Analysis of organic compounds in Javorinka mountain stream in the High Tatra Mountains

T. BURKOŇOVÁ, T. PITOŇÁKOVÁ and J. SOLÁR

Institute of High Mountain Biology, Žilina University, Tatranská Javorina 7, SK-059 56, Slovak Republic; e-mail: tatiana.pitonakova@uniza.sk

Abstract. Research focused on the analysis of organic compounds, namely anthracene, benzo[a]pyrene, chrysene, and fluoranthene, in water samples from Javorinka mountain stream. The research was carried out between 2019 and 2022. Water samples were collected from two sampling sites - Javorina and Podspády and then analysed following extraction using gas chromatography-mass spectrometry (GC/MS). The results showed that seasonality has the greatest influence on the concentrations of PAHs in water. All organic compounds studied showed the highest values during winter and spring seasons, except benzo[a]pyrene, which had the highest values during autumn and winter. Fluoranthene, chrysene and benzo[a]pyrene were significant within seasonality. Significance associated with season was also shown in total dissolved solids (TDS).

Key words: organic compounds, mountain stream, annual comparison, High Tatras

Introduction

Water is a vital element for all living organisms on Earth. The importance of river ecosystems is increasingly recognised as a key issue in global ecology, with the term 'organic compounds' or 'polycyclic aromatic hydrocarbons' (PAHs) posing a serious risk to the environment. Given the ever-increasing demands of the human population, and our dependence on combustion processes, the issue of environmental quality is becoming more and more pressing. The accumulation of organic pollutants in rivers stimulates microbial growth, resulting in reduced oxygen content, and this can disrupt the entire aquatic ecosystem (Sirota *et al.* 2013). These contaminants are transported into the aquatic environment by a variety of pathways, but most commonly by atmospheric deposition. Deposition can take two forms, dry deposition, or deposition of solid particles, as well as wet deposition, when organic compounds bind to water droplets that fall to the ground as rain or snow (Holoubek 2000).

Although mountain rivers are usually less polluted than those in urban areas, there is still a risk of organic matter contamination, particularly when polluting sources such as industrial plants or roads are located close by. Therefore, it is essential to always monitor and control them. It is also essential to focus on all substances that can degrade water for the ecosystem and for people. The previously referenced group of organic substances have toxic, mutagenic, and carcinogenic properties that can cause serious health problems for humans and animals. Therefore, it is appropriate to address and investigate this issue on a regular basis. Persistent or volatile organic pollutants (POPs) are characterized by their low volatility and long-term ability to survive in the environment (Ritter *et al.* 2007). The combination of chemical stability and hydrophobicity (which increases with molecular weight) causes them to persist in soils, sediments, and water sources (Godefroy *et al.* 2005; Jaward *et al.* 2012). Although they are not acutely toxic to biota, their persistence and long-range transport in the broader environment make them a higher priority organic pollutant for periodic monitoring in the environment (ATSDR 2001). Drinking water pollution is one of the most common and serious environmental problems in the world. Contamination of water with various organic compounds is a growing environmental problem that has attracted the attention of many environmental scientists. Most of these pollutants come from industrial waste, textile dyes, pharmaceutical industry products, hormones, and various personal care products that are discharged into wastewater. Not surprisingly, the several chemical, physical, and biological methods have been developed to remove from these pollutants from water sources, or at a minimum to reduce their quantity (Alneyadi *et al.* 2018).

Polycyclic aromatic hydrocarbons are a large group of chemical compounds, consisting of more than 100 organic substances, with different compositions and properties. Two or more benzene nuclei make up these substances, which are bonded together by carbon atoms. According to the way nuclei are arranged, they are divided into linear (e.g., anthracene), clustered (e.g., benzo[a]pyrene) and square (e.g., chrysene). From an environmental point of view, they are classified as persistent organic pollutants and can be identified in any environmental compartment, (i.e., in either water, soil or air). They enter the environment largely due to anthropogenic activities. Many PAHs have toxic, mutagenic and carcinogenic properties, and there-

fore, efforts have historically been devotee to identify a representative group of these polyaromatic hydrocarbons (Lee *et al.* 2012). Under normal conditions, they are colourless, yellow, or white solids, but their physicochemical properties vary depending on their molecular weight. As the molecular weight increases, the boiling point, melting point, and lipophilic properties increase. Their ability to dissolve in water decreases with increasing molecular weight. Although PAHs occur in all types of water, surface waters tend to be the most contaminated. In addition, oil tanker accidents, industrial and municipal wastewater, and natural biological processes can also be sources. As one of their properties is insolubility in water, they are mainly absorbed in suspended particles, which significantly affects their distribution and the stability of aquatic ecosystems. The stability of PAHs in aquatic ecosystems is higher than in air. Their decay time depends on the specific type of PAH, but usually ranges from one week (naphthalene) to two months (dibenzo[a,h]anthracene). Photo-oxidation, chemical oxidation, or biodegradation by bacteria are processes that favour the degradation of PAHs in water. Evaporation, sorption of sediment particles, or bioaccumulation are some of the ways to remove these toxic substances from surface waters (Forsgren 2018).

We determined the concentrations of four polycyclic aromatic hydrocarbons, namely anthracene, benzo[a]pyrene, chrysene and fluoranthene in Javorinka stream, which is a source of drinking water for the municipalities of Tatranská Javorina and Ždiar in the eastern part of the High Tatra Mountains. The Javorinka mountain stream is characterized as a steep gradient river within a spruce-fir stand, in a mixed basin of granitic and limestone-quartz geology (Raven *et al.* 2011). The level of concentration of a given substance in the studied environment is the basis for assessing the quality of the environment regarding PAHs. Good environmental quality exists where the level of PAH pollution is below the limit value set by legislation. The limit values for raw water quality range from 0.1 to 1 mg/L (Decree of the Ministry of Environment SR 636/2004 Col. of laws).

