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Comparison of seasonal accumulation by mountain stream algae *Hydrurus foetidus* and *Oscillatoria* sp.

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Abstract. In the process of biomonitoring, it is crucial to be aware of how species behave and how their metabolic needs influence element accumulation. In this study, we monitored two mountain stream species, *Hydrurus foetidus* and *Oscillatoria* sp. to determine their individual seasonal bio-accumulative abilities and their individual seasonal metabolic traits. Research was carried out at three sites in the foothills of the High Tatras between 2020 and 2023. Samples were collected at monthly intervals and subsequently processed with a fluorescence spectrophotometer. Seasonal changes were largely influenced by the metabolic needs of algae and cyanobacteria. For *Oscillatoria* sp., an increase in S and K was observed during summer, when they reach their metabolic maximum. The metabolic maximum of *H. foetidus* was determined to occur in autumn, based on increased concentration of Cl and Cr. Concentration of Ca and Sr present in *Oscillatoria* sp. exhibited a seasonally dependent pattern of accumulation of these elements during the colder months. The lowest concentrations were found during summer.

Key words: algae, cyanobacteria, accumulation of elements, mountain stream

Introduction

The value of algae as bio-monitors for fresh water has been recognized since the mid-19th century (Cohn 1853). Algae are most known as an indicator of organic pollution such as eutrophication (Palmer 1969), however many studies confirm that they can be used also as bio-accumulators of heavy metal pollution and act as their bio-indicator (Zeraatkar *et al.* 2016; Salama *et al.* 2019; Lin *et al.* 2020). The potential of algae for coping with various environmental crises makes them ideal organisms for biomonitoring water pollution, as well as species with the capacity for removal of pollutants in the process of bioremediation (Roy *et al.* 2022).

Algae have an increased ability to bind elements (Mehta and Gaur 2001). The algae species studied in this research differ in their morphology. *Hydrurus foetidus* of the class Chrysophyceae (golden algae), is a large (up to 10–30 cm), branched, freshwater algae (Wehr and Sheath 2003). Alginates are the main group involved in heavy metal accumulation in brown algae (Kuyucak and Volesky 1989). The negative charge of carboxylic groups plays an important role in binding cations. Polyguluronic and guluronic acids present in alginates (Haug and Smidsrød 1967) showed a high specificity for divalent metal ions such as Pb^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Ca^{2+}, … (Haug *et al.* 1961; Puranik *et al.* 1999). Seki and Suzuki (1998) showed that the biosorption of bivalent metal ions (Fe^{2+}, Ca^{2+}, S^{2+}) by brown algae was due to the presence of carboxylic acids on the alginate groups.

Sheaths of cyanobacteria consist of polysaccharides. Its anionic nature is caused by the presence of uronic acids and/or other charged groups (Mehta and Gaur 2001). Their cell walls provide mainly carboxylic groups for metal binding (Mehta and Gaur 2001; Chojnacka *et al.* 2005). Carboxyl groups serve as a dominant active site for metal ion accumulation. Lipopolysaccharides, lipids, and peptidoglycans are associated with phosphoryl groups. Amino groups are associated with membrane proteins and the peptide component of peptidoglycan (Chojnacka *et al.* 2005).

This research is focussed on differences in accumulation between golden algae *Hydrurus foetidus* and cyanobacteria of the genus *Oscillatoriales* in the mountain stream Javorinka. Selected species for monitoring differ by morphology, physiology, and their life cycle. As such, we predict that changes in element accumulation will be seasonally affected. The goal of the study is to determine how the two species differ in terms of element accumulation and their metabolic needs.

Material and Methods

Location of the research

Research to monitor the accumulation of elements in algae (*Hydrurus foetidus, Oscillatorialles* sp.) is a subsection of broader research undertaken by the Institute of High Mountain Biology of the University of Žilina (IHMB), focused on long-term changes in the levels of elements in the ecosystem of the mountain stream Javorinka.

The Javorinka stream springs at the Žabie Javorové tarn (Javorová dolina, High Tatras) in the northern part of the Eastern Tatras. It is a right-side tributary of the river Białka (Poland). The length of its flow is 19.3 km, and the watershed area is 66 km^2 (Bohuš 1996). Javorinka is a high-mountain stream with a snow-rainfall runoff regime. Most of the riverbed consists of clay, or clay-gravel sediments with boulder-like forms. Parent rocks in the Javorinka watershed area are primarily biotic granodiorites, dolomites, limestones, slates, and sandstones.

