Changes in concentrations of potentially toxic elements during storage of hard river water samples at low temperatures using different sample preservation procedures

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Abstract

Purpose According to standard procedure recommended by the Water Framework Directive (WFD), dissolved concentrations of potentially toxic elements (PTEs) in river water are determined by inductively coupled plasma mass spectrometry (ICP-MS) in filtered (0.45 μm) and acidified (pH 2) samples. Properly prepared and stored composite samples can enhance the temporal representativity of monitoring without increasing analytical costs. For this purpose, the WFD recommends freezing, which can preserve the species integrity and prevent adsorption processes of PTEs.

Methods Low storage temperature in hard water samples can trigger precipitation of calcium carbonate ($CaCO_3$) and subsequent co-precipitation of PTEs. To test and determine to what extent co-precipitation with $CaCO_3$ can influence the determination of PTE concentrations, composite river water samples from two case study catchments in Hungary (Zagyva and Koppány) were prepared following two different sample preservation procedures. To study the behavior of PTEs in river water during storage, in the first procedure, samples were frozen, and they were thawed, filtered, and acidified directly prior to the analysis. In the second procedure, samples were filtered on-site and acidified prior to freezing to prevent precipitation of $CaCO_3$ and then only thawed to carry out the chemical analyses. Concentrations of PTEs were determined by ICP-MS.

Results A statistical evaluation of the results using Student's *t*-test revealed significant differences between the two sample preservation procedures, suggesting that PTEs were largely co-precipitated with $CaCO_3$ if the samples were not acidified prior to freezing.

Conclusion When establishing protocols for sample preservation procedures, the phenomenon of co-precipitation of PTEs with $CaCO_3$ should be considered if the samples were not acidified before freezing. Therefore, to prevent co-precipitation of PTEs with $CaCO_3$, samples should be filtered and acidified before freezing.

Keywords Potentially toxic elements · River water · Hard water samples · Freezing · Precipitation of calcium carbonate · Sample preservation procedures

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1 Introduction

Water is one of the most important resources for sustaining life on our globe. To maintain the water at an adequate quality and make it available and accessible to living beings, it is necessary to use, protect, and manage water resources sustainably (Komatina and Grošelj 2015). In order to control and prevent the pollution of water bodies, national water authorities in European Union (EU) countries conduct regular water monitoring, based on the EU Water Framework Directive (WFD) (Directive 2000/60/EC 2000) and its Daughter Directives (Directive 2008/105/EC 2008; Directive 2013/39/EU 2013; European Communities Environmental Objectives 272/2009 2009; European Communities Technical Report 2009–025 2009; Directive 2013/39/EU 2013). Besides organic micropollutants (Unyimadu et al. 2018; Köck-Schulmeyer et al. 2021), nanomaterials (Sanchís et al. 2018; Vidmar et al. 2022), and microplastics (Piskuła and Astel 2022; Zhao et al. 2022), there is an increasing concern regarding the release of potentially toxic elements (PTEs) from wastewater discharges (Sakson et al. 2018), industrial effluents (Mokarram et al. 2020), and irrigation areas (Meng et al. 2022) into the rivers. Due to their toxic effects associated with health risks (Balali-Mood et al. 2021; Sharma et al. 2022), the presence of PTEs is frequently monitored in river environments (International Commission for the Protection of the Danube River 2015; Milačič et al. 2017; Vidmar et al. 2017; Ali et al. 2018; Kumar et al. 2019; Bhuyan et al. 2019; Simionov et al. 2021; Lučić et al. 2022). Since many rivers are under the pressure of changing flow conditions, investigations on the behavior of contaminants under different flow regimes are increasingly investigated (Milačič et al. 2017, 2019; Superville et al. 2014; Rügner et al. 2019; Zuliani et al. 2022). Extensive information on the pollution of river ecosystems by PTEs and other contaminants is also obtained from various interdisciplinary international projects (Navarro-Ortega et al. 2015; Brack et al. 2015). To estimate the pollution loads and predict the environmental fate of pollutants in river basins, different modelling approaches are used, which require a large number of reliable data on the concentration levels of pollutants in river water (Zessner et al. 2015; Lindim et al. 2016; Jolánkai et al. 2020; Decsi et al. 2020). In order to obtain comprehensive information on the occurrence and concentration level of PTEs in rivers and at the same time reduce the costs of analyses, it is often necessary to optimize the ratio between the resources invested in monitoring and the information obtained. For this purpose, it is possible to analyze composite samples made from subsamples collected under similar river flow conditions. The composite samples cover the temporal variability of pollutant concentrations over a certain period of time. Their advantage is also their representativeness, as they better reflect the actual state of water pollution than the analysis of individual water samples taken at specific time intervals, as for example provided in the WFD, i.e., once a month for priority substances and once every 3 months for other specific pollutants (European Communities Technical Report 2009–025 2009). Composite samples are prepared from subsamples taken at equal time intervals and having the same volume. To obtain reliable results on pollutant concentrations in a composite sample, it is extremely important to prevent loss of analytes or their transformation by redox and/or adsorption processes, degradation of organic matter, and precipitation of carbonates and hydroxides (European Communities Technical Report 2009–025 2009). Therefore, prior to analysis, the sample must be preserved and stored under conditions that ensure the integrity of the analyte, so that the determined concentration of the contaminant represents its true amount in the composite sample. Storing samples at temperatures below -20 °C prevents redox, adsorption, and degradation processes, thus allowing longer sample storage time. However, the risk of precipitation of calcium carbonate ($CaCO_3$) at low temperatures can occur, in particular in samples with high water hardness (Hall 1980; Sander et al. 2006; European Communities Technical Report 2009). Consequently, freezing can lead to analyte co-precipitation with $CaCO_3$ and its loss from the sample analyzed.

