

Seasonal and areal accumulation of heavy metals by algae and cyanobacteria in Javorinka mountain stream

J. TUCHYŇA

*Institute of High Mountain Biology, Žilina University,
Tatranská Javorina 7, SK-059 56, Slovak Republic;
e-mail: tuchyna168@gmail.com*

Abstract. In this study we have compared concentrations of accumulated elements in algae and cyanobacteria from different localities over several years and throughout the seasons. We collected chrysophyceae *Hydrurus foetidus* and cyanobacteria *Oscillatoria* sp. in Javorinka mountain stream over three years (2019 - 2021). A comparison of the concentrations collected from two localities showed that Ba, Fe, Mn and Ti are being accumulated at almost double the rate at the site with lower stream velocity. Depending on the year, we have observed a significant increase in Ca concentrations. We also examined the trend of intense uptake of these elements by algae and cyanobacteria during the summer season, due to the growth of reproductive organs during this season. Analysis was performed using the Kruskal-Wallis test ($p < 0.05$). It has been confirmed that algae and cyanobacteria can be used as bioindicators of spatial and seasonal pollution and element concentration variations.

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

Introduction

The biological value of algae has been known since the mid-19th century. Its greatest importance is as a bio-indicator. Russian scientists have studied the saprobity of algae; the physiological and biochemical characteristics which allow it to live in an environment that contains organic matter, with a certain degree of pollution. Their study was focused on organic pollution in stream and river environments. This system was altered, modified, and expanded over the years by several authors. Because saprobity is defined by the intensity of heterotrophic activity, all photoautotrophic species were finally excluded from the saprobic system when the inorganic nutrient load in rivers became increasingly problematic. During the process of studying saprobity in running water, the trophic classification system was developed for lakes. It is

based on inorganic nutrients which are gained from catchments. In both cases, there is a belief that the presence, absence, or abundance of certain species can reflect the current biochemical, physical, and general state of the environment in which these organisms were located. Such organisms are referred to as bio-indicators. Their specificity is the low ecological valence for living conditions and their specific stress symptoms, which respond to changes in the environment. Thanks to these manifestations of an organism, indicators are not only their presence, absence, but also biomass or growth of algae. Such species are a very reliable indicator of natural pollution. The indicator species then becomes a 'bio-sensor'. During its lifespan, organic and inorganic elements from the environment accumulate, and reflect the level of substances in the environment and exposure to organisms. Such species are labelled as 'bio-accumulators' which can be particularly useful when concentrating very low levels of a substance (Dokulil 2003).

Freshwater algae provide two main types of information about water quality:

- Long-term information; the *status quo*. In the case of a temperate lake, for example, detection of an intense summer bloom of the colonial blue-green algae *Microcystis* is indicative of a pre-existing high-nutrient (eutrophic) status.
- Short-term information; environmental change. In another lake environment, detection of a change in subsequent years from low to high blue-green dominance (with increased algal biomass) may indicate a change in eutrophic status. This may be an adverse transition (possibly caused by human activity) that requires changes in management practice and lake restoration.

In the context of change, bioindicators can thus serve as early-warning markers of the health status of an aquatic system (Bellinger and Sigeo 2015).

Lethal concentration of zinc in algae have been determined to be 30 µg/L. Productivity of several studied algae (green algae (*Ankistrodesmus* and *Scenedesmus*), one diatom (*Navicula*), and natural phytoplankton) at this concentration was reduced by 59, 55, 49, and 52 per cent respectively. At 17 µg/L there was a 37 % reduction in primary production, and 3 µg/L of zinc, there was an 80 % reduction in nitrogen fixation. Zinc is often found in labile or in free ionic form even though Cu, Cd and Pb are in strongly bound compounds. More than 80 % of zinc in water is in free (labile) form (Wong and Chau 1990). Based on the algae species, toxic levels of chromium can range between 20 µg/L to 10,000

$\mu\text{g/L}$. It should be considered that these results are also a function of several additional conditions such as pH, temperature and salinity. The most common symptoms of toxicity were inhibition of growth and photosynthesis inhibition. Toxic concentrations for *Cladophora glomerata* were $250 \mu\text{g/L}$, *Thalassiosira pseudonana* $< 20 \mu\text{g/L}$, and *Skeletonema costatum* at $980 \mu\text{g/L}$ (Nriagu and Nieboer 1988). When the chromium concentration changed it caused a shift in community dominance. When algae was exposed to wastewater with a level of chromium between 1 - 40 mg/L only *Oscillatoria* sp. were present (Nriagu and Nieboer 1988).

The metal sorption ability of algae varies greatly from species to species and even among strains of a single species for any given metal. Each type of heavy metal also uniquely impacts the sorption abilities of different species of algae (Mehta and Gaur 2001).

By studying different species (*Codium vermilara*, *Spirogyra insignis*, *Asparagopsis armata*, *Chondrus crispus*, *Ascophyllum nodosum* and *Fucus spiralis*) from three main algae groups; green algae (*Chlorophyta*), red algae (*Rhodophyta*), and brown algae (*Chromophyta*), we developed a sequence of metal uptake decrease, as follows: $\text{Pb} > \text{Cu} \geq \text{Cd} > \text{Ni} > \text{Zn}$. This sequence has held true for all studied species. Corroborating to this claim, we hypothesize that the binding of metal to active sites of the cell wall is related to their inner metal properties, such as ionic radii and electronegativity of atoms (Romera *et al.* 2006).

On the other hand, brown algae achieved significantly better results with higher uptake capacity. At worst, brown algae reacted two times better than other species, as likely due to alginic groups, brown algae anchors the metal to its biomass. The sequence obtained as a function of the type of algae was: brown $>$ red $>$ green. In conclusion, besides the type of algae, or the physical and chemical properties of the environment, metal uptake is largely dependent on the type of metal to which the algae is exposed (Romera *et al.* 2006).

The cell wall of green algae likely contains two adjacent carboxylic groups to share the bond with one metal cation. Therefore, it shows lower levels of metal recovery. The reason why some biomasses show high affinity for a given metal and low sorption capacity, or vice versa, may be related to the degree of affinity of a specific biomass for each metal (Romera *et al.* 2006).

Many individuals of the *Chlorella* species have excellent sorption capacity, in some cases up to $714 \text{ mg}\cdot\text{g}^{-1}$ when exposed to Cu^{2+} . Generally, its capacity is around $100 \text{ mg}\cdot\text{g}^{-1}$ (Zeraatkar *et al.* 2016). In a study of *Chlorella vulgaris* that contained concentrations of 2.5 ppm, samples were able to remove between 69 % and 80 % of Ni (II) and Cu (II) cations. When the concentration was increased to 10 ppm, the metal removal rate was reduced to 37 % and 42 % (Mehta and Gaur 2001). *Sargassum* sp., as saltwater algae, has a maximum sorption of $72,5 \text{ mg}\cdot\text{g}^{-1}$. *Sargassum* sp. absorb more heavy metal elements and their compounds. This is due to a high alginic content, along with other brown algae. For example, the alginic content of *Sargassum fluitans* is 45 % of its dry weight (Mehta and Gaur 2001).

In general, Chlorophyta, commonly known as green algae, has significant accumulating abili-

ties, especially the *Cladophora* species; one of the best bio-indicators in water environments for nutrient and heavy metal pollution. They generate the majority of algal biomass in some water bodies (Chmielewska and Medved 2001). Using *Cladophora* completely removed arsenic from drinking water (Salama *et al.* 2019).