Research was conducted at three sites along the Javorinka stream, ranging from mountain sites to submountain sites in local villages. The upper-most site was the Control Locality. This sampling site is located at the mouth of the valley Javorová dolina and the valley Zadné Meďodoly (N 49.250419˚; E 20.155494˚; 1180 m a.s.l.). It is 1.5 km from the village of Tatranská Javorina (against the flow) and 2.5 km from the second location – the Upper stream. This sampling site is located between the villages of Tatranská Javorina and Podspády (N 49.271164˚; E 20.152952˚; 960 m a.s.l.). The third sampling site - the Lower Stream in the locality of Vojtasová (N 49.292550˚; E 20.169038˚; 850 m a.s.l.). The sampling localities are depicted in Fig. 1.

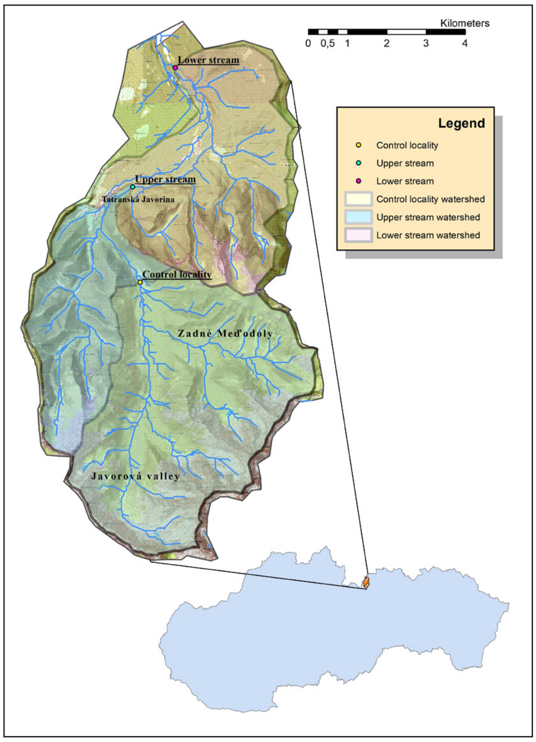

Fig. 1. Map of study area with 3 localities of collecting samples (Lower stream, Upper stream, Control locality). On the map is also shown watersheds corresponding to the exact localities.

Field sampling and sample preparation

Sampling was carried out at regular monthly intervals at all three sampling points from January 2022 to December 2023. Samples were collected within a maximum radius of 100 m downstream of the sampling site.

H. foetidus was collected by hand due to its macroscopic branched thallus. *Oscillatorialles* were scraped of the rocks using tweezers. Approximately 15 mg of each sample was collected. Samples were stored in plastic bottles with a small amount of water from the stream. In the laboratory, the water-deprived samples were placed on a petri dish and stripped of macroscopic contaminants (invertebrates, larger soil particles). The samples were allowed to dry at room temperature for at least 5 days.

Laboratory analyses

The dried samples were homogenized into a fine dust using a CryoMill (Retsch, Germany). It mills each sample for 40 seconds.

Analysis of metallic elements in algae was performed using X-ray fluorescence spectrometry. The samples were analyzed using an ED-XRF Spectrometer DELTA CLASSIC (Innov-X Systems, USA). Each sample was measured for 80 seconds in triplicate; the final value was the average of these measurements. The XRF Spectrometer was calibrated using the INCT-PVTL-6 Polish Virginia Tobacco Leaver standard. Measured were these elements: 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.

Statistical analysis

Only elements that were regularly measured above the detection limit were included in the statistical analysis. Statistica 8 (Data Analysis Software System; Statsoft, Inc.) was used for statistical analysis of the data. Normality of the data was tested by the Shapiro-Wilk test. Comparisons of individual items were made with the nonparametric Kruskal-Wallis H test (K-W H) because the variances between groups were highly heterogeneous. Values with $p < 0.05$ were considered statistically significant.

Results

While 26 metallic elements were spectrometrically monitored in the analysed algae and cyanobacteria samples, only 13 elements (S, Cl, K, Ca, Ti, Cr, Mn, Fe, Zn, Rb , Sr, Ba, Pb) were evaluated for statistical analyses (analysis of variance) comparing seasonal accumulation. These are elements regularly measured above the detection limit of the instrument during analysis. The values of other elements were measured irregularly.