The aim of the present work was to find out how the concentrations of dissolved PTEs, namely lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), nickel (Ni), copper (Cu), and zinc (Zn), can change in hard river water during sample storage at low temperatures. For this purpose, water samples collected in two case study catchments in Hungary (Koppány and Zagyva) were analyzed applying two different sample preservation procedures. Specifically, the procedures were designed to investigate the potential loss of analytes caused by co-precipitation processes and whether carrying out the filtration and acidification steps prior to freezing would lead to significantly better results. Further, it was tested whether such potential differences play a different role for composite samples collected at baseflow and high flow conditions.

2 Materials and methods

2.1 Instrumentation, reagents, and materials

Concentrations of elements were determined using ICP-MS instrument Agilent 7700x (Agilent Technologies, Tokyo,

Japan). ICP-MS operating parameters are presented in Table S1 (Supplementary information).

Ultrapure 18.2 MΩ cm water obtained from a Direct-Q 5 system (Millipore, Watertown, MA, USA) was used for preparation of standard solutions. Suprapur nitric acid (67–70% HNO₃, Carlo Erba Reagents, Val-de-Reuil, Normandie, France) was used for acidification of the river water samples. ICP multi-element standard solution XVI (21 elements in diluted nitric acid, 100 mg L^{-1}) and stock standard solutions of scandium (Sc), germanium (Ge), yttrium (Y), rhodium (Rh), and indium (In) (1000 ± 2 mg L^{-1} in 2–3% HNO₃) purchased from Merck (Darmstadt, Germany) were used to prepare calibration curves and internal standards for the determination of elements by ICP-MS. River water samples were filtered using 0.45 μm Minisart cellulose nitrate membrane filters (Sartorius, Goettingen, Germany). Samples were collected in low density polyethylene wide-mouth bottles (volume 0.5 L) obtained from BRAND (Wertheim, Germany). The certified standard reference material SPS-SW1 (reference material for measurements of elements in surface waters) obtained from Spectrapure Standards (Oslo, Norway) was used for the accuracy check of the determination of elements by ICP-MS. The results of the SPS-SW1 analysis are provided in Table S2 (Supplementary information). A good agreement between the determined elemental concentrations and the certified values was obtained (the differences did not exceed $\pm 3\%$), confirming the accurate determination of PTE concentrations in river waters by ICP-MS.

2.2 Sampling sites

Experiments were carried out in two Hungarian catchments (Koppány and Zagyva) at six sampling sites to evaluate the influence of water hardness on the behavior of PTEs using different sample preservation procedures. These catchments were selected in view of their very hard water, and the sampling campaign included both low flow and high flow conditions. Sampling sites with sampling codes for the 660 km² Koppány catchment and the 1200 km² Zagyva catchment are presented in Fig. 1.

Sampling sites were chosen in such a way that it was possible to cover the differences in the river catchments with regard to emissions of hazardous substances. The Koppány HKH site collects a rural catchment (agricultural fields and villages) with a strong point source impact at the very head of the stream, while the lower station (HKT) shows the effects of dilution on point source (average river flow is about twice the flow of the HKH), representing also rural (predominantly agricultural) emissions, with some extended urban runoff from the Tamási city as a source. In the Zagyva catchment, the HZT sampling site is focused on urban runoff and point source discharge effects, which is set in contrast with the more natural HZN site with much less point source

discharge. The HZH location represents an agricultural location with a lower specific runoff characteristic. The site HZ6 is the outlet of the catchment, where all effects from various sources accumulate. The HZ6 site is the outflow of the catchment, where all effects from various sources accumulate. At all stations, the river discharge is measured, either by existing river gauges or velocity meters to provide the possibility of calculating loads.