Using the cyanobacteria *Gloeotheca* sp., we observed its high susceptibility to Cu^{2+} . Metal concentrations of $1.0 \text{ mg}\cdot\text{L}^{-1}$ led to cell death. Whereas when using Pb^{2+} , similar effects were achieved using a concentration of 40 or $50 \text{ mg}\cdot\text{L}^{-1}$. A bi-metal system, where cyanobacteria were exposed to both Cu^{2+} and Pb^{2+} , caused decrease in removal of each metal. Cu^{2+} removal decreased by 47.2 % and Pb^{2+} removal decreased by 13.8 % (Pereira *et al.* 2011). In cyanobacteria, copper is a micronutrient, functioning as a cofactor and required for structural and catalytic properties of enzymes. It is also a cofactor of the electron transport protein plastocyanin and other proteins associated with thylakoids, which imposes an extra complexity to copper homeostasis in these organisms compared with non-photosynthetic prokaryotes. However, exposure to excessive amounts of copper ions, may lead to cell destruction, similarly to all photosynthetic organisms (Pereira *et al.* 2011).

Accumulation of metals is due to adsorption onto the cell surface (wall, membrane, or external polysaccharides) and binding to cytoplasmic ligands, phytochelatins and metallothioneins, as well as other intracellular molecules. Through electron microscopy and X-ray energy dispersive analysis, studies have been carried out on the localization of heavy metal ions. On the algal cell wall there are many functional groups, such as, hydroxyl (OH), phosphoryl (PO_3O_2), amino (NH_2), carboxyl (COOH), sulphhydryl (SH), etc., which confer a negative charge to the cell surface. Metal ions are absorbed, because generally they exist in water in cationic form (Mehta and Gaur 2001).

The distribution of functional groups varies among algae, therefore, biosorption is different. Metal sorption depends on these groups and if they are present in a given cell. Their presence is dependent on cell wall components, (e.g., peptidoglycan, teichouronic acid, teichoic acids, polysaccharides, and proteins). Conversely, cyanobacteria are dependent on typical carboxylic groups which exist on peptidoglycan, consisting of linear chains of N-acetylglucosamine and β 1, 4-N-acetylmuramic acid with peptide chains. Lipopolysaccharides, lipids, and membrane proteins are also important for sorption. Some studies prove that the carboxyl groups are the primary location for metal binding in cyanobacteria (blue-green algae). Most of the species of this group has a capsule, which tends to have an anionic nature, thus giving them a very high affinity towards metal ions. This could result in significant removal of heavy metals in waste waters (Mehta and Gaur 2001).

In green algae contain significant carboxyl and sulphate groups. The percentage of protein content in cell walls on which these group are attached varies from 10 - 70 %. As previously mentioned, in brown algae the main mean of metal biosorption are alginic groups. Alginate is commonly found in cell wall membranes, where through the sorption is metal binding site (Mehta and Gaur 2001).

Participation of carboxyl groups in adsorption of heavy metal such as Cu, Cd and Pb has been demonstrated on cyanobacterial cell walls, as well as in green and brown algae (*Macrocystis pyrifera*, *Kjellmaniella crassifolia* and *Undaria pinnatifida*). A study of carboxyl groups presented great success in binding Au and bivalent metal ions. Research has even shown binding of Cd and Pb ions, to small binding on sulphate groups. Good sorption of heavy metals by carboxyl groups has been very well demonstrated, even by fungi and higher plants (Mehta and Gaur 2001).

Other groups exhibit a lesser extent of binding compared to carboxyl group. Non-living sargassum has demonstrated binding of Co on carbonyl groups. The amino group plays an important role in the binding of Au in *Chlorella* (Mehta and Gaur 2001).

The above discussion leads to the generalization that carboxyl groups of cell wall polysaccharides play a predominant role in heavy metal sorption by algae and cyanobacteria. The other functional groups, such as sulfonate and amino, play a relatively minor role in metal sorption (Mehta and Gaur 2001).

Binding mechanisms. The process of biosorption includes a number of mechanisms, like ion exchange, complexation, electrostatic attraction and microprecipitation. The most significant of these has been proved to be ion exchange. It was observed in *Spirulina platensis*, that during the binding of Cu^{2+} , Pb^{2+} , Zn^{2+} , Ni^{2+} and Cr^{3+} protons were liberated from biomass, which leads us to the conclusion of ion exchange. We also observed the release of Cu^{2+} and Ca^{2+} by algal biomass while binding Zn^{2+} and Ni^{2+} ions. All these studies suggest that the exchange of metal ions with surface bound protons or cations play a significant role. In heavy metal sorption, though this varies among algal species. We reported the maximum ion exchange capacity (in term of Na^+/H^+ exchange) of five algal species ranging from 41 – 825 $\mu\text{eq g}^{-1}$ dry weight (Mehta and Gaur 2001). Large variability in ion exchange may be due to cell composition, meaning unicellular algae has a higher surface/volume ratio than filamentous forms, and therefore a higher ion exchange capacity. This capacity can be increased by increasing the pH level. However, this mechanism is not the only one and it can be combined with others. In a study of *Sargassum vulgare* it was reported that Cd binding involved chelation, while the binding of Pb consisted of ion exchange and chelation. In the adsorption of Ni and Zn on *Chaetophora elegans* it was observed that electrostatic attraction and covalent binding played a role. In view of the complexity of the composition of algal surfaces, it is possible that various mechanisms operate simultaneously to varying degrees depending on algal species and environmental conditions (Mehta and Gaur 2001). If they are not attached to cell wall, metal ions may get inside the cell and bind to intracellular components or precipitate. In algae cells metals can be detoxified. Their activity is impacted by biological macromolecules and enzymes that are being accumulated in polyphosphate bodies and in intracellular metal binding proteins. In *Ankistrodesmus falcatus*, Sn has been part of about 85 % of cellular polysaccharide fraction, and 15 % in the protein fraction. In some eukaryotic algae, metal solutions were observed within the vacuoles (Mehta and Gaur 2001).

Factors of binding. Results may be affected by several factors, including concentration of metal and biomass, pH, temperature, contact time, presence of competing ions and the metabolic stage of the organism (Mehta and Gaur 2001).

Concentration of metal. Initial concentration of metal ions in the solution is a significant factor of biosorption ability. Biosorption initially increases as the initial concentration of metal ion increases up to the optimal concentration. It was reported, that in dry biomass of *Scenedesmus obliquus*, a 5-fold increase in initial concentrations of Zn^{2+} (from 10 to 50 ppm) boosted the metal ion sorption from 19 to 209.6 mg Zn^{2+} /g. Based on that, the biosorption capacity is increased, but the removal yields of the metal ion are reduced. Therefore, the higher the metal ion concentration, the lower the efficiency and removal yield would be. In another study, *Chlorella vulgaris* in concentrations of 2.5 ppm were able to remove 69 % and 80 % of Ni^{2+} and Cu^{2+} cations. When the concentration was increased up to 10 ppm, the metal removal rate was reduced to 37 % and 42 % (Zeraatkar *et al.* 2016). Getting to know proper concentration is a necessity for efficient growth of algae. Higher rates can inhibit growth or lead to cell and cell structure destruction. For some algae, heavy metals can be beneficial and enhance their growth, while to the others, heavy metal may be toxic at all concentrations. In *Cladophora fracta*, continual increase of lead and cadmium exposure resulted in total chlorophyll content loss, reduced number of chloroplasts, and disintegrated cell walls responsible for cell death and reduced cell growth. Algae have better sorption abilities at lower concentration, because the presence of any excessive toxicity in algal bodies is harmful and can lead to cell destruction (Zeraatkar *et al.* 2016).

pH level. The majority of studies show that biosorption of metal ions is highly dependent on the pH of the solution. Functional groups on cell wall membranes do not have same features at every pH, and therefore it was important to deduce the optimal pH for heavy metal removal. The sorption of Cr^{6+} and Cd on *Padina* sp. and *Sargassum* sp. and Cs sorption on *Padina australis* was optimal at pH 2. In a study of *Durvillaea potatorum*, very little sorption of Cu was observed at a pH below 2, but this sorption increased with a rise in pH. They found maximum Cu sorption between pH 3 and 4, and a plateau was reached at around pH 5. There are numerous studies showing increased metal sorption with increasing pH of the solution. It was suggested that the optimum pH for Cu and Cd sorption by *Laminaria japonica* and *Sargassum kjellmanianum* lies between 4 and 5, while the maximum Cr^{3+} sorption capacity of *Sargassum* sp. was observed at pH 4. The majority of metal binding groups on cell walls are acidic (e.g., carboxyl), and their availability is pH dependent. In acidic pH they generate a charged surface, and since the metal ions are at most charged positively, there is increase in their biosorption. However, at extremely acidic pH (< 2), metal sorption decreased. In general, for metal binding, an acidic pH is more efficient, although this pH level varies (1 - 7) (Mehta and Gaur 2001). It has been clearly shown that algae prefer acidic or slightly acidic environments. However, this prefer-

ence is also dependent on which metal is bonded. The optimum sorption pH of cadmium, nickel and zinc was 6. For copper, the value ranged between 4 and 5, while optimal pH for the sorption of lead was between 3 and 5, based on the type of biomass. The pH value rose between brown algae, through red algae to green algae. Within higher pH values the metal uptake capacity did not show significant differences (Romera *et al.* 2006).