Benzo[a]pyrene

Benzo[a]pyrene (B[a]P) ($C_{20}H_{12}$), with five benzene nuclei is one of the compounds belonging to the group of PAHs. It is normally a solid crystalline substance with a yellow colour, insoluble in water. It is formed by incomplete combustion, at temperatures of 300-600 ° C. This hydrocarbon is the most frequently studied and researched of all PAHs because it is considered to be a highly toxic and mutagenic compound (e.g., Jung *et al.* 2010; Chang *et al.* 2019; Bukowska *et al.* 2022).

B[a]P is a type of PAH found in a variety of environments and work settings. This PAH is produced by the imperfect combustion of organic substances such as coal, gasoline, and wood. It is also found in tobacco smoke and can be released into the environment through industrial processes including coal tar production, and waste incineration (EPA 2017). Exposure to benzo[a]pyrene has been linked

to a range of adverse health effects. Benzo[a]pyrene is a carcinogenic compound that has been linked to lung, skin, bladder, and liver cancer in humans and animals (Bukowska *et al.* 2022). Exposure to benzo[a]pyrene has also been linked to developmental and reproductive problems as well as immunological effects (Bukowska *et al.* 2022). The European Chemicals Agency has set a maximum limit of 5 μg/L (micrograms per litre) for benzo[a]pyrene.

Fluoranthene

Fluoranthene (Flu) is a PAH with the chemical formula $C_{16}H_{10}$. It is a yellowish, crystalline solid that is insoluble in water but soluble in organic solvents such as benzene, toluene, and chloroform. It is found in coal tar and is produced by the incomplete combustion of organic matter including wood, coal, and petroleum (NIH 2023). Fluoranthene is a solid at room temperature, has a melting point of 115-116 ° C, and a boiling point of 332 ° C. It has a molecular weight of 212.25 g/mol and a density of 1.28 g/cm^3. It is a highly aromatic compound and is relatively stable under normal conditions. It can undergo a variety of chemical reactions including oxidation, reduction, and halogenation (ATSDR 2023). Fluoranthene is a compound of relatively low toxicity. However, long-term exposure to fluoranthene has been shown to cause respiratory and skin irritation and DNA damage. It has been classified as a possible human carcinogen by the International Agency for Research on Cancer. It has a variety of industrial uses, including the manufacture of dyes, pigments, and plastics. It is also used as a raw material in the manufacture of semiconductors and in the production of electronic components (NIH 2023).

The World Health Organization (WHO) has still not set a guideline value for fluoranthene in drinking water. In the United States, the Environmental Protection Agency (EPA 2017) has set a maximum contaminant level (MCL) of 500 μg/L for total PAHs (including fluoranthene) in groundwater used for drinking water.

Anthracene

Anthracene (Anth) is a solid at room temperature, has a melting point of 213-215 ° C and a boiling point of 340 ° C. It has a molecular weight of 178.24 g/mol and a density of 1.24 g/cm^3. It is a highly aromatic compound and is relatively stable under normal conditions. It can undergo a variety of chemical reactions including oxidation, reduction, and halogenation (NIH 2023). This compound is of relatively low toxicity, but has been shown to cause skin irritation and may be harmful if swallowed or inhaled in large quantities. However, it is not considered to be a carcinogenic compound (McMurry 2015). Anthracene has a variety of industrial uses, such as the production of dyes, pigments, and plastics (NIH 2023).

The World Health Organization (WHO) has not set a guideline level for anthracene in drinking water. In the United States, the Environmental Protection Agency (EPA) has set a maximum contaminant level (MCL) of 500 μg/L for total PAHs (including anthracene) in groundwater used for drinking water (EPA 2017).

Chrysene

Chrysene (Chry) is a polycyclic aromatic hydrocarbon with the chemical formula $C_{18}H_{12}$. It is a yellowish, crystalline solid that is insoluble in water but soluble in organic solvents such as benzene, toluene, and chloroform. It occurs naturally in coal tar and petroleum and is also produced by the incomplete combustion of organic matter like wood and fossil fuels (NIH 2023). Chrysene is a solid at room temperature, has a melting point of 248-251 ˚ C, and a boiling point of 430 ˚ C. It has a molecular weight of 228.29 g/mol and a density of 1.25 g/cm³. It is a highly aromatic compound and is relatively stable under normal conditions (NIH 2023). This compound is considered a carcinogenic compound and has been associated with a variety of health effects including respiratory and skin irritation and DNA damage. Long-term exposure to chrysene has been shown to increase the risk of lung, skin, and bladder cancer in humans (Brown *et al.* 2017).

The World Health Organization (WHO) has not set a guideline value for chrysene in drinking water. In the United States, the Environmental Protection Agency (EPA 2017) has set a maximum contaminant level (MCL) of 50 μg/L for total PAHs (including chrysene) in groundwater used for drinking water (EPA 2008).

