e10145. https://doi.org/10.1002/ece3.10145
The percentage of Jungové work done on the article: **80%**

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Koláriková L, Svobodová Leišová L, Hanzalová A, Holubec V, **Jungová M,** Esimbekova M (2023) Leaf rust resistance genes in *Aegilops* genus: occurrence and efficiency. European Journal of Plant Pathology.https://doi.org/10.1007/s10658-023-02712-0

Asare MO, Afriyie JO, Hejcman M, **Jungová Krbová M** (2022) Can Wood Ashes of Commonly Planted Tree Species in Ghana be Applied as Fertilizers? Waste and Biomass Valorization 13, 1043–1058. https://doi.org/10.1007/s12649-021-01588-7

6.2 Papers in other scientific journals

Jungová M, Ovesná J, Petrželová I (2022) Validace molekulárních markerů pro identifikaci resistentních rostlin vůči plísni *Hyaloperenospora parasitica* u hospodářsky využívaných druhů *Brassica oleracea*. Úroda 12, roč. LXIX, 2022, vědecká příloha, s. od 51 – do 59. ISSN 0139-6013

6.3 Papers from the scientific conferences in Czech:

Posters:

Jungová M, Hejcman M. Effects of temperature on germination of *Rumex alpinus, Rumex obtusifolius,* and *Rumex longifolius.* Sborník abstraktů z konference *Global Biodiversity Conservation Conference 2018,* FŽP ČZU Praha.

Jungová M, Hejcman M. Effect of temperature on germination of *Rumex alpinus, Rumex obtusifolius* and *Rumex Longifolius.* Sborník abstraktů z konference *Kostelecké inspirování 2018*, FŽP ČZU Praha, ISBN 978-80-213-3222-5

Jungová M, Jurasová Müllerová V, Hlásná Čepková P, Svobodová Leišová L, Svoboda P, Hejcman M. Origin and genetic variability of populations of the invasive plant *Rumex alpinus* L. in the Giant (Krkonoše) Mountains. Sborník abstraktů z konference *Kostelecké inspirování 2021*, FŽP ČZU Praha, ISBN 978-80-213-3222-5

Jungová M, Ovesná J, Petrželová I**.** Validace molekulárních markerů pro identifikaci resistentních rostlin vůči plísni *Hyaloperenospora parasitica* u hospodářsky využívaných druhů *Brassica oleracea*. Sborník abstraktů z konference Aktuální poznatky v pěstování, šlechtění, ochraně rostlin a zpracování produktů. Článek vědecké přílohy časopisu Úroda 12/2022, Brno

Jungová M, Ovesná J, Petrželová I. Validace molekulárních markerů pro identifikaci resistentních rostlin vůči plísni *Hyaloperenospora parasitica* u hospodářsky využívaných druhů *Brassica oleracea*. Sborník abstraktů z konference Kostelecké inspirování 2022, FŽP ČZU Praha, ISBN 978-80-213-3222-5

Jungová M, Leišová Svobodová L, Hanzalová A, Zelba O, Svoboda P. Primer and probe set for the detection and quantification of *Puccinia graminis* and *Puccinia triticina* in association with the *β*-tubulin gene in *Triticum aestivum* L. (wheat) by RT-PCR. Sborník abstraktů z konference Kostelecké inspirování 2022, FŽP ČZU Praha, ISBN 978-80-213-3222-5

Presentation:

Jungová M, Asare MO, Hejcman M, Hakl J, Pavlů V. Distribution and Resorption Efficiency of Macroelements (N, P, K, Ca, and Mg) in Organs of *Rumex alpinus* L. in the Alps and the Giant (Krkonoše) Mountains. Sborník abstraktů z konference *Kostelecké inspirování 2019*, FŽP ČZU Praha

Jungová M, Hejcman M. *Rumex alpinus* as the ancient food plant introduced into the Czech Republic by colonists from the Alps. *Konference environmentální archeologie 2020*, FŽP ČZU Praha

Jungová M, Asare MO, Jurasová Müllerová V, Hejcman M. 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. Sborník abstraktů z konference *Kostelecké inspirování 2022*, FŽP ČZU Praha