Temperature. Temperature affects each algae species differently based on the type of metal to which they are exposed. It is known that metal sorption capacity is a function of temperature. There are several reasons why increasing temperature causes an increase in metal ion biosorption. Increased temperature results in an increased number of active metal binding sites with an increased tendency. Additionally, reduction in mass transfer resistance in the diffusion layer is caused by a reduction of the thickness of the diffusion boundary layer around the adsorbent groups. Along with temperature changes, a complex formation constant that assists metal binding also changes. On the other hand, some studies confirmed higher rate with lower temperature, while others confirmed no significant effect of indicative temperature on biosorption ability. Similarly, several studies have determined temperature-linked changes in metal ion uptake by living algal cells (Zeraatkar *et al.* 2016). These reported a slight increase in cation sorption by powdered seaweed biomass with an increase in temperature from 4 to 55 °C. Similarly, was recorded increased Ni²⁺ biosorption by dried biomass of *Chlorella vulgaris* with an increase of temperature from 15 °C (48.1 mg/g) to 45 °C (60.2 mg/g). This suggests that heavy metal biosorption is most likely an endothermic process, though it has also been suggested that the effect of temperature on sorption is not as certain as that of pH (Mehta and Gaur 2001).

Time. Heavy metal ion biosorption is highly dependent on contact time. Biosorption occurs in two stages. First, for algal biomass, metal ions are passively adsorbed into cell membranes, and biosorption of metal ions occurs rapidly within the first minute. Second, for live algae, active biosorption occurs as the algal cell slowly uptakes the HM ions (Salama *et al.* 2019). Biosorption of uranium by non-living *Chlorella vulgaris* achieved more than 90 % dissolved uranium adsorb during the first 5 min. In other studies, with biomass of *Chlamydomonas reinhardtii*, microalgae rapidly ad-

sorbed free ions biosorption and equilibrium was achieved in 60 min. All these examples show that biosorption of heavy metal ions is passive and occurs relatively rapidly. Higher rates of accumulation occurred with less contact time. In separately harvested *Cladophora fracta*, after 2, 4, 6, and 8 days of Cd and Hg exposure it was found that while the algal growth rate decreased over time, a greater biosorption capacity was obtained in older cultures. These results suggest that while passive heavy metal biosorption commences swiftly in the first moments of contact, a greater level of heavy metal bio removal can be achieved with longer contact times using living algae (Zeraatkar *et al.* 2016).

Our main objective in this work was to follow the annual cycles of some heavy metal pollutants and their abundance in aquatic ecosystems. Salt as well as freshwater algae have been used in the past for bioremediation of heavy metals and the results of these studies have been valuable. We sought to determine how the Javorinka stream changes over the course of the year and which elements fluctuate over time. Secondly, we examined what types of algae live in the aquatic foothills, whether they increase or decrease in volume, and whether biosorption capacity changes through the seasons.

Material and Methods

Sample collection

Samples are collected from attached algae in the mountain stream Javorinka. Two collection sections were chosen based on the findings of previous studies.

The first section is a segment of river situated between two villages, Tatranská Javorina and Podspády (Fig. 1). It is about 3 km long. The altitude range is between 930 - 970 m a.s.l. The stream bed consists of large and small granitic boulders and occasionally limestone. Water in this section is fast moving and rough, with many small, but deep lagoons that serve as an excellent habitat for fish and other aquatic organisms. Due to its dynamic nature, algae coverage of the rocks is sparse and for the most part of the year only microscopic or foliose algae grow. This section is marked as upper stream.

The second section is situated near the village of Vojtasová (N 49.292550°, E 20.169038°). In this section, the stream is significantly milder and slower. Depth is consistent over the length of



Fig. 1. Profile of downstream section (Photo: J. Tuchyňa 2021).



Fig. 2. Rough nature of upstream (Photo: J. Tuchyňa, 2021).

5
*Accumulation
of heavy met-
als by algae and
cyanobacteria*

the river. Altitude here varies between 850 - 865 m a.s.l. Algae growth occurs at a higher rate and tends to contain macroscopic algae. This section is marked as downstream (Fig. 2).

Sample preparation and laboratory analyses

Following sample collection, all invertebrates and large soil particles are removed. Afterwards they are left to dry naturally prior to further processing. In next step we crushed dried samples into fine dust using a cryo-mill (which mills each sample for 40 seconds). Following this, we measured element concentrations using a ED-XRF spectrometer DELTA (Olympus, Innov-x Systems, USA). In approximately 13 minutes results are available. For data analysis, Statistica 8 (StatSoft, USA) software was used. The comparison of individual elements conducted using the nonparametric Kruskal-Wallis test, because variances were highly variable between groups. Values with $p < 0.05$ were considered to be statistically significant. The potential synergic effect of elements was evaluated by principal component analysis (PCA), which is widely used in ecotoxicological studies. It is a variable reduction technique that maximizes the amount of variance accounted for in the observed variables by a smaller group of variables called components or factors.

From all the elements we chose 3 model elements with which we will work more extensively – chromium, zinc, and lead (Cr, Zn, Pb).

During our work we also had to learn from scientific literature, where we mainly focused on algae biology and their processes throughout their lifespan. The algae species were identified with the professional assistance of Mgr. Alica Hindáková, PhD.

Studied species

Hydrurus foetidus (Fig. 3) is a cold-water golden alga (Chrysophyceae) found in fast-flowing rivers mainly during periods of snowmelt. *Hydrurus* is a rheophile, preferring swiftly flowing water. It is also a psychrophile, dependent on low temperature. *Hydrurus* appears to be of specific importance for the early emerging aquatic larvae of chironomids. *Hydrurus* thrives well under seasonal climatic conditions, where there is snow in the winter, cold meltwater, and moderate summer temperatures. Its early emergence during snowmelt makes it visually prominent (Klaveness 2019).

Diatoma ehrenbergii (Fig. 4) are a species of the class bacillariophyceae. It is almost always present in fibers of *Hydrurus foetidus*.

Valves are narrow and elongate becoming lanceolate in smaller valves. Apices are broadly rounded sub-rostrate to capitate. Costae are mostly primary, and number 9 - 15 in 10 μm . Secondary and tertiary costae are commonly present. Frustules are attached to the substratum or joined in zig-zag colonies by mucilage (Kalina and Váňa 2010).

Oscillatoria sp. (Fig. 5). Cyanobacteria can easily handle sudden physical and chemical alterations of light, salinity, temperature, and nutrient composition. These organisms are very efficient for sewage water treatment, remediation of aquatic and terrestrial habitats, chemical industries, industrial



Fig. 3. *Hydrurus foetidus* (Photo: J. Tuchyňa, 2021).