Gas chromatography/mass spectrometry (GC/MS)

GC/MS is a powerful analytical technique that combines two separate techniques: gas chromatography (GC) and mass spectrometry (MS). GC/MS is used to separate, identify, and quantify volatile and semivolatile compounds in a wide range of samples. This technique is widely used in fields such as environmental science, food science, forensic science, and pharmaceuticals (Skoog *et al.* 2017). In GC, the sample is evaporated and injected into a chromatography column that contains a stationary phase. The stationary phase can be a solid, liquid, or gas. The components of the sample separate as they pass through the column due to differences in their physical and chemical properties, including boiling point, polarity, and molecular weight. Separation is achieved through the interaction between the sample components and the stationary phase. The separation of components in GC is detected (Skoog *et al.* 2017). MS is an analytical technique used to identify and characterize compounds based on their mass-to-charge ratio (m/z) and fragmentation patterns. In MS, the sample is ionized, and the resulting ions are separated based on their mass-to-charge ratio using a mass analyzer (Heineman and Cook 2016). Gas chromatography-mass spectrometry combines the separation capabilities of GC with the identification and characterization capabilities of MS. In GC/MS. First the sample is separated by GC and the separated components are then ionized and fragmented by MS. The mass spectrum generated by MS provides information on the molecular weight and chemical structure of the compound, allowing its identification. GC/MS is used in many fields, including environmental science, food science, forensic science, and pharmaceuticals. In environmental sciences, GC/MS is used to identify and quantify pollutants in air, water, and soil samples (Jones 2019).

The aim of the study was to assess river pollution in the high mountain environment in terms of the transport of polyaromatic hydrocarbons from so-called contaminated areas to source areas of minimal contamination.

Material and Methods

Research area and field sampling

The research was carried out on the mountain stream Javorinka, which rises in the northern part of the Eastern Tatras (High Tatras, Western Carpathians) in Slovakia and flows through the villages of Tatranská Javorina and Podspády and continues to Poland, where it is a tributary of the Białka River. It has a specific direction of flow and is 19.3 km long, of which 2 km form the Slovak-Polish border. The Javorinka stream rises below the Javorovský peak, flows upstream through the Zadná Javorová valley, then continues into the Javorová valley. Its lower course flows through Žiarská brána, where the riverbed undulates and creates several narrows.

Water sampling was located at two sampling points on the Javorinka stream. The first sampling point was in the mountain village of Tatranská Javorina (N 49.259272˚; E 20.143610˚; 1027 m a.s.l.), located on the border of the Belianske and High Tatras. The Belianske Tatras are formed by limestone bedrock, in contrast to the High Tatras, which are formed by granite. The second collection point was located outside the village of Podspády (N 49.2964150˚; E 20.1655822˚; 867 m a.s.l.), approximately 4 km away from the first collection point.

Sampling was conducted from January 2019 to December 2022. Water samples from the first sampling point were taken weekly, from the second, every 2 weeks from July 2019. A total of 297 samples were taken from both sampling points.

Physical water parameters such as temperature, pH, salinity, conductivity, TDS (total dissolved solids) and dissolved oxygen were measured directly at the sampling site using a WTW 3430 multimeter (GEOTECH, Weilheim, Germany). For the chemical analysis of the water, which was done in the laboratory, a quantity of 1 litre of water was collected in a dark glass bottle, these samples were processed within 24 hours of collection.

Laboratory analysis of water samples

GC/MS method was used for the analysis. In the first step, the pollutants of interest were concentrated in 1 mL of hexane by extraction. The procedure was as follows: the control substance D-pyrene (30 μL) was added to 1 L of sample, transferred from the tube to an Erlenmayer flask and after closing the flask, the mixture was stirred. The next step was to add 1 mL of n-hexane and stir the sealed mixture for 2 minutes. The mixture was allowed to stand at rest for 2-5 hours depending on laboratory temperature and sample temperature. After equilibration, the separated n-hexane phase (extract) was pipetted from the Erlenmayer flask into a labeled vial, where the amount ranged from 0.5 to 1.0 mL. The extract was allowed to evaporate

to an amount of 0.3 mL. The extract was analysed in a gas chromatograph used for the detection of organic compounds. The mass selective detection (MS) system is a simple mass spectrometry system. Analysis were performed using an Agilent Technologies 5977A stand-alone capillary GC detector (Agilent Technologies 2013) and an Agilent 7890B mass selective detector with MassHunter software. An automated liquid sampler (ALS) was used to inject 2 μL (injection volume) of the extract. We used 6.0 purity helium as the carrier gas, which can form a vacuum system and increases the separation efficiency of the instrument (Vékey 2001). Evaluation of chromatograms was performed using Agilent Technologies software (2013).

Statistical analysis

SHMÚ provided (on the basis of an official request) daily flow data from the Javorinka River, which were supplemented with our data. Statistical evaluation was performed using Statistica 12 software (StatSoft, USA). The Kruskal Wallis H Test (KW-H) was used to test for differences. The confidence interval was 95% (p < 0.05). The principal component analysis (PCA) method was used to determine the main factors of the variables.

Results

During the monitoring period (2019-2022), a total of 297 water samples were collected from two sampling sites in the Javorinka mountain stream.

Significant differences in annual intervals were noted in the variables of flow rate, COD, pH, oxygen (pressure), and oxygen (%). In anthracene (K-W H(3, 282) = 162.6908, p = 0.0001), fluoranthene (K-W H(3, 267) = 27.1342, p = 0.0003) and to a lesser extent chrysene (K-W H(3, 247) = 8.2880, p = 0.0404). The highest levels of anthracene and fluoranthene were recorded in April 2022. Benzo[a]pyrene had non-significant data. Along with the within-season tracers that came out statistically significant, chemical oxygen demand was also significant (K-W H(3, 247) = 8.2880, p = 0.0001). We observe this phenomenon with elevated temperature at the Podspady sampling site.

Fluoranthene (Fig. 1a), chrysene (Fig. 1b), and benzo[a]pyrene (Fig. 1c) were significant within seasonality. Season-related signification was also evident in total dissolved solids (Fig. 1d). The average fluoranthene values were 0.0033 μg/L in spring, 0.0019 μg/L in summer, 0.0025 μg/L in autumn, and 0.0035 μg/L in winter. Mean chrysene concentrations were 0.0033 μg/L in spring, 0.0023 μg/L in summer, 0.0017 μg/L in fall, and 0.0023 μg/L in winter. Average concentrations of of B[a]P were 0.0106 μg/L during spring, 0.0198 μg/L during summer, 0.0278 μg/L during autumn, and 0.3435 μg/L during winter.