The physico-chemical characteristics of the rivers are similar: electrical conductivity (multiannual averages on Zagyva sections: 930–1270 μS cm^{-1}; Koppány: 1006–1120 μS cm^{-1}, dissolved oxygen content (multiannual averages on Zagyva sections: 5.90–8.00 mg L^{-1} O₂ and 52–74% saturated; Koppány: 8.38–8.92 mg L^{-1} O₂ and ~75% saturated), and nutrient status (multiannual averages on Zagyva sections: total nitrogen 2.12–5.37 mg L^{-1} N, total phosphorus 0.20–0.69 mg L^{-1} P; Koppány: total nitrogen 5.4–6.49 mg L^{-1} N, total phosphorus 0.62–1.27 mg L^{-1} P). These river sections were classified as good to moderate. The exception is the nutrient status of the Koppány, which is classified as poor, due to the high point source emission from the municipal WWTP of Balatonlelle, especially of total phosphorus concentration levels.

2.3 Sample collection and analytical procedures

Sampling was carried out over a period of 8 months. At low water discharges, grab sampling was performed weekly, while at high water discharges, flow-proportional sampling was done with automatic samplers. Automatic sampling was triggered by exceeding a predefined river discharge threshold (10 percentile exceedance flow level by default) set by statistical analysis of discharge data. Each automatic sample is a composite from a whole (or the initial part in case of long events) runoff event that is gathered with a continuous sampling with a peristaltic pump with varying rotation speed that is proportional to the increase of river discharge. At low water discharges, the discharges (for the entire sampling period of 8 months) at sampling sites in Koppány catchment ranged from 0.07 to 1.28 m³ s^{-1}, while in Zagyva from 0.01 to 4.15 m³ s^{-1}. At high water discharges, the range of measured mean discharges during the high flow events of the sampling period was from 0.6 to 5.1 m³ s^{-1} and from 0.15 to 8.2 m³ s^{-1} at Koppány and Zagyva sampling sites, respectively.

For the determination of dissolved element concentrations, water samples must be filtered and acidified to a pH below 2 to prevent the adsorption on container walls (Directive 2000/60/EC 2000; European Communities Technical Report 2009–025 2009). The adsorption process can also be prevented by storing samples at low temperatures (-20 °C). In this study, two sets of experiments were conducted in parallel to determine how different sample preservation procedures may affect dissolved element
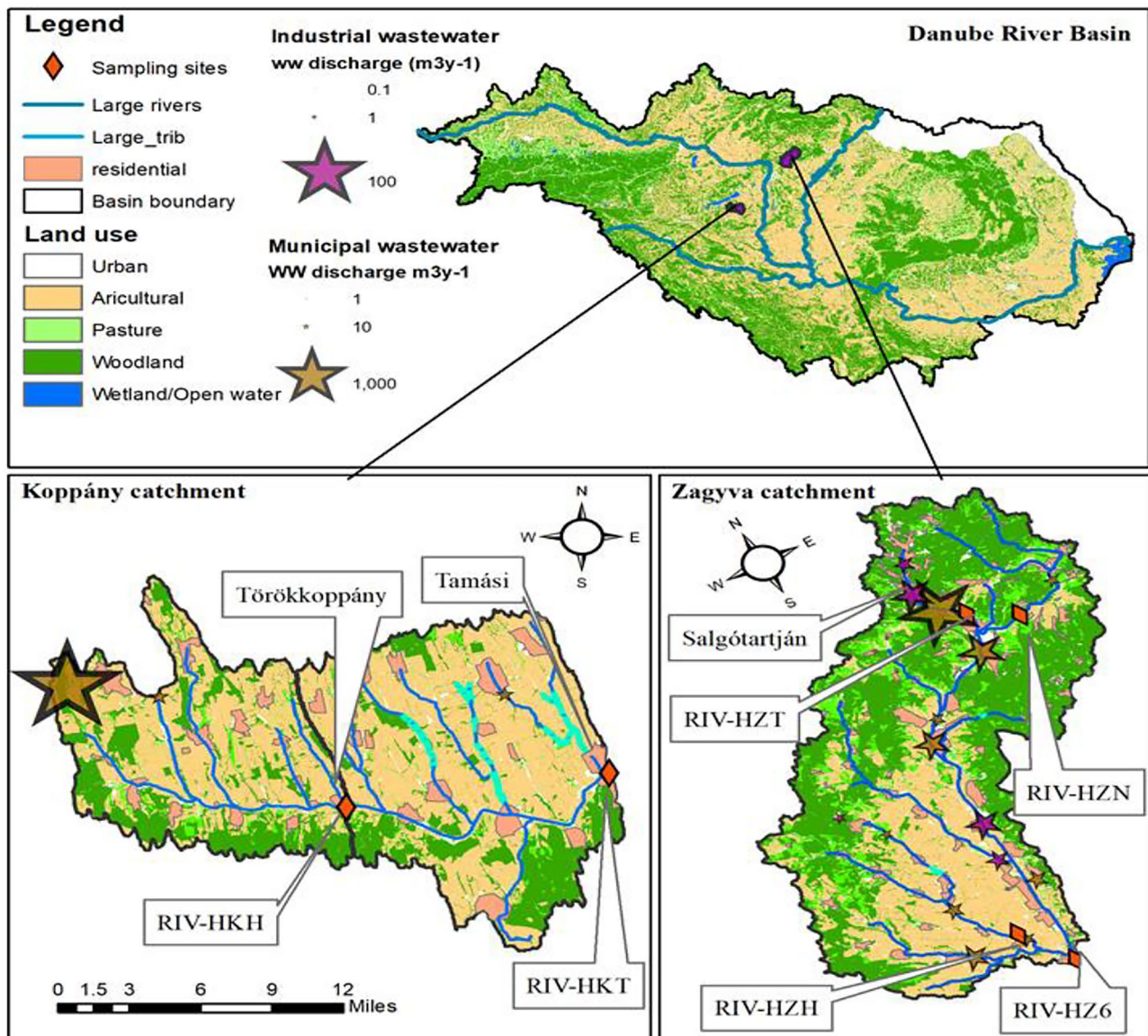
Fig. 1 Sampling sites at Koppány and Zagyva catchments. RIV-HKH, headwater of the Koppány creek outside Törökkoppány village at water gauge station; RIV-HKT, Koppány creek, at the water gauge station within Tamási city; RIV-HZT, Tarjan creek, downstream of the Salgótarjan city and wastewater treatment plant discharge; RIV-HZN, Nemti station at the water gauge on the upper Zagyva river, above the Maconka reservoire; RIV-HZH, Herédi-Bér creek, close to the source of Zagyva creek at the station within Heréd village; RIV-HZ6, Zagyva river within Hatvan city