In this analysis was observed seasonal variations of the two species individually to better understand metabolic needs of the study organisms. In *H. foetidus*, seasonal differences in Cl and Cr accumulation were significant ($p < 0.05$) (Table 1). Both elements reached their maximum in autumn during the highest biological activity of algae (Figs. 2-3). In *Oscillatoriales*, significant seasonal differences were found for 7 (S, K, Ca, Cr, Mn, Sr, Pb) of the 13 elements tested (Table 2). Both S and K showed maximum concentration in summer, during peak productivity of this genus (Figs. 4-5). Ca and Sr had the lowest mean concentration during summer and the highest during winter. However,

Table 1. Characteristic values of analyzed elements accumulated by *Hydrurus foetidus*. Values of elements with significant differences in the spring — winter seasons are highlighted in bold.

	Season	Mean ± SD (n)	Minimum	Maximum	KW - H	p
S	Spring	4828.56 ± 1711.09 (18)	2783	9663	3.27	0.3508
	Summer	5575.65 ± 2510.88 (17)	3159	12584		
	Autumn	5313.71 ± 1646.52 (14)	1205	8021		
	Winter	5451.67 ± 1360.9 (9)	4050	8003		
Cl	**Spring**	**344.28 ± 98.65 (18)**	**150**	**519**	**11.63**	**0.0087**
	Summer	**408.29 ± 152.12 (17)**	**182**	**874**		
	Autumn	**431.5 ± 225.65 (14)**	**150**	**1079**		
	Winter	**445.89 ± 129.49 (9)**	**150**	**579**		
K	Spring	5075.78 ± 2198.2 (18)	1859	8364	1.15	0.7631
	Summer	5460.76 ± 2593.61 (17)	1528	9004		
	Autumn	6529.02 ± 3431.88 (14)	2842	14412		
	Winter	6031.56 ± 2282.03 (9)	3798	10733		
Ca	Spring	21919.39 ± 19136.4 (18)	3116	64629	0.74	0.8631
	Summer	23072.71 ± 28684.6 (17)	3627	107320		
	Autumn	23603.01 ± 21964.5 (14)	3200	73372		
	Winter	33519.11 ± 45429.2 (9)	5841	150254		
Ti	Spring	557.56 ± 744.7 (18)	15	2325	2.79	0.4235
	Summer	640.71 ± 681.41 (17)	15	1993		
	Autumn	922.14 ± 1033.41 (14)	15	3346		
	Winter	578.44 ± 731.01 (9)	48	2207		
Cr	**Spring**	**40.39 ± 24.98 (18)**	**13**	**95**	**17.75**	**0.0005**
	Summer	**71.41 ± 58.26 (17)**	**14**	**187**		
	Autumn	**157.14 ± 128.58 (14)**	**49**	**445**		
	Winter	**82.89 ± 37.96 (9)**	**41**	**167**		
Mn	Spring	114.16 ± 78.97 (18)	34.9	277	4.59	0.2037
	Summer	139.65 ± 85.71 (17)	48	369		
	Autumn	183.57 ± 95.56 (14)	69	344		
	Winter	149.89 ± 112.37 (9)	60	362		
Fe	Spring	5335.61 ± 5717.2 (18)	342	16499	1.89	0.5954
	Summer	6274.29 ± 5524.72 (17)	802	16099		
	Autumn	8919.93 ± 8685.21 (14)	820	31326		
	Winter	5702.67 ± 5610.1 (9)	1164	15331		
Zn	Spring	91.78 ± 39.99 (18)	26	157	2.75	0.4311
	Summer	99.29 ± 52.21 (17)	23	181		
	Autumn	106.57 ± 56.11 (14)	31	188		
	Winter	108.78 ± 63.76 (9)	23	226		
Rb	Spring	12.37 ± 8.11 (18)	5.4	29.8	2.51	0.4722
	Summer	13.01 ± 6.63 (17)	5.8	26.3		
	Autumn	16.86 ± 11.7 (14)	6.7	43.2		
	Winter	13.13 ± 7.92 (9)	7.4	30.7		
Sr	Spring	51.85 ± 44.74 (18)	10	140	0.81	0.8452
	Summer	50.61 ± 45.03 (17)	11.2	146		
	Autumn	65.32 ± 58.28 (14)	11.9	188		
	Winter	68.37 ± 70.43 (9)	13.7	222		
Ba	Spring	159.44 ± 129.08 (18)	45	443	3.31	0.3456
	Summer	188.24 ± 117.81 (17)	57	423		
	Autumn	274.5 ± 213.17 (14)	66	822		
	Winter	190.78 ± 122.39 (9)	86	447		

Pb	Spring	15.94 ± 4.11 (18)	11	25	2.38	0.4957
	Summer	17.29 ± 3.95 (17)	12	24		
	Autumn	19.43 ± 8.73 (14)	12	46		
	Winter	16.22 ± 3.11 (9)	11	22		