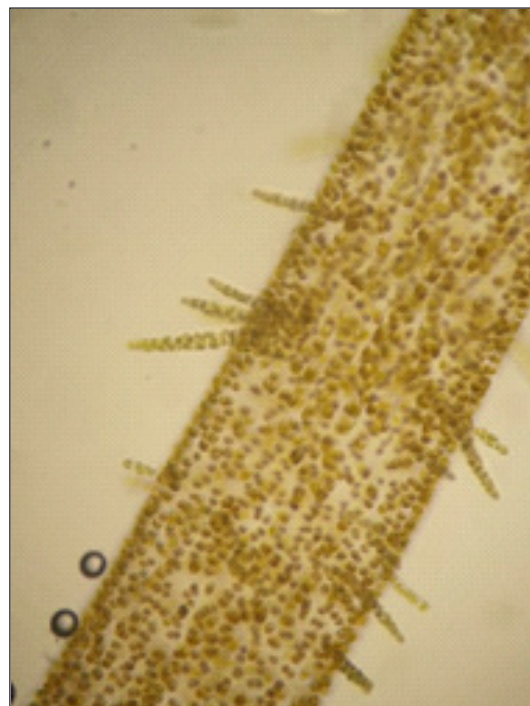


Fig. 4. *Diatoma Ehrenbergii* (Photo: J. Tuchyňa, 2021).



Fig. 5. *Oscillatoria* sp. (Photo: J. Tuchyňa, 2021).

effluents, etc. In addition, organic solutions, nitrate and phosphate compounds, carbon dioxide or alkalinity are important factors in determining the distribution of cyanobacteria (Dubey *et al.* 2011).

Five different cyanobacteria groups were studied (*Oscillatoria* sp., *Synechococcus* sp., *Nodularia* sp., *Nostoc* sp. and *Cyanothece* sp.). All of them were superior degraders and removers of pollutants. They were monitored in 2, 4 and 7 day periods. During the 2nd day, the majority of samples (with the exception of one) were between 92 % and 99.7 % effective at removing pollutants. At their fastest rate, these species could remove almost 99 % of present pollutants while showing high resistance against toxicity (Dubey *et al.* 2011).

Results

In this study we conducted several tests to better understand the seasonality of accumulation in this stream. All our tests could register some changes in the aquatic environment; therefore, we can say that algae and cyanobacteria are suitable for ecotoxicological studies.

There is a trend of higher concentrations at the downstream location compared to the upstream location. This was demonstrated by almost all elements which can be influenced by different dynamics of each part of the stream as well as other physical properties of the stream. Out of all the studied elements, four in particular were notable because of their significant variation between the two localities (Figs. 6 and 7). These significant elements were detected by the KW-H test with a p-value of < 0,05. At the downstream location, mean values were almost double of those upstream. Those elements were Ti, Mn, Fe and Ba.

Changes year to year (Table 1, Fig. 8) between most of the elements where either insignificant, or slight.

Regarding seasonal accumulation, most of the elements exhibited the highest concentrations in summer (Figs. 9 - 17). With the exception of Sr, Ca, and S, every other element showed a significant increase in concentration during summer. In case of Fe (Fig. 11) the rise was almost double the spring values. On the other hand, standard deviation was also relatively high, which could attribute this increase to flash floods and resulting transport of elements and their compounds. Granite bedrock is covered in Fe (OH)₂ and due to flash floods, it is released and adsorbed by algae and cyanobacteria.

During the summer season the level of water in Javorinka stream is generally low and the density is high. Elements are much more concentrated than in any other season.

Altogether we did three univariate statistics and one multivariable statistic (Table 2). We compared how they reacted together throughout the study and if we could find any patterns or interdependency. We chose 5 of 13 factors with a variance greater than 3 %. In the first pattern we can see mutual growth in S, K, Ti, Cr, Mn, Fe, Zn, Rb, Zr, Cd, Ba and Pb. Its variance is 51 % (the highest observed). The second factor is a function where the mutual decrease of S and K affects growth in Sr.

	Year	Mean ± SD (n)	KW-H	p
S	2019	8,636 ± 15,883.5 (10)		
	2020	3,931 ± 4,243 (25)	0.48	0.79
	2021	3,182 ± 1,479 (24)		
Cl	2019	750.77 ± 717.22 (9)		
	2020	494.8 ± 325.82 (15)	0.58	0.74
	2021	403.27 ± 134.69 (16)		
K	2019	13,271 ± 15,006 (10)		
	2020	12,366 ± 10,963 (25)	0.26	0.88
	2021	11,263 ± 6,523 (24)		
Ca	2019	17,845 ± 13,968 (10)		
	2020	34,660 ± 28,282 (25)	9.68	0.008
	2021	54,636 ± 42,408 (24)		
Ti	2019	1,034 ± 932 (9)		
	2020	1,654 ± 1,481 (24)	2.16	0.34
	2021	1,823 ± 1,422 (24)		
Cr	2019	153 ± 127.97 (10)		
	2020	108.6 ± 57.39 (25)	2.45	0.29
	2021	157.59 ± 120.76 (24)		
Mn	2019	394.9 ± 328.43 (10)		
	2020	317.96 ± 324.19 (25)	0.69	0.71
	2021	304 ± 243.18 (24)		
Fe	2019	10,034 ± 8,101 (10)		
	2020	16,084 ± 16,636 (25)	1.88	0.39
	2021	17,792 ± 16,932 (24)		
Cu	2019	18.5 ± 8.31 (6)		
	2020	20.38 ± 5.88 (9)	1.31	0.52
	2021	24 ± 13.54 (9)		
Zn	2019	63.6 ± 47.59 (10)		
	2020	71.76 ± 48.16 (25)	0.99	0.61
	2021	69.45 ± 41.33 (24)		
Rb	2019	20.61 ± 10.37 (10)		
	2020	32.228 ± 29.71 (25)	0.84	0.66
	2021	28.42 ± 22.21 (24)		
Sr	2019	88.35 ± 70.93 (10)		
	2020	105.572 ± 62.37 (25)	2.22	0.33
	2021	121.58 ± 69.01 (24)		
Zr	2019	89.36 ± 94.76 (9)		
	2020	116.26 ± 101.47 (24)	1.24	0.54
	2021	118.60 ± 78.61 (24)		
Ba	2019	1,218 ± 2,189 (10)		
	2020	363.33 ± 265.35 (24)	0.87	0.65
	2021	391.83 ± 236.87 (24)		
Pb	2019	17.2 ± 5.73 (10)		
	2020	20.77 ± 6.99 (25)	3.94	0.14
	2021	23.75 ± 9.87 (24)		

Table 1. Mean values concentrations (± SD) of the measured elements in algae from mountain stream Javorinka during the individual years of the research. Mean concentration values are given in ppm. (KW-H - Kruskal-Wallis test, significant differences are in bold p < 0.05).

7
Accumulation
of heavy met-
als by algae and
cyanobacteria

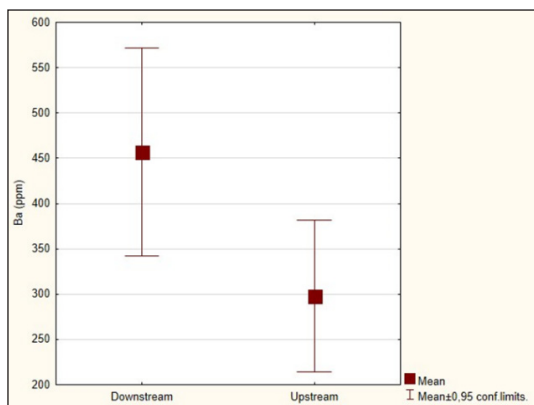


Fig. 6. Mean (\pm 95 c. limits) concentrations of barium in dependence on sample locations. The levels were significantly higher downstream than upstream.

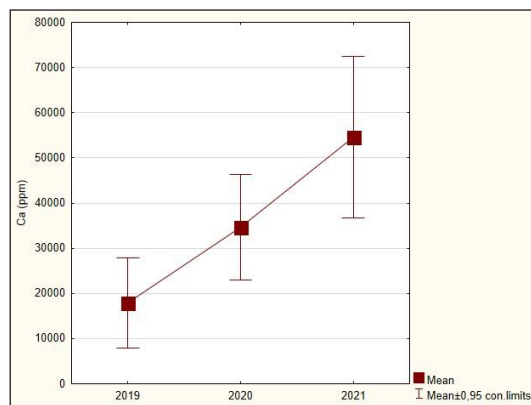


Fig. 8. Mean (\pm 95 c. limits) concentrations of calcium depended on year. The levels were significantly higher by each year.