concentrations in hard water samples. Samples taken at low water discharges were gathered into a 2-month composite sample made of eight subsamples of the same volume (50 mL). The first subsample was frozen at − 20 °C. Then, the next subsample was loaded on top of those previously frozen until the composite sample was finalized. High water composite samples were frozen immediately after collection. Before the samples collected at low and high water discharges were frozen, two sample preservation procedures were applied. In the first one, subsamples were frozen, and prior to PTEs analysis, a composite sample

was thawed, filtered through 0.45 μm filter, and acidified (400 μL of HNO_3 to 400 mL of composite sample). In the second procedure, aliquots of the same subsamples were filtered on-site, acidified (50 μL of nitric acid to 50 mL of sample), and frozen to build a composite sample. Acidification of samples before freezing prevented precipitation of $CaCO_3$. The same double preservation strategy was applied to the flow-proportional high flow samples, which were also frozen for up to 2 months and analyzed together with samples collected under low water flow conditions. After thawing, the concentrations of PTEs in composite

samples at low and high water discharges obtained with the two sample preservation procedures were determined by ICP-MS. Blank samples (MilliQ water) and filtration blanks were prepared on-site, following the same preservation procedures as samples, stored in the same polyethylene bottles and in the same way as samples. Concentrations of PTEs in blank samples did not exceed 5% of the determined elemental concentrations in samples analyzed and were in general below the limits of detection (LODs). LODs for the determination of the dissolved concentrations of elements in river water samples by ICP-MS are provided in Table S3 (Supplementary information).

Water hardness was measured by a standard procedure using a titration with ethylenediaminetetraacetic acid, purchased from Merck. In order to test how conductivity, turbidity, and pH can change in hard water samples, these parameters were measured in river waters from the Koppány and Zagyva catchments on-site before freezing and within 24 h after thawing in the laboratory. In this experiment, the samples were not filtered or acidified.

2.4 Data processing and statistical evaluation

Data were extracted using the Agilent 5.1 MassHunter software and further processed with Microsoft Excel 2019 MSO (Redmond, WA, USA).

A statistical analysis using Student's t-test at a 0.05 level of significance was carried out to test significant differences in the PTE concentrations between composite samples obtained via the two sample preservation procedures. For this purpose, the mean values between individual PTE concentrations determined in river water samples that were filtered and acidified and then frozen, and samples that were first frozen and after thawing, filtered and acidified, were compared for samples collected at low ($n = 24$) and high water discharges ($n = 18$).

3 Results and discussion

3.1 Precipitation of CaCO₃ during storage of hard water samples at low temperatures

The average CaO content measured in the samples at the six sampling sites was for the entire sampling period of 8 months in the Koppány catchment 192 ± 52 mg L^{-1}, while for the Zagyva 175 ± 33 mg L^{-1}, or expressed as German hardness (°dH), 19.2 ± 5.2°dH and 17.5 ± 3.3°dH, respectively (1°dH corresponds to 10 mg CaO per liter of water). According to the German hardness scale, water is considered hard when exceeding 14°dH and very hard above 21.3°dH. This means that the water in samples from the Koppány and Zagyva catchment is hard or very hard.