PCA

The four factors, as determined by principal component analysis (PCA), scored greater than 1 (eigenvalue) and cumulatively explained 69.5% of the data. The values are shown in Table 1.
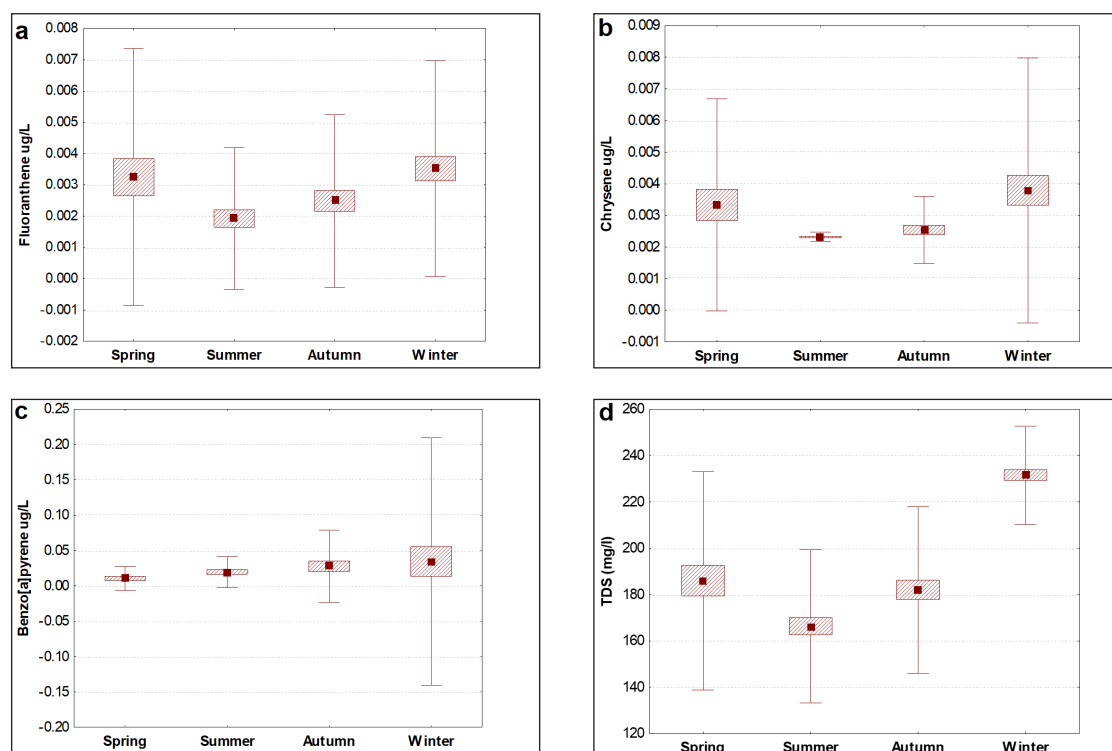

Fig. 1. Significant differences in seasonality. a) Fluoranthene in ug/L: K-W H(3, 264) = 17.5376, p = 0.0005. b) Chrysene in ug/L: K-W H(3, 344) = 83.5397, p = 0.0001. c) Benzo[a]pyrene in ug/L: K-W H(3, 199) = 26.2841, p = 0.0001. d) TDS (mg/L): K-W H(3, 297) = 121.3593, p = 0.0001. (Middle points: means; Boxes: +/- standard errors of mean; Whiskers: +/- standard deviations of mean).

	PC 1	PC 2	PC 3	PC 4
Flow	-0.2017	-0.1059	-0.3160	0.3443
Temperature	**0.8219**	0.2096	0.0428	0.2297
COD	-0.0528	0.2980	0.4019	-0.3036
pH	-0.4295	0.1159	0.1324	-0.1137
Conc. O_2	**-0.8564**	-0.1347	-0.0856	-0.0806
Partial pres. O_2	-0.6453	0.3408	-0.1372	**0.5434**
Saturation O_2	-0.5795	0.3196	-0.1031	**0.6646**
Conductivity	**-0.8185**	-0.2997	-0.1369	-0.2721
TDS	**-0.8199**	-0.2971	-0.1393	-0.2675
Anthracene	-0.3266	**0.7255**	0.3381	-0.1054
Fluoranthene	-0.3773	**0.6037**	0.4230	-0.2004
Chrysene	-0.2048	-0.5071	**0.7184**	0.2046
Benzo[a]pyrene	-0.0725	-0.4620	**0.7105**	0.3815
Eigenvalue	4.0282	1.9337	1.6701	1.4044
Total variance %	30.9868	14.8747	12.847	10.8034

Table 1. Principal component scores for measured variables of water from Javorinka stream. The most significant factor scores are listed in bold.

Principal component 1 (PC1) is a bipolar factor of the effect of temperature on TDS and oxygen content of mountain stream water monitored at two sites. We may refer to it as a physical factor. Its impact did not vary significantly between years (Fig. 2a) but was significantly dependent on seasons (Fig. 3a).

Principal component 2 (PC2) describes elevated levels of anthracene and fluoranthene in the Javorinka mountain stream. It is a unipolar factor. Nearly significant factor difference over the years was found at the Javorina site (Fig. 2b). Differences in the PC2 factor during the seasons were also observed, especially at the Javorina site (Fig. 3b).