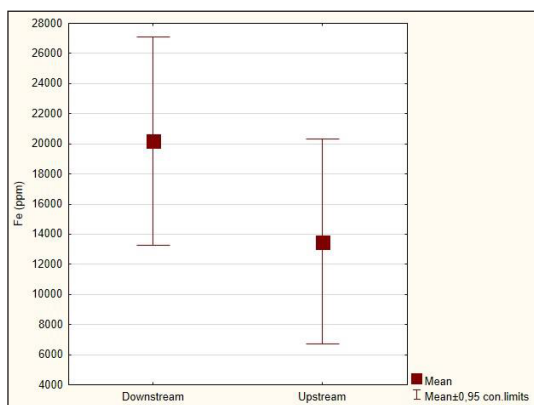


Fig. 7. Mean (\pm 95 c. limits) concentrations of iron in dependence on sample locations. The levels were significantly higher downstream than upstream.

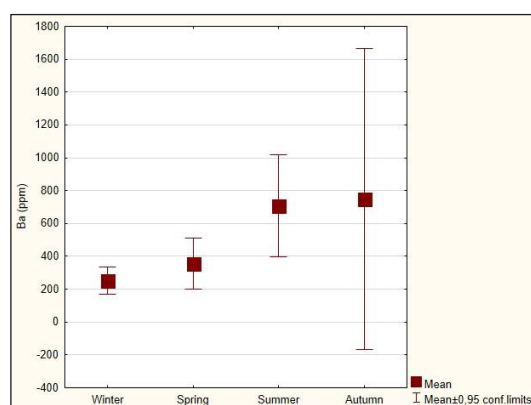


Fig. 9. Mean (\pm 95 c. limits) concentrations of barium depended on season of collection.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
S	0.245	0.800	0.458	0.076	-0.117
K	-0.627	0.568	0.476	0.058	0.111
Ti	-0.941	-0.074	0.007	0.162	-0.095
Cr	-0.673	0.153	-0.123	-0.515	-0.308
Mn	-0.808	0.222	-0.200	0.137	0.064
Fe	-0.972	0.094	-0.070	0.044	0.039
Zn	-0.718	0.219	-0.466	0.076	0.303
Rb	-0.875	0.007	0.154	0.183	0.292
Sr	-0.288	-0.574	0.643	-0.296	0.175
Zr	-0.742	-0.428	0.251	0.299	-0.072
Ba	-0.629	-0.139	-0.076	-0.367	0.291
Pb	-0.680	-0.266	-0.005	0.296	-0.519
Variance in %	51.5	13.3	9.4	7.1	5.9

Table 2. Multivariable statistic for studied elements (in ppm) with their variance (%).

In the third factor, decrease in S, K and Sr results in the growth of Zn level. In the fourth factor, Cr and Ba grow together. The last factor was mainly described by mutual growth of Pb and Cr with an antagonistic effect on Ba and Rb.

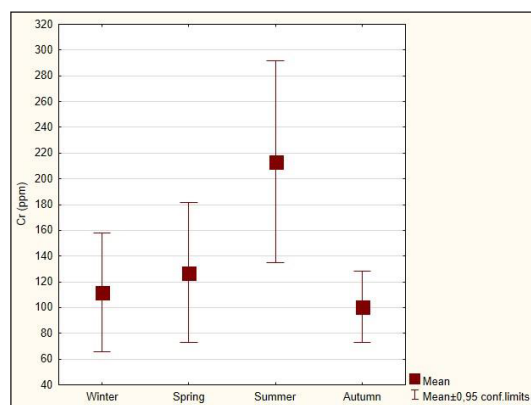


Fig. 10. Mean (\pm 95 c. limits) concentrations of chromium depended on season of collection.

Other factors were not seasonally dependent. Factor 2 of a mutual decrease of S and K, while Sr values growth was not affected by seasonality (KW-H (3,54) = 0.5, $p = 0.91$). Factor 3, which described an accumulation of Zn while S, K and Sr decreased was not affected by seasonality (KW-H (3,54) = 6.18, $p = 0.27$). Factor 4 of mutual accumulation Cr and Ba did not depend on seasonality (KW-H (3,54) = 3.89, $p = 0.27$). Factor 5 of accumulation Pb and Cr while antagonistically decreasing Rb and Ba was not affected on seasonality (KW-H (3,54) = 0.82, $p = 0.85$). Only

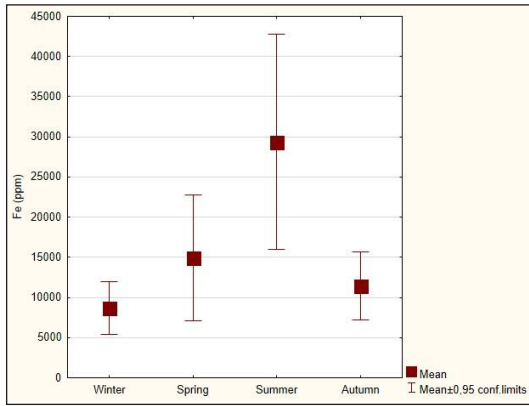


Fig. 11. Mean (\pm 95 c. limits) concentrations of iron depended on season of collection.

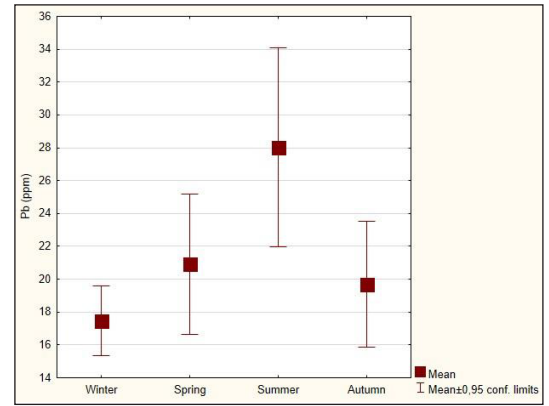


Fig. 14. Mean (\pm 95 c. limits) concentrations of lead depended on season of collection.

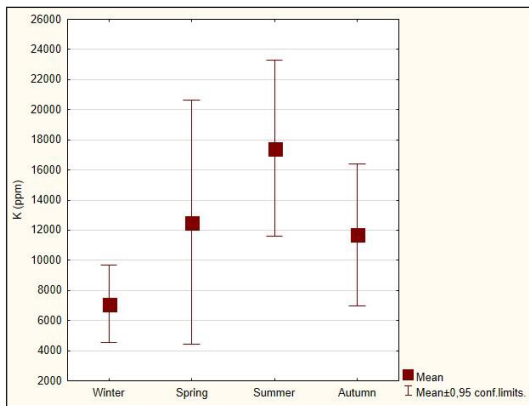


Fig. 12. Mean (\pm 95 c. limits) concentrations of potassium depended on season of collection.

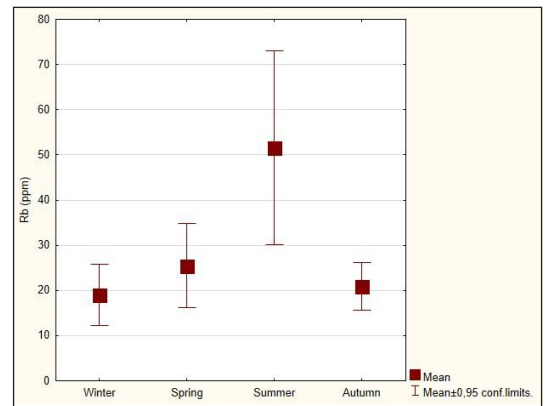


Fig. 15. Mean (\pm 95 c. limits) concentrations of rubidium depended on season of collection.