Due to the high hardness of the water, a tiny precipitate of CaCO₃ was observed in all collected composite samples from the six sampling sites of both rivers after thawing of non-acidified samples. As a result of CaCO₃ precipitation and the lower content of dissolved CaCO₃, the conductivity after thawing was lower (the mean Koppány value dropped from 1131 ± 69 μS cm^{-1} to 873 ± 116 μS cm, while the mean Zagyva value from 1100 ± 42 μS cm^{-1} to 817 ± 44 μS cm^{-1}). On the contrary, the presence of precipitated CaCO₃ particles increased the turbidity of water after thawing (the Koppány mean increased from 71 ± 61 NTU to 101 ± 61 NTU, while the Zagyva mean increased from 14.3 ± 7.74 NTU to 80.1 ± 53.4 NTU). Regarding the pH, it was expected that the pH would decrease due to lower dissolved content of CaCO₃ after thawing. In contrast, the pH in the composite sample increased after thawing. This phenomenon was related to the fact that the time necessary to thaw 0.4 L of the composite sample was about 24 h. During the thawing, carbon dioxide was released from the sample, which resulted to an increase in the pH value. For the Koppány river water, the mean value for pH was increased from 8.23 ± 0.04 before freezing to 8.9 ± 0.22 after thawing, while for the Zagvya river water from 8.21 ± 0.04 to 8.7 ± 0.24.

3.2 Changes in concentrations of PTEs during storage of hard water samples at low temperatures using different preservation procedures

In order to examine how co-precipitation with CaCO₃ may affect the determination of dissolved PTE concentrations, composite river water samples collected at low water discharges and event-driven samples gathered at high water discharges from the Zagyva and Koppány catchments were analyzed using two sample preservation procedures as described in Sect. 2.3. The results of these experiments are presented in Figs. 2, 3, 4, 5, 6, 7, and 8. In assessing the statistical significance of the differences between the two sample preservation procedures, the Student t-test was applied for samples collected at low and high water discharges. The mean values for individual PTE concentrations and the corresponding p values for each set of samples are presented in Table 1, while the raw data used for calculations of p values are shown in Table S4 (Supplementary information).

As shown in Figs. 2, 3, 4, 5, 6, 7, and 8, concentrations of dissolved PTEs in water were generally higher at low water discharges. Lower concentrations at high water discharges were likely due to dilution by rainwater and water from surface runoff. This effect is the most pronounced in the case of Zn, which is present in the highest dissolved concentrations among PTEs analyzed. In Zn, such difference is evident not only between high and low water discharges but also at the same sampling site, between samplings performed at low water discharges. The first and second sets of PTE