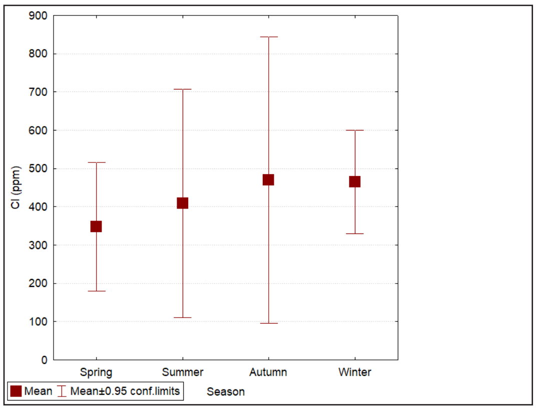

Fig. 2. Mean of case coordinates (±95 c. limits) of chlorine concentrations accumulated by *Hydrurus foetidus* during seasons.

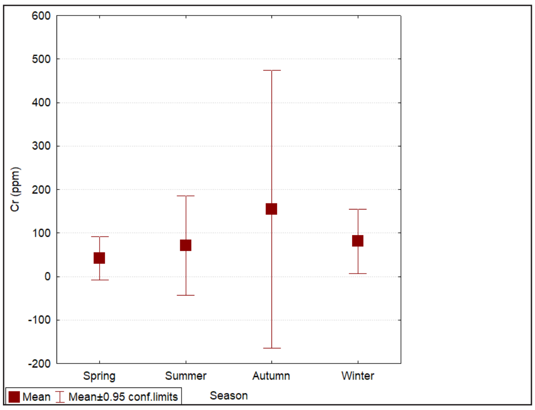

Fig. 3. Mean of case coordinates (±95 c. limits) of chromium concentrations accumulated by *Hydrurus foetidus* during seasons.

Table 2. Characteristic values of analyzed elements accumulated by *Oscillatoria* sp. Values of elements with significant differences in the spring — winter seasons are highlighted in bold.

	Season	Mean ± SD (n)	Minimum	Maximum	KW - H	p
S	**Spring**	**4921 ± 1648.56 (15)**	**2810**	**8373**	**14.51**	**0.0023**
	Summer	**7733 ± 4193.48 (18)**	**2554**	**18812**		
	Autumn	**4832 ± 3430.03 (13)**	**1133**	**12420**		
	Winter	**4872 ± 2148.35 (9)**	**2083**	**7854**		
Cl	Spring	343 ± 77.89 (14)	226	498	6.08	0.1166
	Summer	795 ± 1180.75 (17)	241	5297		
	Autumn	432 ± 209.83 (13)	175	951		
	Winter	392 ± 130.49 (9)	222	620		
K	**Spring**	**11852 ± 3373.94 (15)**	**7319**	**18314**	**11.45**	**0.0095**
	Summer	**15250 ± 6366.01 (18)**	**9077**	**31970**		
	Autumn	**14077 ± 6471.48 (13)**	**9168**	**33415**		
	Winter	**9742 ± 1268.43 (9)**	**8211**	**12192**		
Ca	**Spring**	**72771 ± 61373.1 (15)**	**10799**	**169812**	**10.06**	**0.0181**
	Summer	**28633 ± 20976.5 (18)**	**8215**	**82027**		
	Autumn	**39440 ± 21316 (13)**	**11246**	**71409**		
	Winter	**68408 ± 20141.5 (9)**	**28685**	**90898**		
Ti	Spring	1201.8 ± 966.77 (15)	238	3269	4.25	0.2348
	Summer	1040.39 ± 487.29 (18)	395	2165		
	Autumn	1797.77 ± 1633.31 (13)	593	6986		
	Winter	1003.11 ± 407.65 (9)	465	1566		
Cr	**Spring**	**82.33 ± 84.37 (15)**	**9**	**272**	**9.91**	**0.0193**
	Summer	**112.39 ± 94.56 (18)**	**18**	**337**		
	Autumn	**249.08 ± 271.7 (13)**	**81**	**1050**		
	Winter	**92.56 ± 34.22 (9)**	**61**	**166**		
Mn	**Spring**	**177.27 ± 115.7 (15)**	**52**	**406**	**8.12**	**0.0468**
	Summer	**191.28 ± 56.57 (18)**	**103**	**264**		
	Autumn	**252.38 ± 76.73 (13)**	**156**	**473**		
	Winter	**172.78 ± 49.37 (9)**	**105**	**264**		

