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# Seasonal dynamics of element accumulation in highland rush (*Juncus trifidus*, L.)

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**Abstract.** Seasonal variation influences the elemental composition of alpine plants yet remains understudied due to sampling challenges. This study investigates monthly changes in macro- and micronutrient concentrations in *Juncus trifidus* L., a stress-tolerant alpine species, across leaf blades and basal sheaths over one year in the Low Tatras (Slovakia). Using energy-dispersive X-ray fluorescence spectrometry, 14 elements were consistently detected and analysed. Results revealed clear seasonal and organ-specific differences. Leaf tissues showed higher concentrations of S, Ca, Mn, and Zn, reflecting their role in active metabolism. In contrast, sheaths accumulated more Fe, Pb, and Cl during specific seasons, suggesting storage or detoxification functions. Principal component analysis identified distinct multivariate patterns, with some factors showing inverse relationships between essential and potentially toxic elements, indicating physiological trade-offs and stress responses. Seasonal peaks in elements such as K and Rb in summer, and S and Ca in spring, corresponded with growth phases and environmental conditions. These findings underscore the importance of temporal dynamics in nutrient uptake and allocation, offering insights into alpine plant adaptation and supporting the use of *J. trifidus* as a bioindicator in ecological monitoring.

*Key words: Juncus trifidus*, element content, seasonal differences, leaf blades, sheaths

## Introduction

Seasonality is a key source of ecological variability, yet the seasonal dynamics of elemental concentrations in alpine plants remain underexplored due to logistical constraints in long-term sampling (Březinová and Vymazal 2015; Kandziora-Ciupa *et al.* 2017; Kim and Kim 2018). Recognising seasonal variation is essential for accurate interpretation in biomonitoring programmes (Oliva *et al.* 2012), as variability may reflect physiological differences or biochemical shifts (Viers *et al.* 2013). Alpine ecosystems present a unique set of environmental challenges to plant life, including nutrient-poor soils, extreme temperature fluctuations, high solar radiation, and limited water availability (Sati *et al.* 2024). In response to these stressors, many alpine plant species have evolved specialized physiological and morphological adaptations that enable survival in such harsh conditions. These adaptations include the accumulation of trace elements and metalloids, which play key roles in stress mitigation, metabolic regulation, and ecological resilience (Reeves *et al.* 2018).

Elemental composition in alpine plants is shaped by phylogenetic constraints, environmental factors, and edaphic adaptation, with convergent evolution observed in specialised lineages (Palacio *et al.* 2022). Seasonal dynamics include dramatic increases in carbon-to-nitrogen ratios and species-specific niche partitioning that enhance microbial interactions and biogeochemical cycling (Ruka *et al.* 2023). Selective uptake strategies vary among species, including oxyanion absorption and rare earth element accumulation (Fehlauer *et al.* 2022). Morphological and physiological mechanisms such as specialised roots (Ma *et al.* 2010; Viana *et al.* 2022), mycorrhizal associations (Bueno *et al.* 2017; Sizonenko *et al.* 2020), and leaf adaptations (Liu *et al.* 2020) enhance nutrient acquisition and tolerance to abiotic stressors.

Elemental uptake in plants exhibits a seasonal pattern, with temporal variation playing a significant role in the dynamics of accumulation. In spring, nutrient reserves stored in roots are mobilised to support early growth (Loescher *et al.* 1990; Zapata *et al.* 2004). Summer peaks in uptake and translocation are tied to developmental stages (Schiestl-Aalto *et al.* 2019). In autumn, nutrient absorption slows, and elements are redistributed to basal tissues and roots for overwintering (Chapin and Kedrowski 1983; Babst *et al.* 2018). Winter marks a dormant phase, with minimal metabolic activity and nutrients retained within storage tissues (Kočí 2007).

Environmental factors such as temperature, humidity, wind, and soil moisture regulate nutrient dynamics and are also strongly influenced by seasonal changes (e.g., Feng *et al.* 2012; Dong *et al.* 2018; Tang *et al.* 2022). Low temperatures typical of high-altitude regions can suppress metabolic activity and slow nutrient absorption, while elevated levels of solar radiation may alter nutrient utilisation pathways (Morgan and Connolly 2013). Fluctuations in temperature, precipitation, and soil composition across the growing season can influence both the availability of elements and the physiological demand within the plant. Low temperatures suppress metabolism and nutrient assimilation (Rengel 1999), microbial activity and organic matter decomposition (Zhu *et al.* 2024), while increasing temperature is the most common factor in temporal variability of elemental composition among boreal species (Richmond *et al.* 2021). The mobility and bioavailability of elements are also modulated by water availability and terrain exposure. Precipitation patterns, which vary with altitude and season, influence soil moisture availability. Moderate levels enhance nutrient uptake and microbial activity, whereas excessive rainfall promotes nutrient leaching and loss (Sardans and Penuelas 2012; Shara *et al.* 2021). Under dry conditions, the uptake of certain nutrients may be limited, whereas in more humid environments, increased solubility can enhance absorption (Marschner 2012). Elevated moisture levels reduce transpiration, thereby restricting the movement of water and dissolved nutrients within plants (Gisleröd *et al.* 1987). Wind exposure impacts photosynthetic efficiency and nutrient uptake through physical damage and altered transpiration (Sati *et al.* 2024; Liu *et al.* 2025).

Elemental composition also varies among different plant organs, reflecting their distinct functional roles in growth, reproduction, and maintenance. These organ-specific differences are essential for understanding nutrient allocation and cycling within alpine plants, and they provide insight into physiological adaptations to environmental stress. Leaves concentrate certain elements differently, with calcium being particularly abundant in leaf tissues (Davey and Mitchell 1968). Leaf sheaths may have a higher potential for metal accumulation compared to other organs (Ding *et al.* 2020) and exhibit significant top-to-bottom differences in element distribution (Ishimaru *et al.* 2004).

The target species of the study, *Juncus trifidus* L., a perennial graminoid, is well-adapted to alpine environments and widely distributed across Europe, Siberia, Greenland, and North America (Mossberg *et al.* 2005). It inhabits elevations of 1200–3000 m, favouring exposed, acidic, nutrient-poor soils (Hroudová *et al.* 2010). As a hemicryptophyte, it forms dense clumps in wind-exposed habitats and exhibits tolerance to frost, drought, and solar radiation (Schwabe *et al.* 2017).

Due to its ecological specificity and stress tolerance, *J. trifidus* serves as a bioindicator of acidic, oligotrophic alpine habitats (Kočí 2007; Marhold and Hindák 1998). It accumulates essential and toxic elements, including heavy metals (Adriano 2001), and exhibits seasonal nutrient cycling. Its broad distribution, long lifespan, and measurable responses to environmental stressors make it suitable for biomonitoring (Colesie *et al.* 2020; Oulehle *et al.* 2011). Adapted to oligotrophic conditions, it maintains elemental homeostasis across variable substrates, with nutrient uptake influenced by soil pH, element solubility, root morphology, and microbial associations. Sampling causes minimal disturbance, supporting long-term monitoring (Holt and Miller 2010).

This study examines macro- and micronutrient concentrations in *Juncus trifidus* over the course of a year-long monitoring programme, with monthly sampling conducted on the northern and southern slopes of Chopok (Low Tatras). Elemental content will be analysed in leaf blades and sheaths to evaluate organ-specific differences. We hypothesise that elemental concentrations differ between organs due to their physiological functions, and that seasonal changes influence nutrient content.

## Material and Methods

### *Study area and sampling*

The study was conducted in the Low Tatras Mountains (Western Carpathians), specifically on the slopes of Chopok Peak. The Low Tatras, the second highest mountain range in Slovakia, stretch between the Hron Valley and the Liptov Basin. Chopok (2024 m a.s.l.; 48.9429992° N, 19.5927219° E) is situated in the central part of the range and served as the focal point for sampling.