Principal component 3 (PC3) represents elevated levels of chrysene and benzo[a]pyrene in the monitored flow. It is a unipolar factor that was different at both sites over the years (Fig. 2c). Elevated concentrations of both chrysene and benzo[a]pyrene were recorded in 2021 and 2022. The influence of seasonality on this factor has not been confirmed (Fig. 3c).

Principal component 4 (PC4) is a unipolar factor of oxygen saturation and its pressure in water. Over the years, the highest levels were observed in 2020 at both sites and values decreased in 2022, perhaps due to reduced rainfall (Fig. 2d). Seasonally, this factor was the most pronounced in the summer and fall (Fig. 3d) during 2020 in Tatranská Javorina.

Discussion

Mountain rivers are an important indicator in environmental monitoring, reflecting changes taking place in the environment caused by climatic factors quite rapidly. Based on the physico-chemical and organic properties of the water, it is necessary to regularly assess the state of rivers to determine the extent of the changes that have occurred dur-
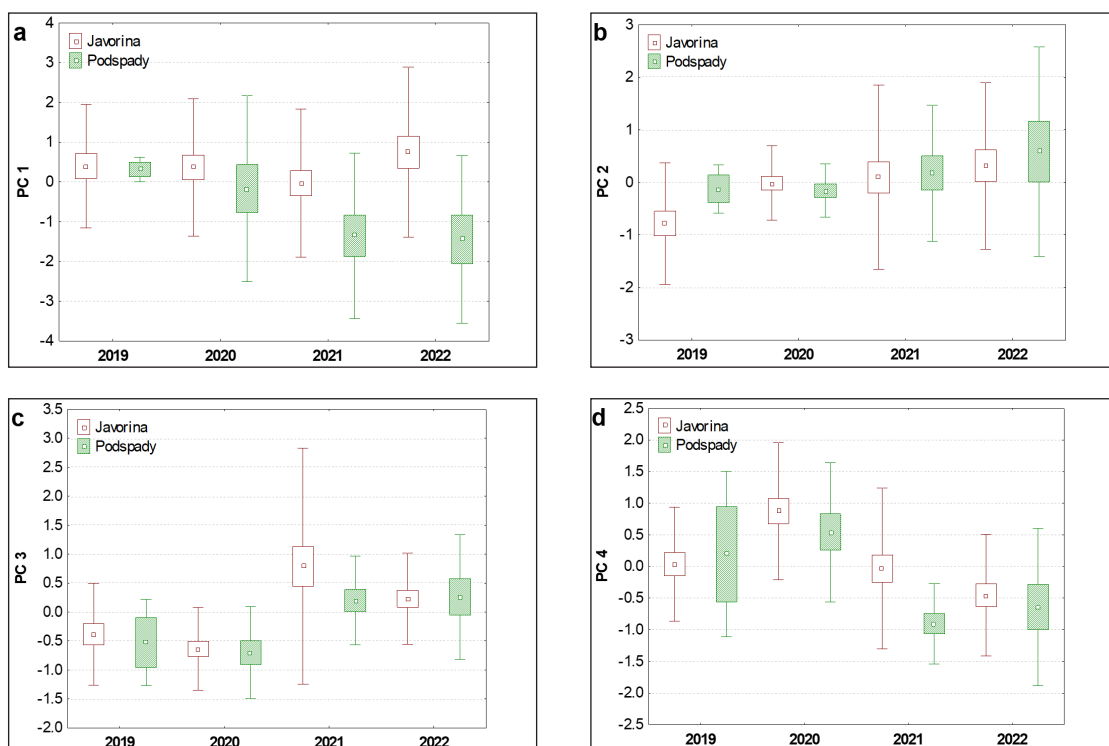