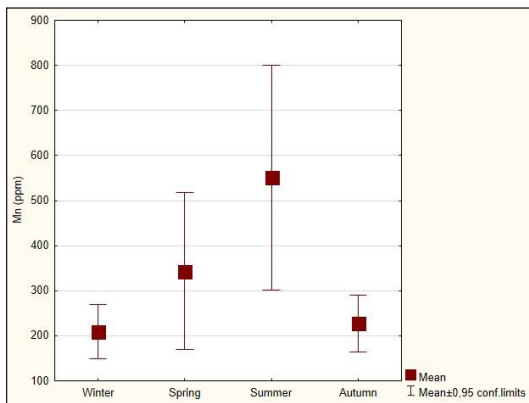


Fig. 13. Mean (\pm 95 c. limits) concentrations of manganese depended on season of collection.

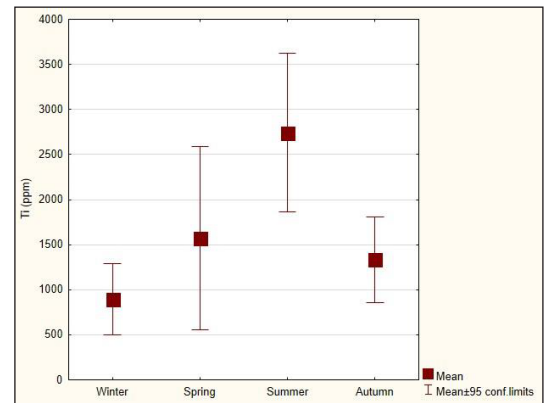


Fig. 16. Mean (\pm 95 c. limits) concentrations of titanium depended on season of collection.

factor 1 showed any dependence on any of variables, namely that the summer season is a period of intense uptake of elements (Fig. 19).

Discussion

Many elements exhibited variation between years. Some elements tended to decreased (e.g., S, Cl, K, ...) while others increased (e.g., Ca, Ti, Pb, ...). Notable changes throughout the years were found only in Ca (KW-H = 9.68, $p = 0.0079$). The concentration of Ca in the stream experienced constant

growth. Grounds for this trend are likely the flash floods, which expose the bedrock and constantly weather the rocks. These trends could also be a direct consequence of global warming and more extreme changes in weather.

Occasional elements

Although we have measured many elements, not all were detectable in tests. Some of them were unable to be detected at all, due to their very low concentration. Among these were Co, Ag and Hg. Accumulation by algae showed a high affinity to-

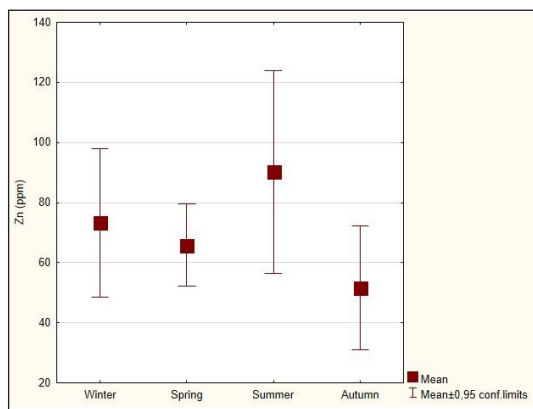


Fig. 17. Mean (\pm 95 c. limits) concentrations of zinc depended on season of collection

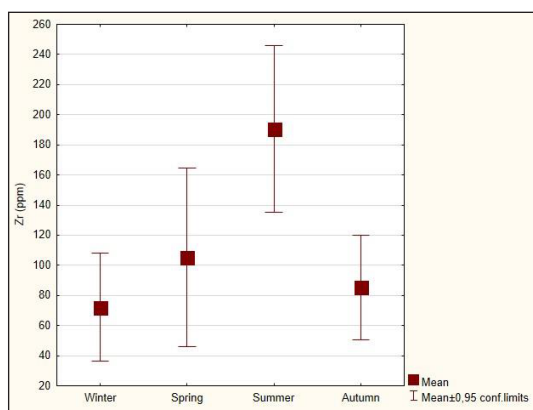


Fig. 18. Mean (\pm 95 c. limits) concentrations of zircon depended on season of collection.

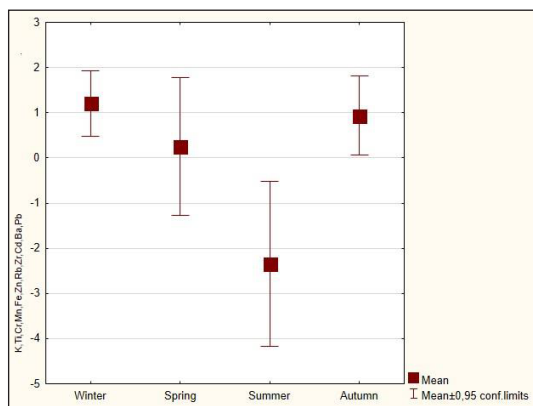


Fig. 19. Mean (\pm 95 c. limits) of principal component rates on factor 1 mutual concentration of K, Ti, Cr, Mn, Fe, Zn, Rb, Zr, Ba, Pb highly depended on seasons. The elements accumulated in algae mainly in summer (KW-H (3,54) = 15.8, $p = 0.0013$).

wards Hg by log K' MS (mean conditional stability constant of M-sites of algae) with a value of 18.0 ± 0.3 (Vasconcelos and Leal 2001). Based on this we can assume that mercury short-term concentrations in Javorinka stream are not high. Other elements were found in some samples, but certainly not often. Some of them were studied more extensively but most remained unused. P, Se, Mo, Sn, Sb were only found in a few samples and were not used in any of the tests. Ni and As, as well as Cu

and Cl were detected in more samples, but not in sufficient amounts for all our tests.

There was some seasonality associated with phosphorus, with the highest values in algae in summer and spring (Francoeur *et al.* 1999). The detectable values were mostly found between June to August, though phosphorus was also present in May and September. We obtained more values from the downstream location due to the slower stream.

Nickel has its highest accumulation in algae in autumn months (Haritonidis and Malea 1995; Vilares *et al.* 2002). The most common were values present during late summer and autumn months, from July to November. However, the most significant results occurred between August and October.

Astatine was detected throughout the year in relative equality. It was more abundant in July and August, but this might be attributable to the higher density of the stream. Astatine is a radioactive and highly unstable element with a minute natural abundance (Al-Adilah *et al.* 2022; Sturges and Barrie 1988), therefore its values are often more-or-less random.

Selenium was found only between December and March. Organic selenium in water is at its peak in late summer, but total selenium exhibits maximum concentrations in winter (Sherrard *et al.* 2004). We were able only to detect selenium during winter months, due to the significant seasonal growth during that period.

Molybdenum was only present in summer, between June and August, presumably due to a higher density of elements in the stream during this period. We only found tin 5 times out of 59 samples, and most often in January samples.

Antimony follows the same cycle patterns as selenium (Pelachaty *et al.* 2004) and most of the measurable values were detected in late autumn, winter, and early spring, (October to March). Neither Cu nor Cl had any visible seasonal pattern in their accumulation.

Univariate statistics

Concentration of elements in relation to the location. We tested 17 elements and their mean concentration depended on the locality of sample collection (downstream and upstream). In general, uptake of elements was higher at the downstream location, with the exception of Zn and Ni. Four elements (Ba, Fe, Mn, Ti) exhibited significant variation between localities. Their concentration nearly doubled at the downstream location compared to the upstream location. Standard deviation varied among all 17 elements. Some had higher deviation at the downstream location, while others had higher deviation at the upstream location.

Uptake of barium is related to its concentration in the environment; the higher the concentration the higher the uptake. *Oscillatoria* sp. were able to remove 10 % of Ba in the medium. Cell size or pH of the cultures did not affect this accumulation (Fisher *et al.* 1991). Brown and green algae have many alginate groups with a high affinity to barium. Accumulation takes place through ion exchange. This process occurs rapidly at first and then takes time to reach its equilibrium (Fontao *et al.* 2020). Barium has a minimal affinity toward minerals (Dong *et al.* 2003)

that leave its compounds unattached. Additionally, natural levels of Ba are the result of bleaching and eroding of sedimentary rocks (Fontao *et al.* 2020).