Fe	Spring	12133.27 ± 10412.3 (15)	2219	33603	3.53	0.3164
	Summer	10467.72 ± 4685.39 (18)	4235	21454		
	Autumn	14384.85 ± 8257.67 (13)	7104	39947		
	Winter	8999.56 ± 3041.8 (9)	5107	15226		
Zn	Spring	45.53 ± 21.58 (15)	19	81	3.94	0.2677
	Summer	53 ± 14.38 (18)	31	82		
	Autumn	48.38 ± 15.18 (13)	31	84		
	Winter	41.56 ± 12.63 (9)	30	67		
Rb	Spring	20.49 ± 11.91 (15)	8.4	44.1	6.11	0.1059
	Summer	18.21 ± 6.09 (18)	11.8	35		
	Autumn	23.73 ± 7.16 (13)	13.2	32.7		
	Winter	18.54 ± 4.77 (9)	12.7	24.7		
Sr	**Spring**	**116.82 ± 68.58 (15)**	**23.7**	**230**	**9.25**	**0.0261**
	Summer	**69.42 ±59.87 (18)**	**17.1**	**190**		
	Autumn	**115.28 ± 75.67 (13)**	**20**	**245**		
	Winter	**136.56 ± 47.44 (9)**	**54**	**194**		
Ba	Spring	302.33 ± 184.77 (15)	100	668	5.11	0.1639
	Summer	289.11 ± 103.3 (18)	139	455		
	Autumn	455.08 ± 338.45 (13)	210	1549		
	Winter	260.56 ± 70 (9)	146	380		
Pb	**Spring**	**18.8 ± 7.2 (15)**	**9**	**33**	**9.33**	**0.0252**
	Summer	**20.83 ± 4.62 (18)**	**14**	**33**		
	Autumn	**23.85 ± 3.11 (13)**	**18**	**31**		
	Winter	**19 ± 3.87 (9)**	**13**	**26**		

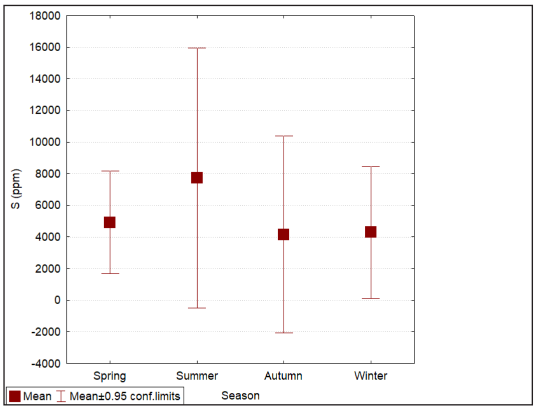

Fig. 4. Mean of case coordinates (±95 c. limits) of sulfur concentrations accumulated by *Oscillatoria* sp. during seasons.

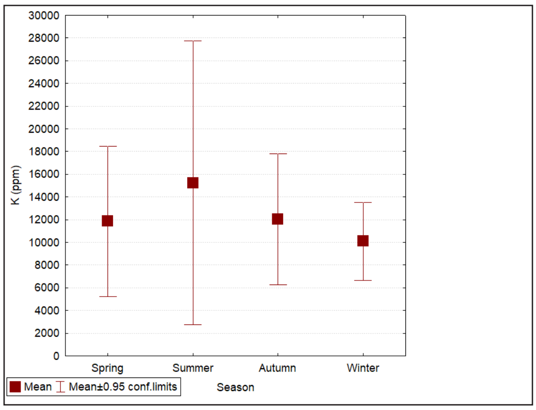

Fig. 5. Mean of case coordinates (±95 c. limits) of potassium concentrations accumulated by *Oscillatoria* sp. during seasons.

total maximum was reached in autumn (Figs. 6-7). Mn and Pb had the greatest variance during spring snowmelt season. Maximum concentration of Mn and Pb was reached during autumn (Figs. 8-9). Cr accumulated in great variance from spring until autumn, and similarly to *H. foetidus* it reached its peak during autumn (Fig. 10).

Discussion

Chlorine in Hydrurus foetidus

It was observed that many organisms including algae (Gribble 1994; Nzengung *et al.* 1999; La Barre *et al.* 2010) produce chlorinated compounds as secondary metabolites. Nzengung *et al.* (1999) and Küpper *et al.* (2008) proposed that the transformation of halogenated compounds is processed through sequestration, phytoreduction, partial exudation of the metabolites, phytooxidation of the sequestered phytoreduction products, and assimilation.