Fig. 2. Representation of the influence of factors over the years on the Javorinka mountain stream in the Javorina and Podspády. a) PC1: Javorina K-W H(3;117) = 3.2343, p = 0.3569. Podpády K-W H(3;46) = 4.0305, p = 0.2582. b) PC2: Javorina K-W H(3;117) = 11.9142, p = 0.0077. Podspády K-W H(3;46) = 1.1991, p = 0.7532. c) PC3: Javorina K-W H(3,117) = 40.7569, p = 0.0001. Podspády K-W H(3;46) = 11.9181, p = 0,0077. d) PC4: Javorina K-W H(3;117) = 24.0108, p = 0.00002. Podspády K-W H(3;46) = 16.0556, p = 0.0011 (Middle points - means; Boxes - +/- standard errors of mean; Whiskers - +/- standard deviations of mean).
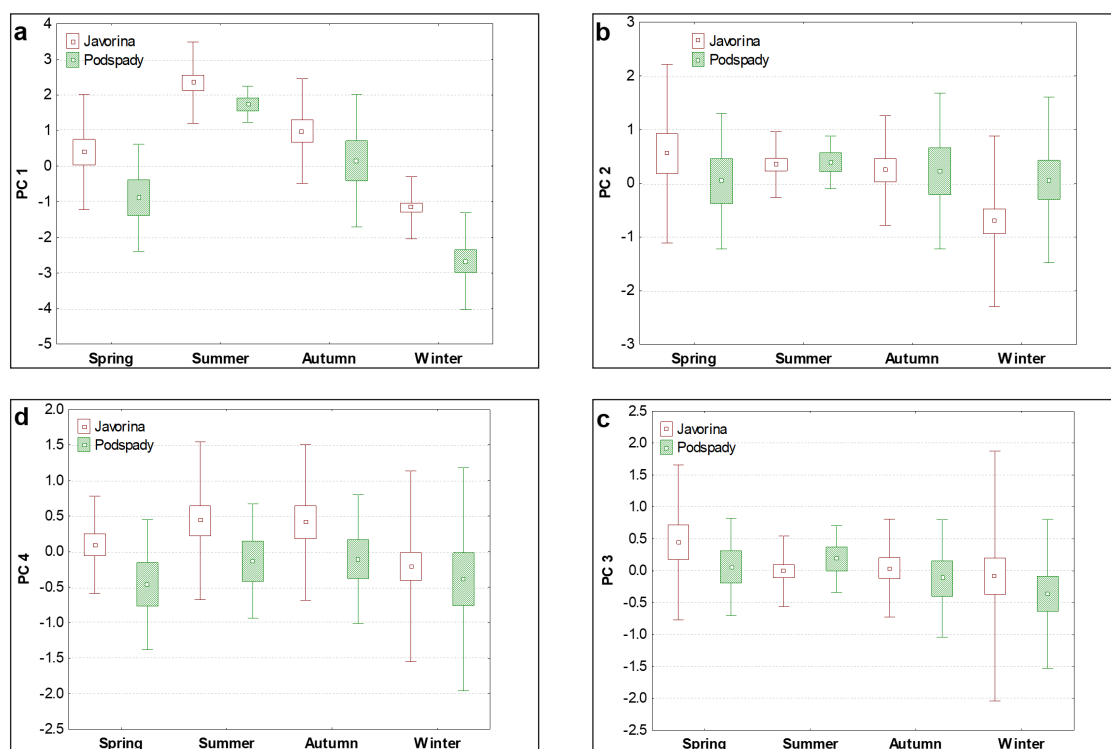
Fig. 3. Seasonal variations of four main principal components in the two sample sites (Javorina, Podspády). a) PC1: Javorina K-W H(3;117) = 63.4619, p = 0.0001. Podspády K-W H(3;46) = 27.239, p = 0.0001. b) PC2: Javorina K-W H(3;117) = 20.9856, p = 0.0001. Podspády K-W H(3;46) = 4.212, p = 0.2395. c) PC3: Javorina K-W H(3;117) = 6.341, p = 0.0961. Podspády K-W H(3;46) = 4.4412, p = 0.2176. d) PC4: Javorina K-W H(3;117) = 13.4025, p = 0.0038. Podspády K-W H(3;46) = 1.5273, p = 0.6760. (Middle points - means; Boxes - +/- standard errors of mean; Whiskers - +/- standard deviations of mean).

ing different seasons, to identify cycles that recur at regular intervals, and to analyse the evolution of events that have occurred unexpectedly and have significantly affected the usual dynamic trends (Potecká 2021; Zápotočný 2022). Almost all studies in the world focus on the determination of persistent organic pollutants in sediments, as the determination of these pollutants in waters is often challenging due to their low solubility and low concentration (Pozo *et al.* 2007; Schmid *et al.* 2011). The analysis of samples from the Javorinka stream in the Tatra Mountains revealed the presence of several substances from a group of organic pollutants. Seasons are a major factor influencing the magnitude of concentrations of these substances in river systems (Pozo *et al.* 2007). The highest concentrations of these compounds at our site were found during winter and spring, which may be because this period is characterized by precipitation in the form of snow, to which potentially present organic compounds bind (Arellano *et al.* 2011). Some substances from the group of polycyclic aromatic hydrocarbons are not on the list of priority pollutants because they are highly toxic, however, in our work we have quantitatively evaluated benzo[a]pyrene, anthracene, fluoranthene, and chrysene, which have confirmed toxic, carcinogenic, and mutagenic effects and can be absorbed relatively rapidly by water particles (Mitra *et al.* 1999; Oliveira *et al.* 2012). Our results showed elevated concentrations of fluoranthene, chrysene and benzo[a]pyrene in the environment during the winter and spring seasons; therefore, we believe that the higher measured concentrations of these substances may also

positively correlate with rising temperatures. This type of increase of substances in the ecosystem is reported in a study by Hrivnáková *et al.* (2020). The main sources of these pollutants in the environment are the various industries where they are produced as a product of imperfect combustion. As such, industrial areas including Ostrava (west; Czech Republic), Zakopane, Nowy Targ (Poland), or Košice (south; Slovak Republic) are potential sources of these pollutants (Van Drooge 2004). These industrial facilities are located several tens of kilometers away from the Tatra Mountains, but due to their ability to be transported long distances in the atmosphere (Morales-Baquero *et al.* 2013), they can also have an effect on areas not in their close proximity (Landlová 2006). Higher abundance of PAHs detected through sediments in winter and autumn, but not during spring, is described by Dong *et al.* (2018) in the Yangtze River in China. They suggest that the increase may have been due to higher production and discharge from industrial sources during the winter. This increased occurrence of PAHs during winter and autumn in China is confirmed by Zhang *et al.* (2019), whose research was conducted in an urban area. Another study from northern China on the Luan River showed the abundance of chrysene in sediments to be higher in summer than in winter (Zhang *et al.* 2013). In autumn and spring, increased PAHs were confirmed in the Vltava River in 2003 and 2004. The most frequently detected substances were fluoranthene, pyrene, benzo[a]pyrene and indeno[1,2,3-cd]pyrene (Kozáková and Pokorný 2007). Seasonal effects on polycyclic aromatic hydrocarbons, including chrysene, fluoranthene,

and benzo[a]pyrene, have also been monitored in the Yamuna River (India), and confirmed during the monsoon/summer season (Kumar *et al.* 2016). Increased seasonal abundance during summer and low abundance in winter has also been confirmed in the Alster River, Germany, (Hollert *et al.* 2002), in the Po River, Italy, (Di Guardo *et al.* 2010) and in the Huaihe River, China (Li *et al.* 2015), in the case of fluoranthene, specifically. The authors link this phenomenon to changes in temperature and changes in flow rates. Our observed increased occurrence of PAHs in the river during winter and spring is confirmed by Kučerová *et al.* (2015), who sampled the Váh River once in each season, finding significantly higher concentrations during winter and spring than in summer and autumn. The study by Han *et al.* (2017) also confirms the increased occurrence of anthracene in winter and in spring in northern China.