Field sampling was conducted monthly from November 2023 to November 2024, resulting in a total of 108 specimens of *Juncus trifidus*. Sampling sites were arranged along transects on both the northern and southern slopes of Chopok, spanning from the lowest elevations where the species occurs, up to the summit. Four altitudinal levels were selected, including the ridge crest.

In total, samples were collected from nine distinct locations: three sites on the northern slope (1700, 1800, and 1900 m), three on the southern slope (1700, 1800, and 1900 m), one at the summit (2000 m), and two additional sites on the eastern and western ridges (Fig. 1). At each site, a portion of the plant tuft (approximately 3 cm) was excised using a sterile knife, placed into labelled plastic bags, and transported for analysis. Each tuft included leaves, stems, and sheaths, allowing for tissue-specific comparisons. During the winter months, samples were retrieved from beneath the snow cover at known growth locations (Fig. 2).

### *Laboratory analysis*

Leaf and sheath samples were dried separately in Petri dishes at room temperature for 4–5 days. Following desiccation, each sample was homogenised into a fine powder using a laboratory mill (RETSCH CryoMill, Germany). Grinding was performed for 1.5–3 minutes at a frequency of 30 Hz, with approximately 0.5 g of dry material processed per sample.

Elemental composition was determined using energy-dispersive X-ray fluorescence spectrometry (ED-XRF). Measurements were conducted with a DELTA CLASSIC spectrometer (Innov-X Systems, USA). Each sample was analysed in three consecutive runs, each lasting 80 seconds, and the final concentration was calculated as the mean value of these replicates. To ensure analytical accuracy, the reference standard INCT-PVTL-6 Polish Virginia tobacco leaf (Labmix24 GmbH, Germany) was remeasured prior to the first sample and after every tenth measurement. The following elements were quantified:

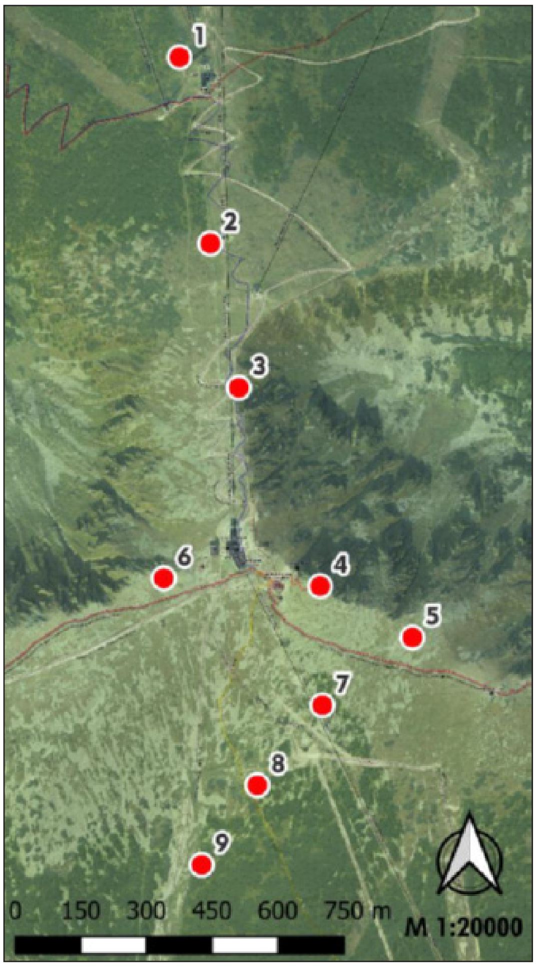

**Fig. 1.** Map of sampling locations around Chopok peak (Low Tatras, Slovakia). Red dots (1–9) indicate sampling sites distributed across different slope orientations and altitudinal levels. Sites 1–3 are located on the northern slope, while sites 7–9 are situated on the southern slope. Site 4 corresponds to the Chopok summit, representing one of the highest elevations, along with sites 5 (east) and 6 (west). Sites 1 and 9 mark the lowest elevation points within the sampling design.

P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Zr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Pb.

Calibration was performed by calculating a calibration coefficient, defined as the ratio between the certified value of each element in the reference material and the value measured prior to sample analysis. Final concentrations in the plant samples were standardised by applying this coefficient to the raw data.

### *Statistical analysis*

Statistical evaluation was performed using Statistica Version 12 (StatSoft, Inc.). Only those elements that were consistently measured above the detection limit, namely S, Cl, K, Ca, Ti, Cr, Mn, Fe, Cu, Zn, Rb, Sr, Ba, and Pb, were included in the analysis.

The Shapiro–Wilk test indicated that the data did not follow a normal distribution. To compare the differences in the average values of the element content in the analyzed plant parts (in the leaves and sheath parts), between seasons, the non-parametric Mann-Whitney U Test (95% confidence level; $p < 0.05$) was used. To assess differences in elemental concentrations between seasons, the Kruskal–Wallis H test was applied (confidence level 95%; $p < 0.05$). This non-parametric method allowed for the comparison of multiple groups without assuming normality.

To explore simultaneous relationships among elements, a principal component analysis (PCA) was conducted. PCA reduces dimensionality by identifying components that explain the majority of variance in the dataset, thereby revealing underlying patterns and associations among ecological variables (Jolliffe 2002). The first four principal components, which together accounted for 83% of the total variance and had eigenvalues greater than 1, were further tested using the Kruskal–Wallis H test (confidence level 95%; $p < 0.05$) to evaluate differences in multivariate structure across seasons, altitudes, and slope orientations.

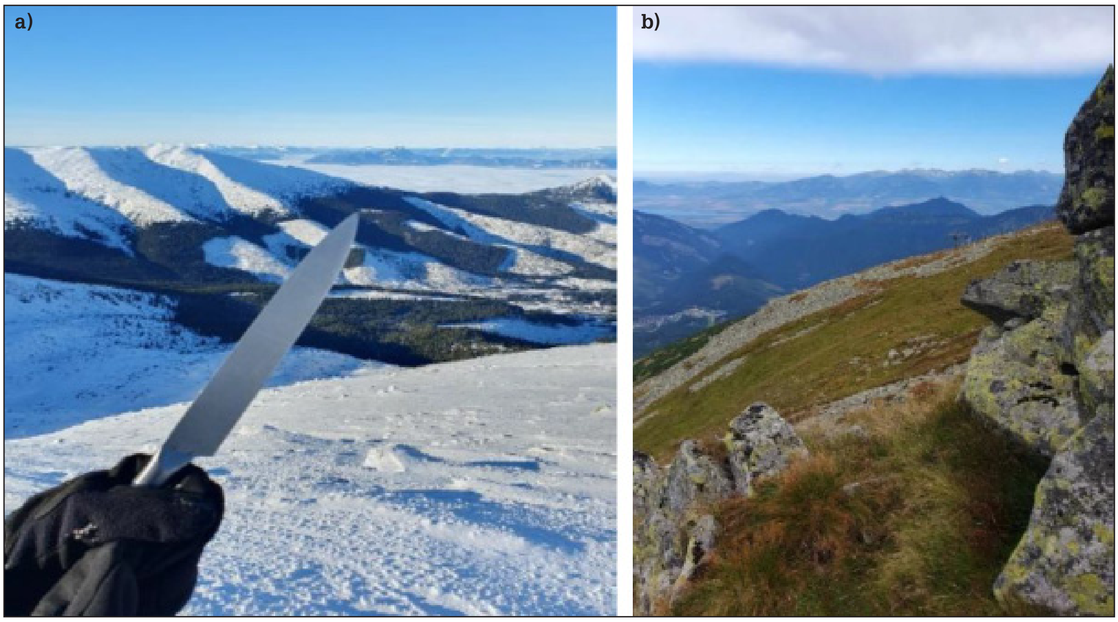

**Fig. 2.** Habitat of *Juncus trifidus* during winter (a), and summer (b) on the slopes of Chopok.(Photo: E. Hortobágyiová, 2025)

## Results

### *Differences in element levels between parts of the plant*

To assess the influence of environmental factors on elemental accumulation in *Juncus trifidus*, we compared the concentrations of selected macro- and micronutrients in leaves and sheaths across seasons, altitudes, and slope orientations. Periodically, 14 elements: sulfur (S), chlorine (Cl), potassium (K), calcium (Ca), titanium (Ti), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), rubidium (Rb), strontium (Sr), barium (Ba), and lead (Pb), were measured above the detection limits of the instrument. The highest concentrations (>1000 ppm) were detected for the biogenic elements S, K, Ca, Fe. The lowest concentrations (>100 ppm) were found for the elements Cu, Rb, Sr, Ba, Pb.