H. foetidus experiences its highest biological activity from autumn until late winter (Kann 1978; Klaveness 2019). During this time period, the highest mean Cl concentrations (431.5 ppm in autumn and 445.81 ppm in winter) were also observed. During this period, the highest iodine content as a product of halogenation in the brown algae *Laminaria digitata* was also observed. It can be assume that

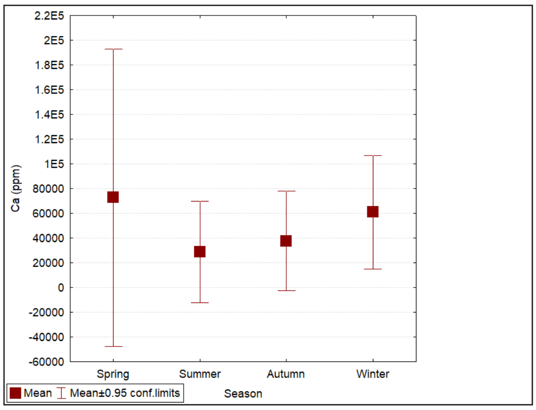

Fig. 6. Mean of case coordinates (±95 c. limits) of calcium concentrations accumulated by *Oscillatoria* sp. during seasons.

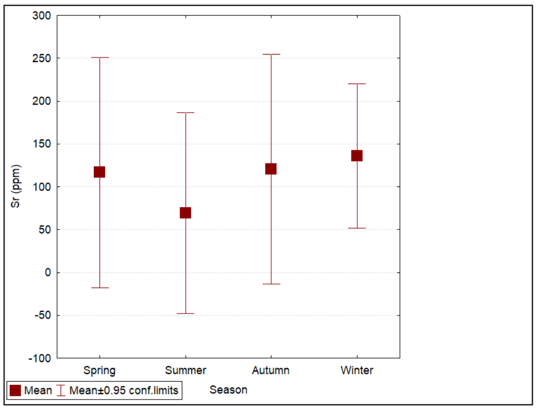

Fig. 7. Mean of case coordinates (±95 c. limits) of strontium concentrations accumulated by *Oscillatoria* sp. during seasons.

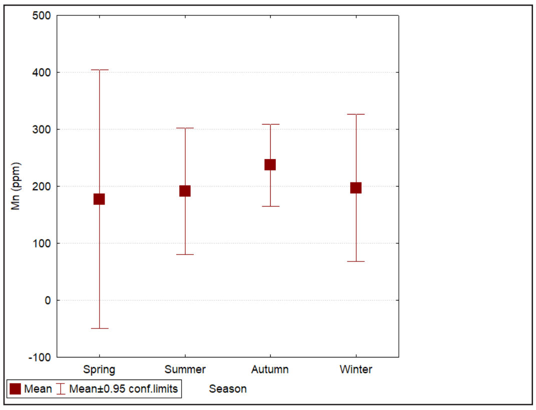

Fig. 8. Mean of case coordinates (±95 c. limits) of manganese concentrations accumulated by *Oscillatoria* sp. during seasons.

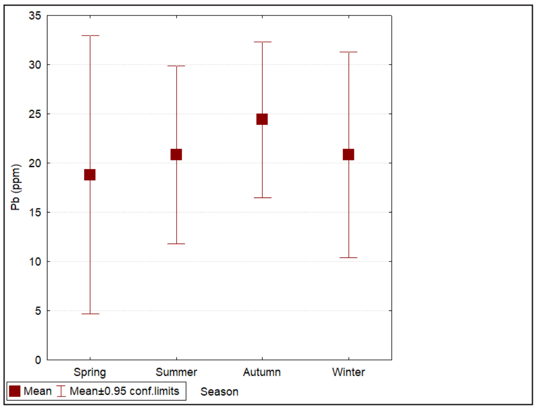

Fig. 9. Mean of case coordinates (±95 c. limits) of lead concentrations accumulated by *Oscillatoria* sp. during seasons.

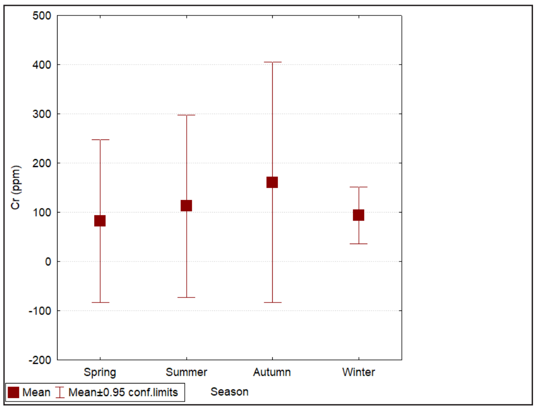

Fig. 10. Mean of case coordinates (±95 c. limits) of chromium concentrations accumulated by *Oscillatoria* sp. during seasons.

the increase of Cl content in *H. foetidus* throughout these seasons is caused by its high metabolic activity, as it produces chlorinated compounds during photosynthesis.