Fig. 2 Dissolved Pb concentrations in river water samples from the Koppány and Zagyva catchments at low and high water discharges, using two sample preservation procedures. Pb concentrations were determined by ICP-MS. Measurement uncertainty is better than ± 3%
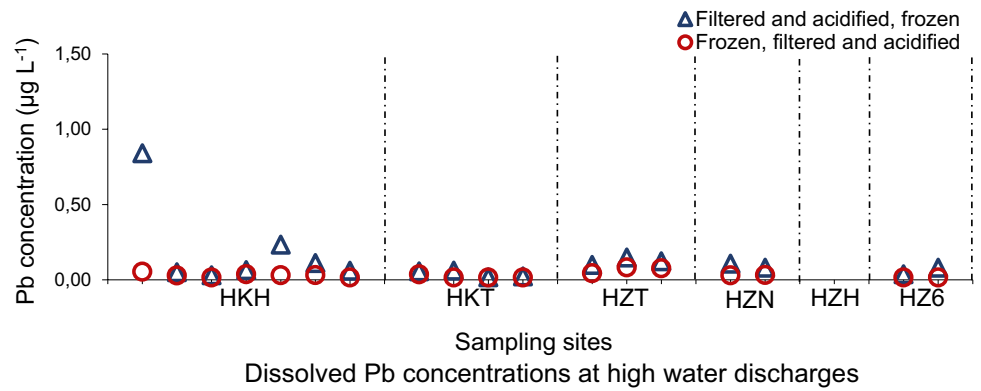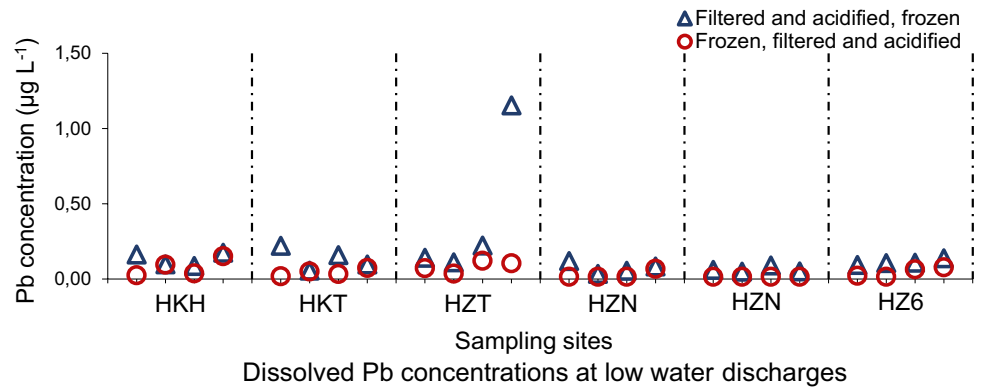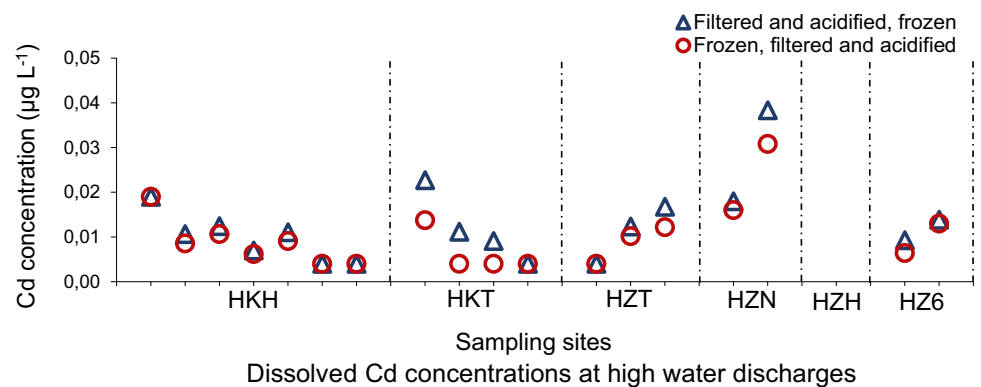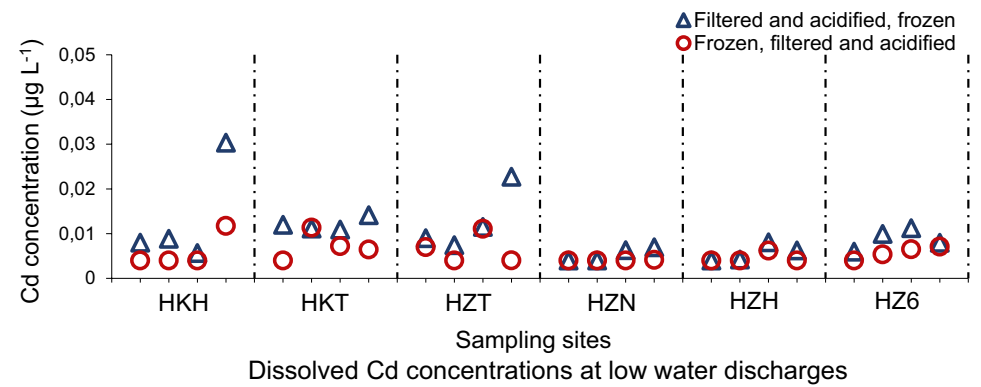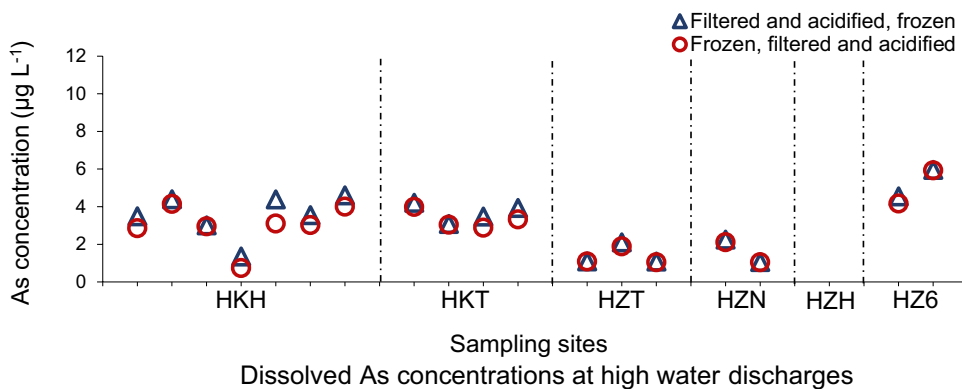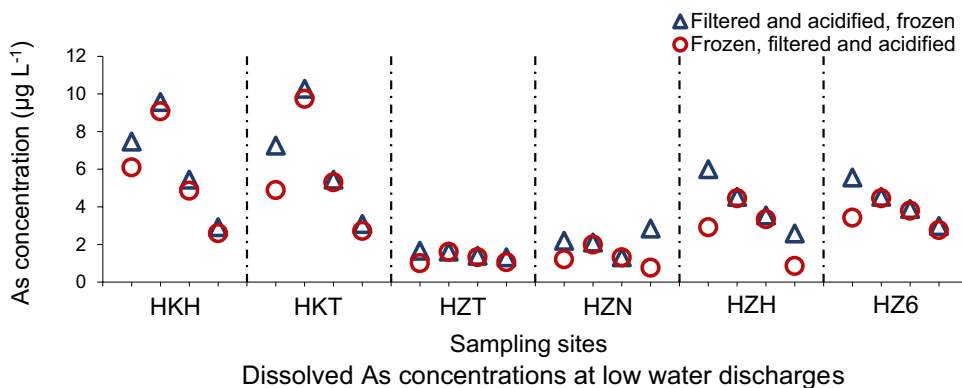