### *Seasonal variation in elemental content*

We hypothesized that the levels of elements vary throughout the year in the plant, and that their content differs between the above-and below-ground parts of the plant. Significantly, the elemental levels among these plant parts differed during all seasons for the elements S, K, Ca, Mn, Zn, Rb, and Sr (Table 1). In spring, the content of S, Ca, Mn, Zn, and Sr was higher in the leaves, while K and Rb were higher in the sheaths. In summer, S, K, Ca, Mn, Zn, and Rb were higher in the leaves, whereas Sr, Cr, Ba, and Pb were higher in the sheaths. In autumn, S, Ca, Mn, Zn, and Sr were higher in the leaves, while K and Rb were higher in the sheaths. In winter, S, Ca, Mn, Zn, Sr, and Cr were higher in the leaves, while K, Rb, and Pb were higher in the sheaths.

Iron (Fe) levels differed significantly between leaves and sheaths in spring, summer, and autumn, and in all three seasons Fe was consistently higher in the sheaths. In winter, the difference was not significant. Among the elements, Cr, Ba and Pb showed significant differences specifically between summer and winter. Copper (Cu) levels did not differ significantly between leaves and sheaths throughout the year.

We hypothesized that elemental concentrations in *J. trifidus* would vary with season. Statistical analysis confirmed that seasonality was a key factor influencing the elemental variability of all analyzed elements. Significant seasonal differences ($p < 0.05$) were observed in both leaves and sheaths for S, Cl, Ca, Cr, Fe, Rb, Sr, Ba, and Pb. In contrast, K, Mn, Cu, and Zn showed significant seasonal variation only in leaves, suggesting a stronger response in photosynthetically active tissues. Specifically, the contents of sulfur (S), chlorine (Cl), calcium (Ca), chromium (Cr), iron (Fe), rubidium (Rb), strontium (Sr), barium (Ba), and lead (Pb) differed significantly between seasons in both plant organs.

Sulfur concentrations peaked in winter and spring in both leaves and sheaths, indicating early-season uptake and metabolic activation. A gradual decline followed in summer and autumn (Fig. 3). Chlorine concentrations exhibited clear seasonal variation in both leaves and sheaths. In leaves, Cl levels were highest in summer, reflecting increased physiological activity and osmotic regulation during peak growth. Concentrations declined in autumn and reached their lowest values in winter, followed by a moderate increase in spring (Fig. 4). In sheaths, the seasonal pattern was less pronounced, but Cl still peaked in summer, with lower concentrations in winter and autumn, and a slight rise in spring. These trends suggest that Cl uptake and redistribution are closely linked to photosynthetic activity and water transport dynamics, particularly in green tissues. Calcium showed elevated concentrations in leaves during cooler seasons (spring and winter), with a sharp decline in summer. In sheaths, Ca also peaked in winter and spring, though seasonal differences were less pronounced (Fig. 5). Chromium reached maximum concentrations in spring in both organs. In leaves, Cr levels declined during summer, while in sheaths, the lowest values were recorded in winter (Fig. 6). Iron content in leaves was highest in spring and lowest in summer, reflecting seasonal shifts in photosynthetic demand. In sheaths, Fe increased from winter to summer (Fig. 7). Rubidium exhibited a consistent seasonal pattern, with significantly higher concentrations in summer in both organs. In leaves, summer values were up to five times higher than in other seasons, indicating strong seasonal uptake (Fig. 8). Strontium levels in leaves fluctuated irregularly, peaking in autumn and spring. In sheaths, a gradual increase was observed from winter to autumn, suggesting progressive accumulation (Fig. 9). Barium concentrations in leaves were highest in spring and lowest in summer, while in sheaths, seasonal differences were less distinct, with slightly elevated values in spring and summer (Fig. 10). Lead concentrations were highest in spring, likely due to snowmelt-driven mobilization of atmospheric deposits. Levels declined in summer, remained low in autumn, and reached a minimum in winter, especially in sheaths (Fig. 11).

Among the monitored elements, potassium (K), manganese (Mn), copper (Cu), and zinc (Zn) demonstrated statistically significant seasonal fluctuations exclusively in leaf tissues, while their concentrations in sheaths remained relatively stable.

Potassium levels reached their maximum during the summer months, which likely reflects intensified metabolic processes and elevated nutrient requirements associated with the peak of vegetative growth (Fig. 12). In contrast, manganese concentrations were highest in winter, followed by a gradual decline through spring and summer, suggesting its potential involvement in cold stress adaptation or seasonal storage mechanisms (Fig. 13). Copper exhibited its highest concentrations in spring, with a progressive decrease observed in summer and autumn. This pattern may indicate a role in early-season physiological functions, such as enzymatic activation or redox regulation (Fig. 14). Similarly, zinc levels peaked in spring, while markedly lower values were recorded in summer and winter, possibly reflecting its contribution to photosynthetic efficiency and enzyme activity during periods of active growth (Fig. 15).