Higher uptake of Ba at the downstream location was presumably caused primarily by differences in physical properties of the stream, lower velocity and by bleaching of limestone and slates. With lower velocity and higher abundance of Ba in the water, there was more time and enough element for algal cell walls to react with and reach equilibrium of biosorption.

Algae accumulate iron mainly in Fe^{2+} form, the concentration of which is controlled by the solubility of FeCO_3 , $\text{Fe}(\text{OH})_2$ and FeS . Most of it is connected to the cell wall (Mahasneh 1988). Sources of iron in surface waters are primarily from bedrock and soil, (i.e., granite and granodiorite) (Erel *et al.* 1990; Romanescu *et al.* 2016).

The granite bedrock of Javorinka river may contribute to the higher concentration of iron at the downstream location. Continual weathering of these rocks creates higher concentrations at lower localities.

Accumulation of manganese is mostly processed by the cell wall, where it is absorbed by alginate structures. It is an element present in the Earth's core and is naturally occurring in the environment. Anthropogenic sources include fertilizers, mining, metal manufacturing industries (Kaonga *et al.* 2008), and municipal sewage (Moore 2012). When algae is exposed to Mn pollution it can exhibit higher values depending on velocity of the stream. In seasons where stream had lower velocity, its values increased up to $4.203 \text{ (ppm)} \pm 0.805$. During seasons with higher velocity at both locations, its concentration was only $1.438 \text{ (ppm)} \pm 0.196$ (Kaonga *et al.* 2008). Other studies, such as Scott *et al.* (2002), came to the conclusion that, in lower localities less manganese was accumulated in the stream than in higher localities. This is most likely due to plant uptake. Manganese accumulation takes place in two phases, rapid and slow. Its rapid phase can absorb up to 25 % of accumulated metal (Garnham *et al.* 1992).

Titanium, similarly to other metals, transfers within aquatic environment in oxides form, which has a high sedimentary rate (Kulacki *et al.* 2012). Therefore at lower velocity, it begins to sediment at higher rates (Lin *et al.* 2015). Similarly to other metals, titanium uptake undergoes 2 phases, rapid and slow (Kulacki *et al.* 2012). Most of the elements did not differ based on whether the algae were collected in higher located and faster moving parts of the stream, or at a lower and slower locality. Variation was only detected in Ba, Fe, Mn and Ti. This could also be related to lower velocity, varying transfer processes in aquatic environment, and different physical properties of water in lower lying areas.

Concentration of elements between years.

Calcium was the only element that showed significant growth over the year. Calcium is dependent on bedrock and parental geology. Weathering of the soil and rocks helps maintain natural levels of calcium in the environment (Ohta *et al.* 2018). In July of 2018, the High Tatra expe-

rienced an extensive flood which resulted in a great decrease in both nutrients and sediments in water environments (Hrivnáková *et al.* 2020). According to Meurant (2012), the concentration of calcium does not decrease immediately after a flood decreases, contrary to other elements. Flooding does not affected its cycles significantly. Calcium concentration mainly affects plant uptake, discharge, and bedrock. According to Munn and Meyer (1990), the lowest uptake of calcium occurred in gravelly soils, whereas high uptake of calcium occurred at rock outcrops during early spring. Higher flushing rates through the sediments may result in a less stable and less productive epibenthic community, resulting in a lower nutrient demand.

Our results suggest that higher concentration of calcium in the stream is likely due to the massive flood that occurred in 2018. Flood exposed and accumulated bedrock results in flushing calcium through the stream. Continual erosion of the riverside and its sediments exacerbates this phenomenon and the concentration of calcium in the stream grows.

Almost all of elements (S, K, Ca, Ti, Cr, Mn, Fe, Zn, Rb, Sr, Zr, Ba and Pb) that we studied using this test had significant seasonality variance ($p < 0.05$), except S, Ca and Sr. Other seasonal patterns were noted, but none were as distinct as the first. S and Ca were the only elements to experience a peak during autumn, while the rest of elements were recorded at their peak during summer.

Other studies (Skriptsova 2016; Imbs *et al.* 2009; Villbrandt *et al.* 1991) proved that the highest uptake of elements in water streams occurs during summer or early autumn. In summer, season algae acquire mature reproductive organs. Intensive growth and development of reproductive structures causes an intense uptake of nutrients (Skriptsova 2016). Gross primary production in summer months ranged from 5.8 to $11.3 \text{ g O}_2/\text{m}^2$ per day during the summer and algal respiration from 1.9 to $2.7 \text{ g O}_2/\text{m}^2$ per day. Photosynthesis/respiration ratios depending on month were ranging from 1.59 in December to 5.04 in May (Stockner 1968). Physical characteristics, such as temperature and irradiation affect these processes to a significant extent. Warmer water during summer months, and higher irradiance increase the tendency of the cell wall structures that absorb metals (Zeraatkar *et al.* 2016). According to our results 7, out of 10 elements were metals, which confirm their higher affinity for the algae cell wall during summer.

In terms of space-time these critical phenomena for heavy metal accumulation in algae and cyanobacteria seem to occur during the summer season. During this time of intense growth, they are absorbing elements (K, Ti, Cr, Mn, Fe, Zn, Rb, Zr, Ba and Pb) and nutrients from their surroundings, including both biogenic as well as non-biogenic elements (Pb). In summer, higher contamination of water and the necessity of element uptake for the growth of reproductive structures occur.

The smallest variance in values was observed during winter. Many elements are stored in snow and ice in the mountains. Thus, the composition of water in colder months is more stable and there is no disturbance present.

Synergic accumulation depended on season

The results clearly indicate significant variance based on season, mainly during summer. Synergic effects proved a notable increase in accumulation of K, Ti, Cr, Mn, Fe, Zn, Rb, Zr, Ba and Pb during the season. An increased tendency of cell wall structures (Zeraatkar *et al.* 2016) and the need for growth of algae and their reproductive organs enhances metal uptake during this period (Skriptsova 2016).

Acknowledgements

I would like to thank to all the staff from the Institute of High Mountain Biology who provided me with laboratory equipment for monitoring and measurements and especially thanks to Mgr. M. Kompiš, PhD. and Mgr. M. Haas, PhD., for professional help in the field. I would like to thank Mgr. A. Hindáková, PhD. who willingly helped me with the identification of algae samples. I would like to thank also to Prof. RNDr. M. Janiga, CSc. for performing statistical analysis and interpretation of results.