Dissolved Pb concentrations at low water discharges

Dissolved Pb concentrations at high water discharges

Fig. 3 Dissolved Cd concentrations in river water samples from the Koppány and Zagyva catchments at low and high water discharges, using two sample preservation procedures. Cd concentrations were determined by ICP-MS. Measurement uncertainty is better than ± 3%

Dissolved Cd concentrations at low water discharges

Dissolved Cd concentrations at high water discharges

Fig. 4 Dissolved As concentrations in river water samples from the Koppány and Zagyva catchments at low and high water discharges, using two sample preservation procedures. As concentrations were determined by ICP-MS. Measurement uncertainty is better than $\pm 3\%$
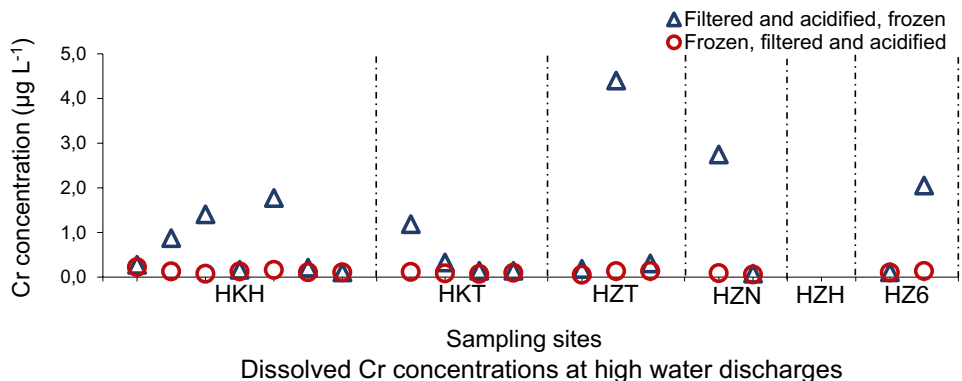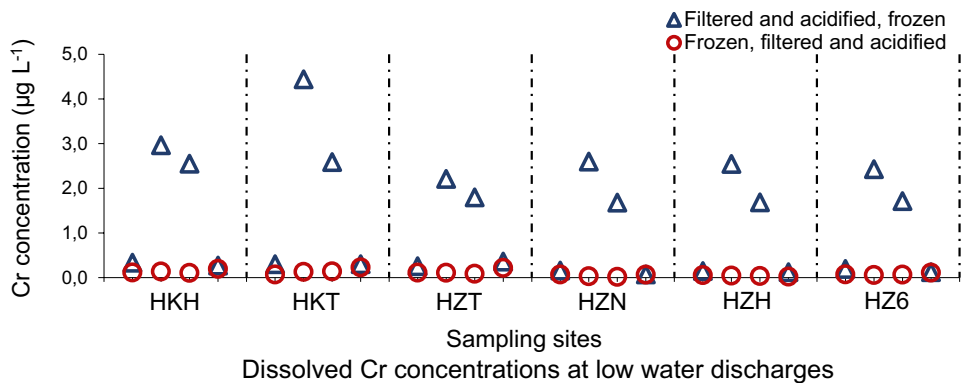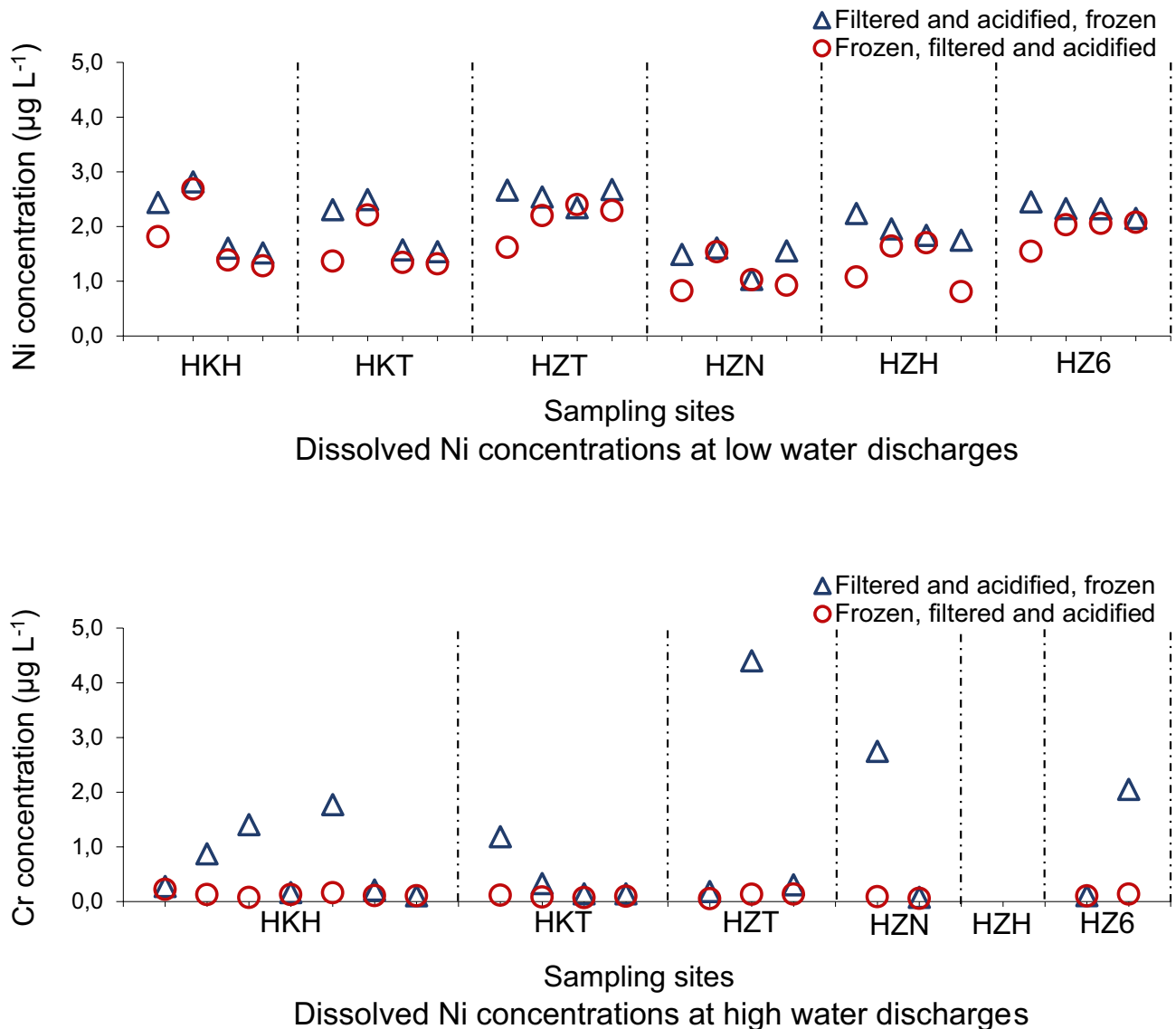