| | | Spring | | | Summer | | | Autumn | | | Winter | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **N** | **Median (SD)** | **M-W U test** | **N** | **Median (SD)** | **M-W U test** | **N** | **Median (SD)** | **M-W U test** | **N** | **Median (SD)** | **M-W U test** |
| **S** | L | 27 | 2602.88 (409.86) | **0.0000** | 27 | 1982.73 (570.02) | **0.0000** | 27 | 1329.33 (358.43) | **0.0006** | 27 | 2029.46 (945.13) | **0.0048** |
| | S | 27 | 1847.29 (317.92) | | 27 | 1116.42 (313.52) | | 27 | 1057.92 (226.53) | | 27 | 1394.82 (811.97) | |
| **Cl** | L | 27 | 515.10 (130.2) | **0.0001** | 27 | 2511.60 (1117.85) | **0.0000** | 27 | 405.84 (947.29) | 0.0836 | 27 | 667.08 (366.35) | 0.6907 |
| | S | 27 | 715.83 (324.42) | | 27 | 881.50 (277.15) | | 27 | 575.64 (217.66) | | 27 | 651.42 (514.88) | |
| **K** | L | 27 | 2286.60 (2981.77) | **0.0001** | 27 | 19238.98 (7049.95) | **0.0000** | 27 | 3059.00 (4911.89) | **0.0035** | 27 | 4407.37 (3275.87) | **0.0466** |
| | S | 27 | 6428.23 (2596.17) | | 27 | 6300.00 (2084.01) | | 27 | 6010.20 (1687.99) | | 27 | 4569.08 (4350.62) | |
| **Ca** | L | 27 | 6312.18 (2150.25) | **0.0000** | 27 | 2616.96 (1162.19) | **0.0000** | 27 | 4264.74 (1153.52) | **0.0000** | 27 | 4949.91 (3628.13) | **0.0000** |
| | S | 27 | 2316.72 (433.61) | | 27 | 1754.98 (455.81) | | 27 | 1699.98 (714.49) | | 27 | 1980.58 (2303.26) | |
| **Cr** | L | 27 | 146.88 (41.12) | 0.2572 | 27 | 44,16 (33.83) | **0.0000** | 27 | 98.56 (56.08) | 0.6038 | 27 | 114.24 (63.52) | **0.0147** |
| | S | 27 | 129.54 (76.79) | | 27 | 105.60 (83.22) | | 27 | 102.08 (39.92) | | 27 | 70.38 (55.03) | |
| **Mn** | L | 27 | 401.44 (215.65) | **0.0000** | 27 | 299.04 (107.79) | **0.0000** | 27 | 337.31 (202.46) | **0.0000** | 27 | 510.64 (274.70) | **0.0001** |
| | S | 27 | 179.92 (42.02) | | 27 | 162.96 (61.18) | | 27 | 132.61 (157.64) | | 27 | 216.32 (278.81) | |
| **Fe** | L | 27 | 1637.60 (724.51) | **0.0018** | 27 | 522.00 (468.07) | **0.0000** | 27 | 1174.70 (1325.29) | **0.0046** | 27 | 1275.00 (1101.15) | 0.5564 |
| | S | 27 | 2529.16 (1810.77) | | 27 | 2828.37 (2162.15) | | 27 | 2420.34 (1345.56) | | 27 | 1068.98 (1449.55) | |
| **Cu** | L | 22 | 21.00 (4.12) | 0.7959 | 20 | 18.00 (10.74) | 0.9127 | 21 | 16.00 (4.90) | 1.0000 | 14 | 14.50 (2.44) | 0.1194 |
| | S | 26 | 20.50 (5.50) | | 23 | 16 .00 (7.18) | | 26 | 17.50 (4.92) | | 20 | 19.00 (7.39) | |
| **Zn** | L | 27 | 164.16 (44.20) | **0.0000** | 27 | 133.45 (32.63) | **0.0000** | 27 | 164.80 (45.99) | **0.0004** | 27 | 135.36 (101.67) | **0.0003** |
| | S | 27 | 92.80 (28.03) | | 27 | 78.2 (34.31) | | 27 | 100.30 (62.41) | | 27 | 83.52 (110.61) | |
| **Rb** | L | 27 | 9.80 (11.64) | **0.0000** | 27 | 51.39 (14.16) | **0.0000** | 27 | 13.38 (7.99) | **0.0000** | 27 | 12.07 (10.45) | **0.0001** |
| | S | 27 | 23.716 (12.30) | | 27 | 28.91 (9.80) | | 27 | 24.48 (7.95) | | 27 | 21.92 (9.16) | |
| **Sr** | L | 27 | 4.77 (1.31) | **0.0000** | 26 | 2.49 (1.13) | **0.0221** | 27 | 4.86 (2.56) | **0.0035** | 26 | 3.15 (0.89) | **0.0233** |
| | S | 26 | 2.83 (1.99) | | 25 | 3.14 (4.72) | | 26 | 3.38 (2.52) | | 18 | 2.06 (1.40) | |
| **Ba** | L | 27 | 139.00 (25.21) | 0.1301 | 27 | 69.00 (28.14) | **0.0000** | 27 | 108.00 (29.20) | 0.8491 | 27 | 130.00 (38.90) | **0.0313** |
| | S | 27 | 132.00 (37.59) | | 27 | 128.00 (41.06) | | 27 | 114.00 (31.64) | | 27 | 110.00 (43.28) | |
| **Pb** | L | 27 | 42.00 (22.75) | 0.5564 | 27 | 25.00 (19.66) | **0.0002** | 27 | 36.00 (22.76) | 0.8153 | 27 | 28.00 (9.57) | **0.0341** |
| | S | 27 | 47.00 (16.21) | | 27 | 35.00 (30.06) | | 27 | 33.00 (27.37) | | 27 | 35.00 (13.79) | |

**Table 1.** Median concentrations of selected elements in leaf (L) and sheath (S) parts of the plant across seasons. N – number of samples; SD – standard deviation; M–W U test – Mann–Whitney U test; values of $p < 0.05$ indicate statistically significant differences between plant parts.

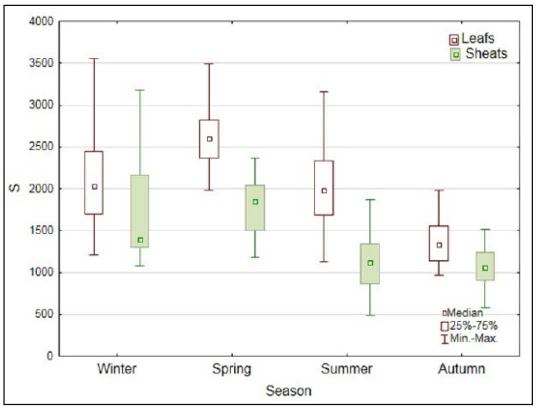

**Fig. 3.** Seasonal variation in sulfur (S) concentration in leaf and sheath tissues. Significant differences were confirmed both parts: leaf (KW-H (3;108) = 52.4781, p < 0.0001) and sheath (KW-H (3;108) = 56.0863, p < 0.0001). Median values and standard deviations indicate a clear decline in sulfur levels from spring to autumn, with partial recovery in winter.

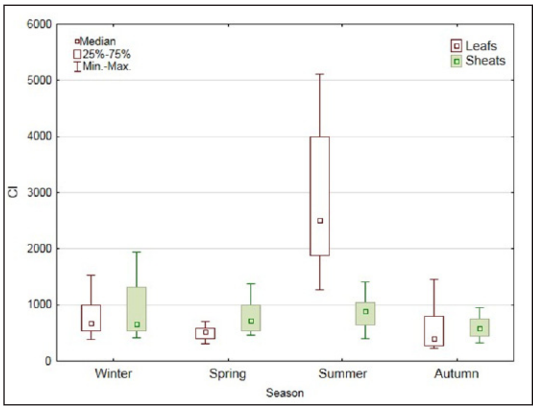

**Fig. 4.** Seasonal changes in chlorine (Cl) concentration in the leaf and sheath. Significant differences were confirmed both parts: leaf (KW-H (3;108) = 60.41, p < 0.0001) and sheath (KW-H (3;108) = 15.50, p = 0.0014), indicating dynamic redistribution of Cl throughout the year.

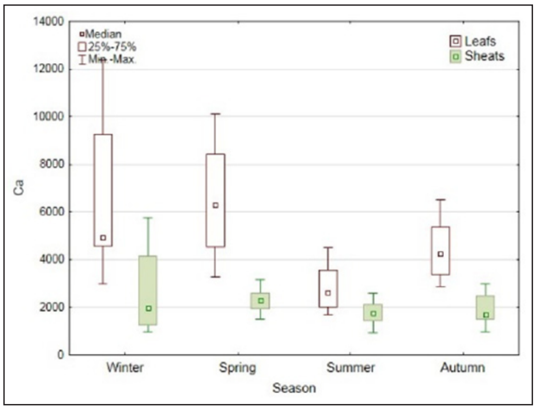

**Fig. 5.** Seasonal changes in calcium (Ca) concentration in leaves and sheaths. Significant differences were found in both tissues: leaf (KW-H (3;108) = 51.97, p < 0.0001), sheath (KW-H (3;108) = 10.46, p = 0.0151).