References

- Al-Adilah, H., Feiters, M.C., Carpenter, L.J., Kumari, P., Carrano, C.J., Al-Bader, D. and Küpper, F.C. 2022: Halogens in seaweeds: Biological and environmental significance. *Phycology*, **2**: 132-171.
- Bellinger, G.E. and Sigeo, D.C. 2015: Algae as bioindicators. Freshwater algae: Identification, Enumeration and Use as Bioindicators. John Wiley & Sons.
- Dokulil, M.T. 2003: Algae as ecological bio-indicators. In: *Trace Metals and other Contaminants in the Environment, Volume 6, Bioindicators & Biomonitoring, Principles, Concepts and Applications*, (eds. B.A. Markert, A.M. Breure and H.G. Zechmeister), pp. 285-327. Elsevier.
- Dong, D., Li, Y., Zhang, J. and Hua, X. 2003: Comparison of the adsorption of lead, cadmium, copper, zinc and barium to freshwater surface coatings. *Chemosphere*, **51**: 369-373.
- Dubey, S.K., Dubey, J., Mehra, S., Tiwari, P. and Bishwas, A.J. 2011: Potential use of cyanobacterial species in bioremediation of industrial effluents. *Afr. J. Biotechnol.*, **10**: 1125-1132.
- Erel, Y., Morgan, J.J. and Patterson, C.C. 1991: Natural levels of lead and cadmium in a remote mountain stream. *Geochim. Cosmochim. Acta*, **55**: 707-719.
- Fisher, N.S., Guillard, R.R.L. and Bankston, D.C. 1991: The accumulation of barium by marine phytoplankton grown in culture. *J. Mar. Res.*, **49**: 339-354.
- Fontao, N.C., Hackbarth, F.V., Mayer, D.A., Mazur, L.P., de Souza, A.A.U., Vilar, V.J.P., Guelli, U. and de Souza, S.M.A. 2020: A step forward on mathematical modeling of barium removal from aqueous solutions using seaweeds as natural cation exchangers: batch and fixed-bed systems. *J. Chem. Eng.*, **401**: 6-10.
- Francoeur, S.N., Biggs, B.J., Smith, R.A. and Lowe, R.L. 1999: Nutrient limitation of algal biomass accrual in streams: seasonal patterns and a comparison of methods. *J. North Am. Benthol. Soc.*, **18**: 242-260.
- Garnham, G., Codd, G. and Gadd, G. 1992: Kinetics of uptake and intracellular location of cobalt, manganese and zinc in the estuarine green alga *Chlorella salina*. *App. Microbiol. Biotechnol.*, **37**: 270-276.
- Haritonidis, S. and Malea, P. 1995: Seasonal and local variation of Cr, Ni and Co concentrations in *Ulva rigida* C. Agardh and *Enteromorpha linza* (Linnaeus) from Thermaikos Gulf, Greece. *Environ. Poll.*, **89**: 319-327.
- Hrivnáková, K., Janiga, M. and Pogányová, A. 2020: Effects of flooding on the physical and chemical water composition of the alpine lake Kolové pleso (High Tatra, West Carpathians). *Oecologia Montana*, **29**: 23-27.
- Chmielewska, E. and Medved, J. 2001: Bioaccumulation of heavy metals by green algae *Cladophora glomerata* in a refinery sewage lagoon. *Croat. Chem. Acta*, **74**: 135-145.
- Imbs, T.I., Shevchenko, N.M., Sukhoverkhov, S.V., Semenova, T.L., Skriptsova, A.V. and Zvyagintseva, T.N. 2009: Seasonal variations of the composition and structural characteristics of polysaccharides from the brown alga *Costaria costata*. *Chem. Nat. Compd.*, **45**: 786-791.
- Kalina, T. and Váňa, J. 2005: Heterokontophyta. In: *Sinice, řasy, houby, mechorošty a podobné organismy v současné biologii*. Karolinum, Praha.
- Kaonga, C.C., Chiotha, S.S., Monjerezi, M., Fabiano, E. and Henry, E.M. 2008: Levels of cadmium, manganese and lead in water and algae; *Spirogyra aequinoctialis*. *Int. J. Environ. Sci. Tech.*, **5**: 471-478.
- Klaveness, D. 2019: *Hydrurus foetidus* (Chrysophyceae): an update and request for observations. *Algae*, **34**: 1-5.
- Kulacki, K.J., Cardinale, B.J., Keller, A.A., Bier, R. and Dickson, H. 2012: How do stream organisms respond to, and influence, the concentration of titanium dioxide nanoparticles? A mesocosm study with algae and herbivores. *Environ. Toxicol. Chem.*, **31**: 2414-2422.
- Lin, M., Tseng, Y.H. and Huang, C.P. 2015: Interactions between nano-TiO₂ particles and algal cells at moderate particle concentration. *Front. Chem. Sci. Eng.*, **9**: 242-257.
- Mahasneh, I.A. 1988: Iron accumulation by blue-green algae from saline environments. Ph.D. Thesis, Durham University, UK, 19-22.
- Mehta, S.K. and Gaur, J.P. 2005: Use of algae for removing heavy metal ions from wastewater: progress and prospects. *Crit. Rev. Biotechnol.*, **25**: 113-152.
- Meurant, G. 2012: Flooding and Plant Growth. Academic Press, London, UK.
- Moore, J.W. 2012: Inorganic contaminants of surface water: research and monitoring priorities. Springer-Verlag, New York.
- Munn, N.L. and Meyer, J.L. 1990: Habitat-specific solute retention in two small streams: An intersite comparison. *Ecol.*, **71**: 2069-2082.
- Nriagu, J.O. and Nieboer, E. 1988: Chromium in the natural and human environments. John Wiley & Sons, Ontario, Canada.
- Ohta, T., Shin, K.C., Saitoh, Y., Nakano, T. and Hiura, T. 2018: The effects of differences in vegetation on calcium dynamics in headwater streams. *Ecosystems*, **21**: 1390-1403.
- Pelachaty, M., Pelachata, A., Niedzielski, P., Siepak, M. and Sobczyński, T. 2004: Analysis of the spatial and seasonal variability of inorganic species of arsenic, antimony and selenium in a Shallow Lake subjected to moderate anthropopressure. *Pol. J. Environ. Stud.*, **13**: 185-190.
- Pereira, S., Micheletti, E., Zille, A., Santos, A., Moradas-Ferreira, P., Tamagnini, P. and De Philippis, R. 2011: Using extracellular polymeric substance (EPS) - producing cyanobacteria for the bioremediation of heavy metals: do cations compete for the EPS functional groups and accumulate inside the cell. *Microbiology*, **157**: 451-458.
- Romanescu, G., Miftode, D., Pintilie, A.M., Stoleriu, C.C. and Sandu, I. 2016: Water quality analysis in mountain freshwater: Poiana Uzului Reservoir in the Eastern Carpathians. *Rev. Chim.*, **67**: 2318-2326.
- Romera, E., González, F., Ballester, A., Blázquez, M.L. and Muñoz, J.A. 2006: Comparative study of biosorption of heavy metals using different types of algae. *Bioresour. Technol.*, **98**: 3344-3353.
- Salama, E., Roh, H., Dev, S., Khan, M.A., Abou-Shanab, R.A.I., Chang, S.W. and Jeon, B. 2019: Algae as a green technology for heavy metals removal from various wastewater. *World J. Microbiol. Biotechnol.*, **35**: 1-19.
- Scott, D.T., McKnight, D.M., Voelker, B.M. and Hrcir, D.C. 2002: Redox processes controlling manganese

- fate and transport in a mountain stream. *Environ. Sci. Technol.*, **36**: 453-459.
- Sherrard, J.C., Hunter, K.A. and Boyd, P.W. 2004: Selenium speciation in subantarctic and subtropical waters east of New Zealand: trends and temporal variations. *Deep-Sea Res. I: Oceanogr. Res. Pap.*, **51**: 491-506.
- Skriptsova, A.V. 2016: Seasonal variations in the fucoidan content of brown algae from Peter the Great Bay, Sea of Japan. *Russ. J. Mar. Biol.*, **42**: 351-356.
- Stockner, J.G. 1968: Algal growth and primary productivity in a thermal stream. *J. Fish. Res. Board Can.*, **25**: 2037-2058.
- Sturges, W.T. and Barrie, L.A. 1988: Chlorine, bromine and iodine in arctic aerosols. *Atmos. Environ.*, **22**: 1179-1194.
- Vasconcelos, M.T.S.D. and Leal, M.F.C. 2001: Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. *Mar. Chem.*, **74**: 65-85.
- Villares, R., Puente, X. and Carballeira, A. 2002: Seasonal variation and background levels of heavy metals in two green seaweeds. *Environ. Poll.*, **119**: 79-90.
- Villbrandt, M., Krumbein, W.E. and Stal, L.J. 1991: Diurnal and seasonal variations of nitrogen fixation and photosynthesis in cyanobacterial mats. *Plant and Soil*, **137**: 13-16.
- Wong, P.T.S. and Chau, Y.K. 1990: Zinc toxicity to freshwater algae. *Toxicity Assessment*, **5**: 167-177.
- Zeraatkar, A.K., Ahmadzadeh, H., Talebi, A.F., Moheimi, N.R. and McHenry, M.P. 2016: Potential use of algae for heavy metal bioremediation, a critical review. *J. Environ. Manage.*, **181**: 817-831.

Received 8 May 2022; accepted 5 June 2022.