Chromium in Hydrurus foetidus

Great increase of Cr during the autumn will be most probably caused by increased metabolic activity of the algae. Prado *et al.* (2010) described that highest concentrations of Cr during the summer observed in aquatic fern were caused by increased activity of the plant and its active uptake of the elements. Miranda *et al.* (2012) observed increased accumulation of Cr by living algae than dead. This also suggests that chromium was actively accumulated during the growth of algae.

Sources of Cr can be partially attributed to granodiorite, which contains a small amount of it (Izbicki *et al.* 2008). Other sources of Cr in the area include burning of coal in south Poland which emits chromium particles into the atmosphere (Cheng *et al.* 2014; Molik *et al.* 2018.) Seasonal changes observed in *H. foetidus* are primarily a result of the metabolic needs of the algae, though this is likely not the singular factor.

Sulphur and potassium in Oscillatoria sp.

Both sulfur and potassium are elements indicating higher biological activity in algae (Subhashini and Kaushik 1986; Ometto 2018; Rashel and Patiño 2019; Ptak *et al.* 2021). Sulfur is one of the main components in algal cells (Giordano 2013) and one of the most important macronutrients for photosynthetic organisms (Giordano and Prioretti 2016). Its main source for algae is SO_4^{2-}, which is the most common form of inorganic S in the environment (Giordano and Raven 2014).

Algae show greater dependence on S assimilation from photosynthesis compared to vascular plants (Schmidt 1979). Most S assimilation processes take place in chloroplasts (Brunold and Schiff 1976) where it is crucial for assimilation of cysteine, methionine, and production of dimethylsulphoniopropionate (DMSP) (Giordano and Raven 2014). A study by Rashel and Patiño (2019) found that the algae *Prymnesium parvum* increased its growth rate when exposed to $MgSO_4$. Though Mg^{2+} enhanced growth performance, SO_4^{2-} stimulated growth of golden alga in a concentration-dependent manner and independently of salinity.

Likens *et al.* (1967) described increased uptake of K during the vegetation period of algae, while Subhashini and Kaushik (1986) found that with increasing concentration of K, protein synthesis and overall biomass of cyanobacteria rose to 0.13 mg K/mL. Photosynthesis is also a potassium dependent process. When algae is deprived of potassium, the volume of produced O_2 drops drastically, and even with the subsequent addition of potassium, only slight recovery was observed (Clendenning *et al.* 1956; Jackson and Volk 1968).

Exchangeable K varies from < 100 ppm to several thousand ppm. This form of soluble K, available to many organisms, usually constitutes a few ppm of the dry weight of the soil that leaches into the water (Pratt 1965). Its natural sources in this area are mostly derived from the crystalline core of the Tatra Mountains. Primary rocks with high potassium concentrations include quartz, feldspar, and mica (Żelazny *et al.* 2011).

Maximum mean concentrations of both elements were measured during the peak biological activity of *Oscillatoria* sp., during summer. Based on these observations, it can be assume that sulfur and potassium can be a suitable bioindicator of high biological activity and photosynthesis in these species. Seasonal changes in concentrations of these elements are primarily caused by their metabolic needs and not through anthropogenic impact or geochemical processes.

Calcium and strontium in Oscillatoria sp.

In calcium and strontium, we observed similar behavior. Both decrease during summer and increase throughout autumn and winter. Calcium also experiences large variance in the spring. Snowmelt has been shown to alter concentrations of stream elements in a significant way. Ca^{2+} can be stored in snow from mineral dust and is consequently released due to high mobility (Bohleber *et al.* 2018; Avak *et al.* 2019). Other studies have confirmed calcium increases in streams during the spring snowmelt (Stottlemyer and Troendle 1992; Campbell *et al.* 1995; Moon *et al.* 2014).

However, in recent years, due to increased physical erosion caused by acid rain, weathering of apatite was observed as a rising source of Ca^{2+} in the Tatra Mountains (Kopáček *et al.* 2019), and could be washed downstream during snowmelt (Yu *et al.* 2019). Sr and Ca tend to bioaccumulate in strong correlation (Dabbagh *et al.* 2007; Segovia-Campos *et al.* 2023). This correlation was also observed in our research. Strontium does not follow the same pattern during spring, which is indicative of another, more abundant calcium source during this season. This favors the hypothesis of increased apatite dissolution.