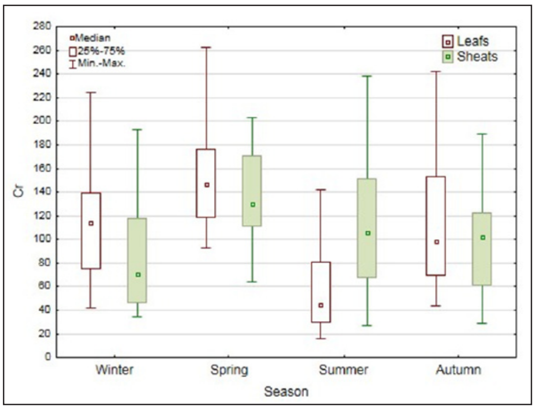

**Fig. 6.** Seasonal variation in chromium (Cr) concentration in leaves and sheaths. Significant differences were found in both tissues: leaf (KW-H (3;108) = 45.01, p < 0.0001), sheath (KW-H (3;108) = 17.40, p = 0.0006).

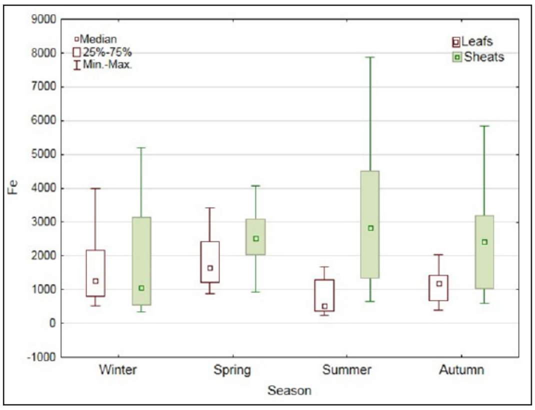

**Fig. 7.** Seasonal variation in iron (Fe) concentration in leaves and sheaths. Significant differences were found in both tissues: (KW-H (3;108) = 32.27, p < 0.0001) and sheath (KW-H (3;108) = 12.56, p = 0.0057), reflecting seasonal shifts in Fe uptake and allocation.

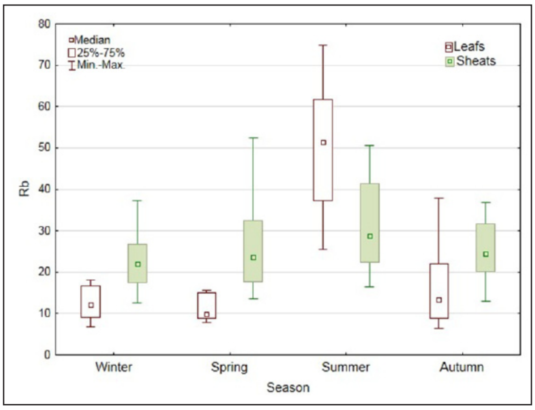

**Fig. 8.** Seasonal variation in rubidium (Rb) concentration in leaves and sheaths. Significant differences were found in both tissues: leaf (KW-H (3;108) = 53.05, p < 0.0001) and sheath (KW-H (3;108) = 8.69, p = 0.0337).

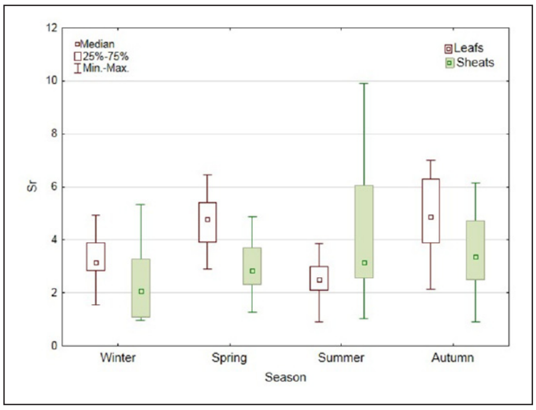

**Fig. 9.** Seasonal variation in strontium (Sr) concentration in leaves and sheaths. Significant differences were found in both tissues: leaf (KW-H (3;106) = 52.74, p < 0.0001) and sheath (KW-H (3;95) = 9.59, p = 0.0224).

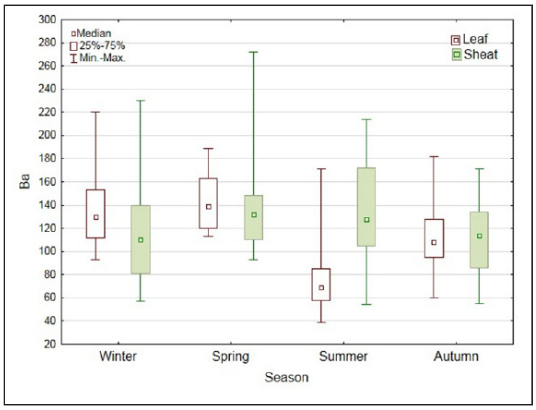

**Fig. 10.** Seasonal variation in barium (Ba) concentration in leaves and sheaths. Significant differences were found in both tissues: leaf (KW-H (3;108) = 53.00, p < 0.0001) and sheath (KW-H (3;108) = 8.76, p = 0.0326).

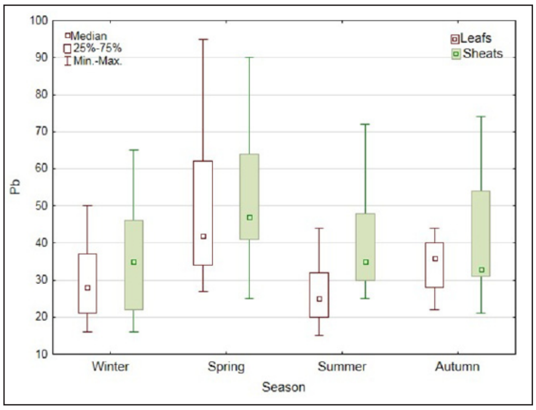

**Fig. 11.** Seasonal variation in lead (Pb) concentration in leaves and sheaths. Significant differences were found in both tissues: leaf (KW-H (3;108) = 32.24, p < 0.0001) and sheath (KW-H (3;108) = 11.20, p = 0.0107).

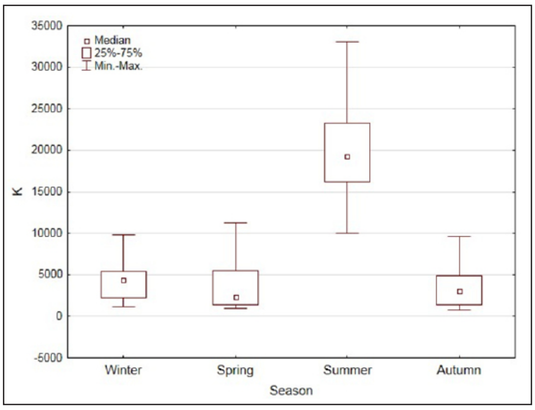

**Fig. 12.** Seasonal variation in potassium (K) concentration in leaves and sheaths. Significant differences were found in leaves (KW-H (3;108) = 59.30, p < 0.0001), while differences in sheaths were not statistically significant (KW-H (3;108) = 6.66, p = 0.0836), suggesting that seasonal dynamics of K are more pronounced in the photosynthetically active tissues.

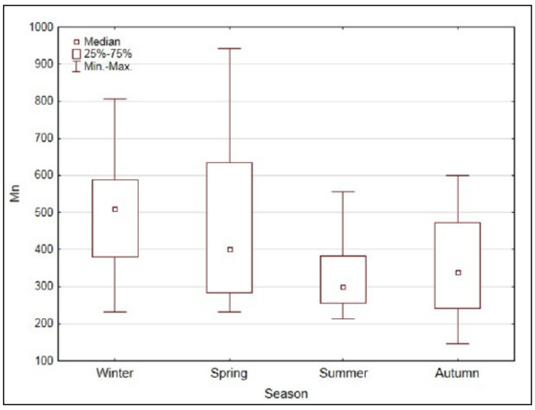

**Fig. 13.** Seasonal variation in manganese (Mn) concentration in leaves and sheaths. Significant differences were found in leaves (KW-H (3;108) = 19.12, p = 0.0003), while differences in sheaths were not statistically significant (KW-H (3;108) = 5.48, p = 0.1396), indicating that Mn dynamics are more pronounced in photosynthetically active tissues.