From these findings, we can conclude that the dependence of PAH concentrations varies between locations and the associated differences in climate and season. According to our findings, fluoranthene and chrysene were most abundant in the spring (0.0033 g/L) and winter (Fl = 0.0035 g/L; Chry = 0.0023 g/L). Research by (Manzetti 2013), which mapped PAH measurements and concentrations globally, but had an emphasis on China, India, and former Easter Block countries, verified that seasonality influences PAH concentrations outside of water. This study discovered that as spring proceeds, PAH levels in soil, water, and sediments rise due to the release of significant amounts of PAHs trapped in snow and ice near roadways and industrial locations.

Acknowledgements

References

ATSDR (Agency for Toxic Substances and Disease Registry) 2001: Public health statement, polycyclic aromatic aydrocarbons. Department of Health and Human Service, Atlanta.

ATSDR (Agency for Toxic Substances and Disease Registry) 2023: Toxic substances and disease registry. Department of Health and Human Service, Atlanta.

Alneyadi, A.H., Rauf, M.A. and Ashraf, S.S. 2018: Oxidoreductases for the remediation of organic pollutants in water - a critical review. *Crit. Rev. Biotechnol*, **38**: 971-988.

Arellano, L., Fernández, P., Tatosova, J., Stuchlik, E. and Grimalt, J.O. 2011: Long-range transported atmospheric pollutants in snowpacks accumulated at different altitudes in the Tatra Mountains (Slovakia). *Environ. Sci. Technol.*, **45**: 9268-9275.

Brown, T.E., LeMay, H.E.H., Bursten, B.E., Murphy, C., Woodward, P. and Stoltzfus, M.E. 2017: Chemistry: The central science plus mastering chemistry with eText. 14th Ed. Pearson, London.

Bukowska, B., Mokra, K. and Michałowicz, J. 2022: Benzo [a]pyrene - environmental occurrence, human exposure, and mechanisms of toxicity. *Int. J. Mol. Sci.*, **23**: 6348.

Di Guardo, A., Zacco, A. and Morgante, M. 2010: Seasonal variation of polycyclic aromatic hydrocarbons (PAHs) in surface waters of the Po River (Italy). *Environ. Monit. Assess.*, **168**: 171-185.

Dong, X., Li, H., Li, J., Li, C., Sun, C., Li, L. and Li, Y. 2018: Seasonal variations and ecological risk assessment of PAHs in sediments from the Yangtze Estuary and its adjacent areas. *Environ. Sci. Pollut. Res.*, **25**: 19634-19643.

EPA (Environmental Protection Agency) 2008: Update of human health ambient water quality criteria: Chrysene 218-01-9. Washington, DC.

EPA 2017: Toxicological Review of Benzo[a]pyrene. Executive Summary [CASRN 50-32-8]. Washington, DC.

Forsgren, A.J. 2018: Wastewater treatment: Occurrence and fate of polycyclic aromatic hydrocarbons (PAHs). CRC Press, Boca Raton.

Godefroy, S.J., Martincigh, B.S. and Salter, L.F. 2005: Measurements of polycyclic aromatic hydrocarbons and genotoxicity in soot deposited at a toll plaza near Durban, South Africa. *S. Afr. J. Chem.*, **58**: 61-66.

Han, D. and Currell, M.J. 2017: Persistent organic pollutants in China's surface water systems. *Sci. Total Environ.*, **580**: 602-625.

Heineman, W.R. and Cooks, R.G. 2016: Mass spectometry: principles and applications. 3rd ed. John Wiley & Sons, Chichester.

Holoubek, I. 2000: Persistentní, bioakumulativní a toxické látky v prostředí. *Klinická onkologie. Brno*, **13**: 21-23.

Hollert, H., Dürr, M., Westrich, B., Cofalla, C. and Brinkmann, M. 2002: Seasonal variation of polycyclic aromatic hydrocarbon concentrations in water and sediment samples from the river Alster, Germany. *Sci. Total Environ.*, **286**: 81-91.

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). *Oecol. Mont.*, **29**: 23-27.

Chang, Y., Siddens, L.K., Heine, L.K., Sampson, D.A., Yu, Z., Fischer, K.A., Lohr, C.V. and Tilton, S.C. 2019: Comparative mechanisms of PAH toxicity by benzo [a] pyrene and dibenzo [def, p] chrysene in primary human bronchial epithelial cells cultured at air-liquid interface. *Toxicol. Appl. Pharmacol.*, **379**: 114644.

Jaward, F.M., Alegria, H.A., Galindo Reyes, J.G. and Hoare, A. 2012: Levels of PAHs in the waters, sediments, and shrimps of Estero de Urias, an estuary in Mexico, and their toxicological effects. *Sci. World J.*, **2012**: Article ID 687034

Jones, M. 2019: Gas chromatography-mass spectrometry. American Chemical Society. Online: https://www.acs.org/education/whatischemistry/landmarks/gas-chromatography-mass-spectrometry.html (retrieved: 19 March 2023).

Jung, K.H., Yan, B., Chillrud, S.N., Perera, F.P., Whyatt, R., Camann, D., Kinney, P.L. and Miller, R.L. 2010: Assessment of benzo(a)pyrene-equivalent carcinogenicity and mutagenicity of residential indoor versus outdoor polycyclic aromatic hydrocarbons exposing young children in New York City. *Int. J. Environ. Res. Public Health*, **7**: 1889-1900.