Decreases in Sr and Ca concentrations during summer and increases in colder months can be explained by algal symbionts and their response to sea surface temperatures (SSTs). Sr/Ca ratios in reef coral skeletons are used as a paleothermometer to estimate SSTs. Cohen *et al.* (2002) found that algal symbionts influence Sr/Ca content. Corals with algal symbionts also had inverse correlation with the SSTs but with greater variance during the summer, when the concentration of Sr and Ca are influenced photosynthesis. Wei *et al.* (2004) discussed that though algal symbionts are more sensitive to SSTs compared to coral, Sr/Ca ratios can yield more precise information about SSTs. It can be suggest that blue-green algae of the order *Oscillatoriales* follows the same pattern. Based on the correlation coefficients (r = -0.15 – -0.18 (Sr); -0.24 – -0.37 (Ca)) developed for US and LS we predict that accumulation of these elements is inversely influenced by the water temperature.

The metabolism of *Oscillatoria* sp. also plays a role also in the accumulation of Sr and Ca as they are vital elements for cell growth and the growth of cell structures (Ray and Bagchi 2002; Dabbagh *et al.* 2007; Shi *et al.* 2013; Sharma 2019; Chen *et al.* 2021; Segovia-Campos *et al.* 2023).

Based on these observations, it can be concluded that the majority of calcium accumulated during spring is released from weathered apatite and mineral dust trapped in snow. Decreases in mean concentration in summer and further increases in autumn and winter is likely driven by sensitivity of the Sr/Ca ratio to changes in water temperature with inverse correlation.

Manganese and lead in Oscillatoria sp.

Similar results to calcium were observed in manganese and lead. They had the greatest variance (Mn – 115.7 ppm, Pb – 7.2 ppm) during the spring. Changes in discharge and influx from melting snow varied concentrations of these elements. Manganese and lead occur also naturally in the mountains. Manganese is present in the Tatra Mountains in the form of rhodochrosite, whereas manganese oxides (manganite, pyrolusite) (Pawlikowski and Wróbel 2013). During physical weathering it can form MnO_2 (Ostwald 1988). From these compounds, it can enter streams in the form of manganese cations, which are available to algae (Haug *et al.* 1961; Puranik *et al.* 1999; Das *et al.* 2015). Lead is a trace element naturally occurring in granodiorites, limestones and their soils (Erel *et al.* 1991; Alloway 2012). Its concentrations are regulated by physical weathering (Erel *et al.* 1991; Romanescu *et al.* 2016).

Though snow movement, including avalanches and snowmelt promote bedrock and soil weathering (August *et al.* 2002; Fortner *et al.* 2009) and consequently the release of Mn^{2+} and Pb^{2+}, we believe, based on previous studies, that the source of these is not only a result of natural sources. Both Mn and Pb are produced during burning of fossil fuels, which can travel long distances (Joselow *et al.* 1978; Janiga 2001; Parmalee and Aschner 2016).

Kopáček *et al.* (2006) and Kwapuliński *et al.* (2012) proved that the source of lead found in Tatra mountain soils was long-range vehicle, industrial, and residential fume emissions. Manganese is also produced by burning gasoline (Joselow *et al.* 1978; Miah *et al.* 2020) and was found by Joselow *et al.* (1978) to be in strong correlation with Pb concentrations.

Large variance of these elements in the spring is likely due to high accumulation of Mn and Pb pollution during late autumn and winter. In the process of snow melting, they are released from the snow and the top layers of soil into the stream as bivalent cations.

Chromium in Oscillatoria sp.

We observed an increase of Cr in *Oscillatoria* sp. between spring and autumn, where it had exhibited the greatest mean and overall concentrations. During winter, its mean concentration decreased. Different seasonality of Cr uptake by the two species was caused by their differing peaks of biological activity, which greatly contributed to uptake (Prado *et al.* 2010). *Oscillatoria* species were observed by Miranda *et al.* (2012) to accumulate Cr in both its most stable oxidative states, Cr^{3+} and Cr^{6+}.

Increase of Cr during autumn, when *Oscillatoria* do not experience high metabolic activity is likely due to external factors. This could be caused by emissions from coal burning, which are atmospherically transported and deposited in the Tatra Mountains (Cheng *et al.* 2014; Molik *et al.* 2018). This pollution can be later washed away into the stream.

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