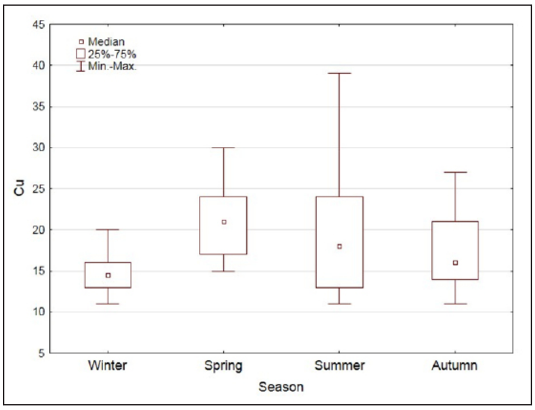

**Fig. 14.** Seasonal variation in copper (Cu) concentration in leaves and sheaths. Significant differences were found in leaves (KW-H (3;77) = 15.76, p = 0.0013)), while differences in sheaths were not statistically significant (KW-H (3;95) = 7.59, p = 0.0552).

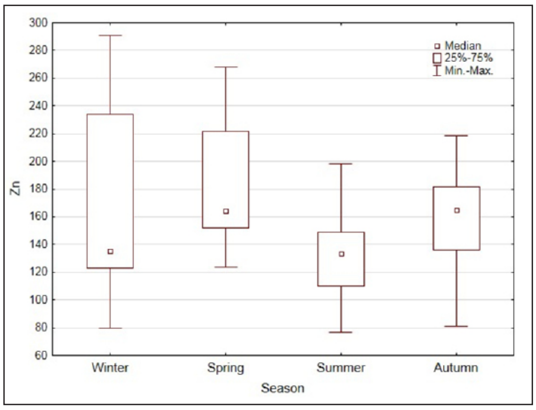

**Fig. 15.** Seasonal variation in zinc (Zn) concentration in leaves and sheaths. Significant differences were found in leaves (KW-H (3;108) = 13.89, p = 0.0031)), while differences in sheaths were not statistically significant (KW-H (3;108) = 4.56, p = 0.2067).

### *Multivariate patterns in elemental composition*

To investigate the interrelationships among the analysed elements, a principal component analysis (PCA) was performed. This multivariate approach revealed four principal factors, each representing distinct patterns of elemental co-occurrence (Table 2).

Factor 1 represents a bipolar axis, characterised by a positive association of barium (Ba) and chromium (Cr), contrasted with a negative correlation involving chlorine (Cl), potassium (K), and rubidium (Rb). This opposing relationship may indicate a functional trade-off between elements involved in stress response and those linked to metabolic activity. Factor 2 also exhibits a bipolar structure, with iron (Fe) and lead (Pb) increasing concurrently, while concentrations of sulphur (S), calcium (Ca), manganese (Mn), and zinc (Zn) decline. This pattern could reflect physiological adjustments under environmental stress or seasonal redistribution of nutrients. Factor 3 is unipolar, marked by a general decrease in the concentrations of Cl, K, Cr, Fe, Rb, and Ba, potentially associated with seasonal depletion or reduced uptake during dormancy. Factor 4 again displays a bipolar configuration, with a positive correlation for Zn and a negative one for S, suggesting contrasting seasonal dynamics or competitive uptake mechanisms between these elements.

| | **Factor 1** | **Factor 2** | **Factor 3** | **Factor 4** |
|---|---|---|---|---|
| **S** | 0.1546 | **-0.6665** | -0.1779 | **-0.6467** |
| **Cl** | **-0.6941** | -0.3740 | **-0.4848** | -0.0955 |
| **K** | **-0.7298** | -0.2941 | **-0.5737** | -0.0806 |
| **Ca** | 0.4690 | **-0.8076** | 0.0826 | -0.1384 |
| **Cr** | **0.6974** | 0.1131 | **-0.5636** | 0.0732 |
| **Mn** | 0.2670 | **-0.8513** | 0.0150 | 0.2693 |
| **Fe** | 0.5308 | **0.4416** | **-0.6529** | 0.1664 |
| **Zn** | 0.1845 | **-0.6686** | 0.0314 | **0.5632** |
| **Rb** | **-0.6716** | 0.0309 | **-0.5617** | 0.2219 |
| **Ba** | **0.7946** | -0.1359 | **-0.4608** | -0.0667 |
| **Pb** | 0.3679 | **0.4723** | -0.1300 | -0.1993 |
| **Eigenvalue** | 3.35 | 2.94 | 1.89 | 0.97 |
| **Total variance %** | 30.45 | 26.77 | 17.21 | 8.81 |
| **Cumulative %** | 30.45 | 57.22 | 74.43 | 83.24 |

**Table 2.** The most significant factors based on PCA, with coefficients for each variable. The value represents the "strength" with which the given element contributes to the effect of the factor. For each factor, the values of the variables that contribute most to the effect of the factor (strong correlations) were selected in the range of minimum and maximum values (-0.8513 - -0.6467 and 0.4690 - 0.7946, respectively), and are given in bold.

Each factor captures a distinct dimension of elemental variability, offering a comprehensive perspective on their distribution across tissues and seasons.

Factor 1, defined by a positive association of Ba and Cr, and a negative association of Cl, K, and Rb, varied markedly in leaves across seasons. During summer, concentrations of Cl, K, and Rb increased, while Ba and Cr declined. In spring, autumn, and winter, the expression of this factor was less pronounced (Fig. 16). In contrast, Factor 1 did not show significant seasonal changes in sheaths (KW-H (3;108) = 5.6729; p = 0.1287), suggesting a tissue-specific expression of this component. Factor 2, characterised by a positive association of Fe and Pb and a negative association of S, Ca, Mn, and Zn, also showed significant seasonal variation in leaves. From winter to autumn, concentrations of Fe and Pb gradually increased, whereas S, Ca, Mn, and Zn declined, with autumn exhibiting the highest levels of Fe and Pb (Fig. 17). Factor 3, representing a unipolar decline in Cl, K, Cr, Fe, Rb, and Ba, demonstrated significant seasonal differences in both leaves and sheaths. The lowest values were recorded in winter and autumn, while spring and summer showed relatively higher concentrations of these elements (Fig. 18). Factor 4, defined by a positive correlation with Zn and a negative correlation with S, also varied significantly across seasons in both tissues.

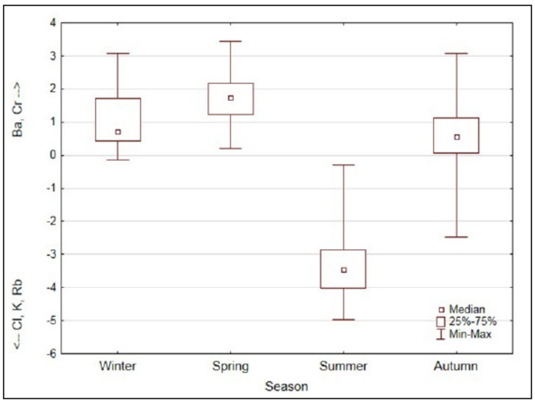

**Fig. 16.** The results indicate a pronounced effect of Factor 1 (KW-H (3;108) = 68.93; p < 0.0001) across seasons in the leaves of *J. trifidus*, suggesting statistically significant differences between seasonal groups.

In leaves, sulphur concentrations were lowest in winter, followed by an increase in spring, whereas zinc exhibited the opposite trend (Fig. 19).