Kozáková, R. and Pokorný, P. 2007: Dynamics of the biotopes at the edge of a medieval town: Pollen analysis of Vltava river sediments in Prague, Czech Republic. *Preslia*, **79**: 259-281.

Kučerová, J., Findoráková, L., Šútorová, B. and Masaryk, M. 2015: Seasonal variations of selected PAH concentrations in the Váh River (Slovakia). *J. Environ. Sci. Health A.*, **50**: 617-625.

Landlová, L. 2006: Sníh jako médium pro akumulaci perzistentních organických polutantů-sledování kontaminace sněhu v průmyslových a pozaďových lokalitách a jejich změn v čase. Bachelor's thesis, Masaryk University, Brno.

Lee, M.L., Novotný, M.V. and Bartle, K.D. 2012: Analytical chemistry of Polycyclic Aromatic Compounds. 2nd ed. Academic press, London.

Li, J., Cui, B., Liu, X. and Wang, X. 2015: Seasonal varia-

tion, distribution and ecological risk assessment of polycyclic aromatic hydrocarbons in water and sediment from the Huaihe River, China. *Ecotoxicol. Environ. Saf.,* **122**: 137-143.

Manzetti, S. 2013: Polycyclic aromatic hydrocarbons in the environment: Environmental fate and transformation. *Polycycl Aromat Compd.*, **33**: 311-330.

McMurry, J. 2015: Organická chemie. Vutium, VŠCHT Praha.

Mitra, S., Dickhut, R.M., Kuehl, S.A. and Kimbrough, K.L. 1999: Polycyclic aromatic hydrocarbon (PAH) source, sediment deposition patterns, and particle geochemistry as factors influencing PAH distribution coefficients in sediments of the Elizabeth River, VA, USA. *Mar. Chem.,* **66**: 113-127.

Morales-Baquero, R., Pulido-Villena, E. and Reche, I. 2013: Chemical signature of Saharan dust on dry and wet atmospheric deposition in the south-western Mediterranean region. *Tellus B: Chem. Phys. Meteorol.*, **65**: 18720.

NIH (National Institutes of Health) 2023: PubChem: A database of chemical compounds that provides information about the structure, properties, and uses of aromatic hydrocarbons, National Institutes of Health.

Oliveira, M., Gravato, C. and Guilhermino, L. 2012: Acute toxic effects of pyrene on *Pomatoschistus microps* (Teleostei, Gobiidae): Mortality, biomarkers and swimming performance. *Ecol. Indic.*, **19**: 206-214.

Potecká, D. 2021: Restoration of physical and chemical properties of water in the lake Kolové pleso after the flood in the year 2018. Bachelor thesis, IHMB, University of Žilina.

Pozo, K., Urrutia, R., Barra, R., Mariottini, M., Treutler, H.C., Araneda, A. and Focardi, S. 2007: Records of polychlorinated biphenyls (PCBs) in sediments of four remote Chilean Andean Lakes. *Chemosphere*, **66**: 1911-1921.

Raven, P., Holmes, N., Dawson, H., Lawniczak, A., Bulánková, E., Topercer, J. and Lewin, I. 2011: River habitat and macrophyte surveys in the high Tatra mountains of Slovakia and Poland. Results from 2010. Online: https://www.riverhabitatsurvey.org/wp-content/uploads/2013/02/RiverHabitat-Slovakia-Poland-2011.pdf (retrieved: 20 March 2023).

Ritter L; Solomon KR; Forget J; Stemeroff M; O'Leary C. 2007: Persistent organic pollutants. An assessment report on: DDT-Aldrin-Dieldrin-Endrin-Chlordane, Heptachlor-Hexachlorobenzene, Mirex-Toxaphene, Polychlorinated Biphenyls. The International Programme on Chemical Safety (IPCS) within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC).

Dioxins and FuransSchmid, P., Bogdal, C., Blüthgen, N., Anselmetti, F.S., Zwyssig, A. and Hungerbühler, K. 2011: The missing piece: Sediment records in remote mountain lakes confirm glaciers being secondary sources of persistent organic pollutants. *Environ. Sci. Technol.,* **45**: 203-208.

Sirota, J., Baiser, B., Gotelli, N.J. and Ellison, A.M. 2013: Organic-matter loading determines regime shifts and alternative states in an aquatic ecosystem. *Proc. Natl. Acad. Sci.*, **110**: 7742-7747.

Skoog, D.A., Holler, F.J. and Crouch, S.R. 2017: Principles of instrumental analysis. Cengage learning, Boston.

Van Drooge, B.L. 2004: Long-range atmospheric transport and fate of persistent organic pollutants in remote mountain areas. PhD. thesis. Faculty of Sciences, Autonomous University of Barcelona

Vékey, K. 2001: Mass spectrometry and mass-selective detection in chromatography. *J. Chromatogr. A*, **921**: 227-236.

Zápotočný, M. 2022: Changes in physicochemical parameters of the mountain stream Javorinka. Bachelor thesis, IHMB, University of Žilina.

Zhang, L., Li, X., Li, Y. and Li, L. 2013. Seasonal variation of PAHs in riverine sediments from the Luan River, North China. *Environ. Monit. Assess.*, **185**: 7151-7161.

Zhang, L., Qi, S., Huang, H., Lu, G., Zhang, Y., Liu, H. and Wang, Y. 2019: Seasonal variation, source, and health risk assessment of polycyclic aromatic hydrocarbons (PAHs) in urban rivers: A case study of the Qinhuai River Basin, China. *Sci. Total Environ.*, **650**: 2085-2093.