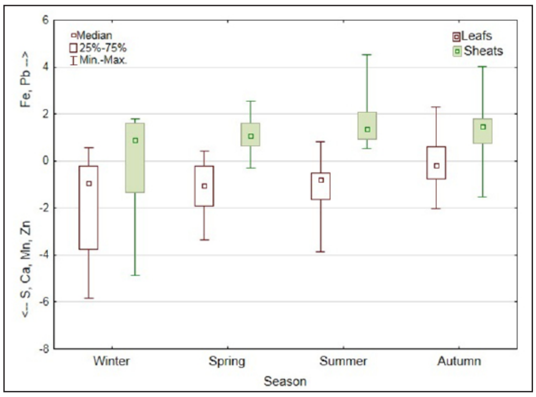

**Fig. 17.** Analysis revealed a significant seasonal variation in Factor 2 (KW-H (3;108) = 14.8297; p = 0.0020) in the leaves of *J. trifidus*, indicating notable differences between seasonal groups. A second comparison showed a weaker effect (KW-H (3;108) = 7.8819; p = 0.0485), suggesting only marginal statistical significance.

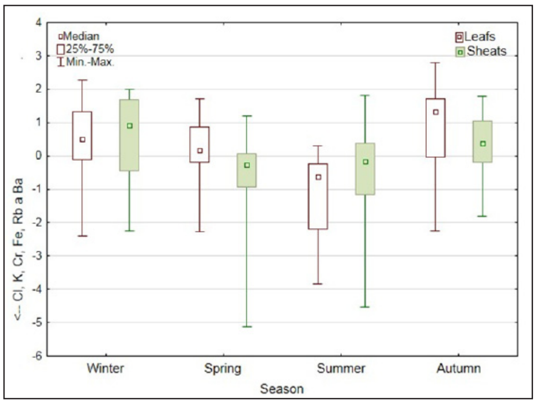

**Fig. 18.** Statistical analysis demonstrated a clear seasonal effect in Factor 3 (KW-H (3;108) = 35.38; p < 0.0001) in the leaves of *J. trifidus*, indicating significant differences between seasonal groups. A secondary comparison also revealed a statistically significant effect (KW-H (3;108) = 16.18; p = 0.0010), supporting the presence of seasonal variation.

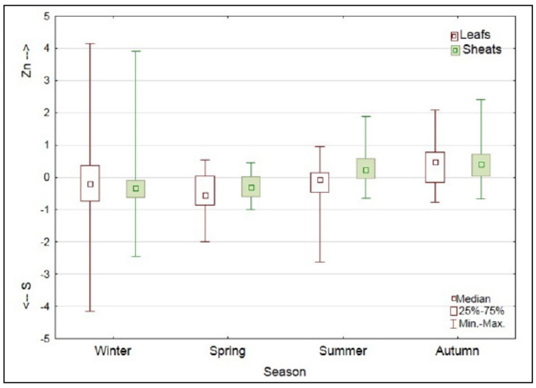

**Fig. 19.** Seasonal variation in Factor 4 was statistically confirmed (KW-H (3;108) = 19.94; p = 0.0002) in the leaves of *J. trifidus*, indicating significant differences between seasonal groups. A second comparison revealed an even stronger effect (KW-H (3;108) = 31.22; p < 0.0001), further supporting the presence of marked seasonal variation.

## Discussion

### Tissue-specific elemental dynamics and seasonality

Our findings confirmed that elemental concentrations differ significantly between leaf blades and basal sheaths across most seasons. Leaves, as photosynthetically active tissues, consistently showed higher levels of S, Ca, Mn, and Zn, reflecting their role in metabolism and growth.

Sulphur (S) was notably elevated in green tissues throughout the year. Absorbed as sulphate via roots, it is transported to leaves where it supports amino acid synthesis, chlorophyll production, and stress tolerance (Li *et al.* 2020; Jonsson *et al.* 2019). Its higher concentration in leaves compared to structural organs is a common pattern across species (McNaught and Chrisstoffels 1961), indicating its link to metabolic activity. Sulphur concentrations were consistently higher in green tissues. In both leaves and sheaths, peak levels occurred in winter and spring. This may result from reduced leaching and mineralisation during colder months (Kopriva *et al.* 2019), as well as atmospheric deposition mobilised by snowmelt in alpine environments (Li *et al.* 2017).

Calcium (Ca) is a key biogenic element in plants, essential for cell wall structure, mechanical stability, signal transduction, and enzyme activation (Lecourieux *et al.* 2006). It is most abundant in leaf tissues (Davey and Mitchell 1968), as confirmed by our results. Leaves contained significantly more calcium than storage organs, likely due to limited redistribution within the plant (Bangerth 1979). Leaf calcium content also correlates with transpiration rate and leaf lifespan (Funk and Amatangelo 2013), highlighting its role in physiological regulation.

Manganese (Mn) concentrations were consistently higher in leaves than in sheaths, a pattern also reported by Huang *et al.* (2024). Fernando *et al.* (2006) demonstrated that photosynthetic tissues are primary sites of manganese sequestration in several tree species, likely due to tissue-specific transport and accumulation mechanisms. These involve specialised transport proteins and reflect the metabolic demands of photosynthetic cells. Supporting this, McCain and Markley (1989) showed differential manganese accumulation in sun- and shade-exposed leaves, with chloroplasts identified as key storage sites.

Zinc (Zn) is an essential micronutrient involved in growth, protein synthesis, and enzyme activation. Although present in low concentrations, its deficiency can impair leaf development. Leaves, particularly epidermal cells, serve as key storage sites. Küpper *et al.* (1999) reported that epidermal vacuoles may contain Zn at levels 5–6.5 times higher than mesophyll cells, a pattern also observed by Tian *et al.* (2009), especially in hyperaccumulator species.

Krendželák (2017) reported similar patterns for Ca, Mn, and Zn in *Juncus trifidus*, with lower concentrations in green tissues during summer compared to autumn. Calcium is relatively immobile and accumulates in young tissues, while manganese and zinc are transported via xylem but poorly redistributed (Millaleo *et al.* 2010; Rasheed *et al.* 2024). As a result, these elements tend to accumulate in ageing leaves. Their elevated levels in colder seasons may also reflect physiological responses to low temperatures, such as increased calcium in mesophyll cells (Carpaneto *et al.* 2007).

Our results showed significantly higher potassium (K) concentrations in leaves during summer, while in other seasons, levels were higher in sheaths. These differences likely reflect physiological changes across growth stages and environmental conditions. Xue *et al.* (2016) observed that K distribution between leaves and sheaths varies with developmental phase, with early growth favouring upper leaves and later stages shifting accumulation to lower tissues. Similarly, Wang *et al.* (2013) reported that sheaths function as storage tissues, with mature leaves and sheaths becoming key potassium reservoirs, consistent with our findings.

Rubidium (Rb), a chemical analogue of potassium, showed significantly higher concentrations in leaves during summer, while in other seasons it was more abundant in sheaths. It behaves similarly to potassium and can be passively absorbed via the same transport pathways. Studies show that rubidium mimics potassium uptake and transport mechanisms (White and Broadley 2001) and may occasionally be taken up in place of $K^+$ due to its chemical similarity.

Both elements exhibited a consistent seasonal pattern with maximum concentrations in leaves during summer. This trend likely reflects increased metabolic activity and photosynthetic demand in the growing season.

Strontium (Sr) showed peak concentrations in sheaths during summer, while in other seasons it was more abundant in leaves. Its accumulation is linked to chemical similarity with calcium, despite lacking a known essential function. Seasonal differences between organs may reflect changes in ion uptake, cellular metabolism, and water transport. Storey and Leigh (2004) reported that strontium moves apoplastically from xylem into cells, with mesophyll cells accumulating more than sheath or epidermal tissues.

Chromium (Cr) and barium (Ba) showed similar seasonal trends, with significantly higher concentrations in leaves during winter and in sheaths during summer. These differences likely reflect shifts in physiological processes between growth and dormancy phases. Fan *et al.* (2019) demonstrated seasonal changes in chromium mobility in sediments, suggesting broader environmental influences. Such variation may be linked to changes in cellular metabolism, water transport, and tissue activity across seasons.

In both leaves and sheaths, the highest chromium concentrations were recorded in spring. In alpine environments, seasonal changes (particularly spring snowmelt) can mobilise atmospheric chromium deposits into the soil (Kuklová *et al.* 2016), potentially explaining elevated plant Cr levels during this period. Lower summer concentrations may result from reduced Cr mobility under dry conditions, as limited soil moisture restricts availability and uptake (Ertani *et al.* 2017).

Chlorine (Cl) concentrations were significantly higher in sheaths during spring and summer. This may be due to specialised tissue mechanisms that enable selective chloride accumulation and regulation. Studies on grasses by Boursier *et al.* (1987, 1989) show that leaf sheaths possess unique physiological adaptations for chloride storage. Large parenchyma cells function as reservoirs, while epidermal cells can accumulate high chloride levels, helping maintain low concentrations in photosynthetically active inner leaf tissues.

Iron (Fe) showed significantly higher concentrations in leaf sheaths during spring, summer, and autumn. This seasonal pattern likely reflects a combination of environmental factors and internal regulatory mechanisms. Zhang *et al.* (2012) found that Fe levels in leaves correlate with annual temperature and precipitation, which influence soil availability. Krohling *et al.* (2016) and Kobayashi *et al.* (2019) highlight that plants regulate Fe uptake and transport via specific chelators and transporters responsive to seasonal conditions. Sheaths may function as storage tissues, accumulating Fe during active growth phases.

Lead (Pb) concentrations were significantly higher in sheaths during summer and winter. This pattern aligns with findings by Krendželák (2017), who reported elevated Pb levels in roots compared to aerial parts, suggesting plant strategies to limit Pb translocation and protect photosynthetic tissues. Endodermal cells with Casparian strips function as barriers, preventing Pb entry into the vascular system (Gupta *et al.* 2024). Additionally, Pb binds to carboxyl groups in root cell walls, reducing mobility (Sharma and Dubey 2005).

Seasonally, peak Pb levels in spring may result from atmospheric deposition during winter, as snow captures airborne Pb and releases it into the soil upon thawing (Nowack *et al.* 2001). Increased nutrient demand in spring may also enhance Pb uptake alongside essential elements (Gupta *et al.* 2024).

### *Seasonal dynamics of element groups*

Factor 1 (increase in Ba and Cr with a simultaneous decrease in Cl, K, and Rb) was significant only in leaves across seasons. Descriptive analysis showed that K, Cl, and Rb concentrations in leaves peaked in summer and declined in other seasons. As Factor 1 is bipolar, reduced levels of these elements corresponded with increased Ba and Cr.

Potassium and chlorine are key to ionic balance and osmoregulation (Marschner 2012; Flowers 1988), while rubidium, a potassium analogue, can substitute for $K^+$ in cellular functions (Alda *et al.* 2015; Shtangeeva *et al.* 2021). Potassium supports turgor pressure, stomatal movement, and nutrient transport (Hawkesford *et al.* 2012; Rawat *et al.* 2022). Chloride contributes to photosynthesis and cell elongation (Kobayashi *et al.* 2006; Chen *et al.* 2016), with uptake linked to water flow (Moya *et al.* 2003). Excess $Cl^-$ is sequestered in vacuoles (Li *et al.* 2006) and tends to accumulate in older, basal leaves (Li *et al.* 2002).

Since Cl, K, and Rb share similar physiological roles, their sequestration into vacuoles and cell walls during leaf senescence may explain higher concentrations in winter. In contrast, Ba and Cr—elements with potential toxicity—tend to increase as metabolic activity declines. Barium, considered a contaminant, can disrupt plant growth depending on compound solubility (Kabata-Pendias 2010; Lu *et al.* 2019; Sleimi *et al.* 2021). Chromium, though a trace element, influences enzyme synthesis and may promote growth at low levels but becomes toxic at higher concentrations, particularly in its $Cr^{3+}$and $Cr^{6+}$ forms (Samantaray *et al.* 1998; Patra *et al.* 2024).

Elemental stress can alter the distribution of other nutrients (Baxter *et al.* 2012). Thus, increased Ba and Cr may suppress uptake of essential elements like Cl, K, and Rb. This bipolar pattern in Factor 1 likely reflects reduced demand for metabolic elements during dormancy, alongside increased accumulation of stress-related or toxic elements.

Factor 2, characterised by increased Fe and Pb and decreased S, Ca, Mn, and Zn, may reflect stress from the bioaccumulation of toxic elements, like Factor 1. Iron is essential for DNA synthesis, respiration, and photosynthesis, but excess levels can cause oxidative stress and disrupt nutrient uptake (Rout and Sahoo 2015; Hamzah *et al.* 2024; Lacoma 2019). Lead similarly impairs growth and nutrient transport, leading to deficiencies in Ca, Mg, and K (Mitra *et al.* 2019; Nas and Ali 2018).

Sulphur, calcium, manganese, and zinc are vital for enzyme activation, photosynthesis, and nutrient regulation, especially during active growth phases, as reflected in their seasonal peaks in spring and summer (Alejandro *et al.* 2020). Fe and Pb uptake are influenced by soil conditions and tends to accumulate in leaves over time, with autumn showing the highest concentrations due to seasonal accumulation and reduced redistribution (Nas and Ali 2018; Patra *et al.* 2024).

Factor 3 showed a consistent trend in both leaves and sheaths of *Juncus trifidus*, with simultaneous reductions in Cl, K, Cr, Fe, Rb, and Ba during summer. Although this group overlaps with Factor 1, Factor 3 reflects a unidirectional decline. As the factors are statistically independent, elemental accumulation may be influenced by both environmental conditions and the plant's physiological state.

Summer reductions in element content may result from elevated temperatures and intense sunlight accelerating metabolism and depleting nutrients (VanDerZanden 2008). Drought can further limit uptake due to reduced soil moisture (Yu *et al.* 2023). Seasonal shifts in microbial activity also affect nutrient cycling (Ibáñez *et al.* 2023). Rapid summer growth increases nutrient demand, potentially lowering concentrations of certain elements. During peak photosynthetic activity, plants may prioritise uptake of nitrogen and phosphorus, reducing levels of other nutrients (Uchida 2000).

Factor 4 reflects an inverse relationship between sulphur and zinc, with Zn increasing as S decreases, and vice versa. This trend was consistent across seasons in both leaves and sheaths, with the lowest S and highest Zn levels observed in autumn. Both elements are essential for plant metabolism and stress response, contributing to photosynthesis, enzyme activation, and protein synthesis (Fariduddin *et al.* 2022; Abou Seeda *et al.* 2020).

Sulphur uptake is highest in spring and summer, supporting active growth and metabolic activity (Kopriva *et al.* 2019). Its decline in autumn likely reflects reduced physiological demand. Zinc demand also rises during rapid growth, but environmental stressors such as drought and heat can limit its uptake and transport (Hafeez *et al.* 2013; Hamilton *et al.* 1993), contributing to seasonal fluctuations.

Our findings confirm that seasonal changes are a key factor shaping the elemental profile of *J. trifidus*. Understanding these temporal variations is essential for accurate interpretation in biomonitoring programmes, as fluctuations may reflect physiological shifts or biochemical responses. In the Low Tatras, where *J. trifidus* grows under extreme conditions (cold, nutrient-poor soils, high radiation), seasonal cycles, such as dormancy, snowmelt, and peak vegetation, are closely linked to the dynamics of element uptake and allocation.

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