Dissolved As concentrations at low water discharges

Dissolved As concentrations at high water discharges

Fig. 5 Dissolved Cr concentrations in river water samples from the Koppány and Zagyva catchments at low and high water discharges, using two sample preservation procedures. Cr concentrations were determined by ICP-MS. Measurement uncertainty is better than $\pm 3\%$

Dissolved Cr concentrations at low water discharges

Dissolved Cr concentrations at high water discharges

Fig. 6 Dissolved Ni concentrations in river water samples from the Koppány and Zagyva catchments at low and high water discharges, using two sample preservation procedures. Ni concentrations were determined by ICP-MS. Measurement uncertainty is better than ± 3%

concentrations at each sampling site at low water discharges represent composite samples collected under extremely dry conditions, i.e., May–June and July–August, when river water discharges were also very low (around 0.07 $m^3 s^{-1}$ in Koppány and 0.01 $m^3 s^{-1}$ in Zagyva), which means very small volumes of river water. Consequently, at extremely low water flows, Zn concentrations were the highest. The third and the fourth sets of PTE concentrations at each sampling site are composite samples collected in September–October and November–December, when there were more rainy periods and consequently higher water discharges (around 1.2 $m^3 s^{-1}$ in Koppány and 4.1 $m^3 s^{-1}$ in Zagyva). Periods of heavy rain were excluded from these samplings. From ranges of mean PTE concentrations presented in Table 1, it

can be seen that dissolved PTEs can be 20 to 50% lower due to the dilution factor at high water discharges. Exceptions were higher dissolved Cu concentrations observed in water samples collected at high water discharges at sampling sites HKH, HZN, and HZ6, which were most likely related to a more extensive release of Cu from the suspended particulate matter associated with fulvic acids. Fulvic acids are present in sediments and suspended particles as a decay of plant and animal remains and as a product of microbial metabolism. They form strong negatively charged complexes with Cu^{2+} ions, which leads to increased solubility of Cu in natural waters (Britannica 2016). This effect is mostly more pronounced during sediment perturbation at high water discharges, which can desorb Cu from suspended particles
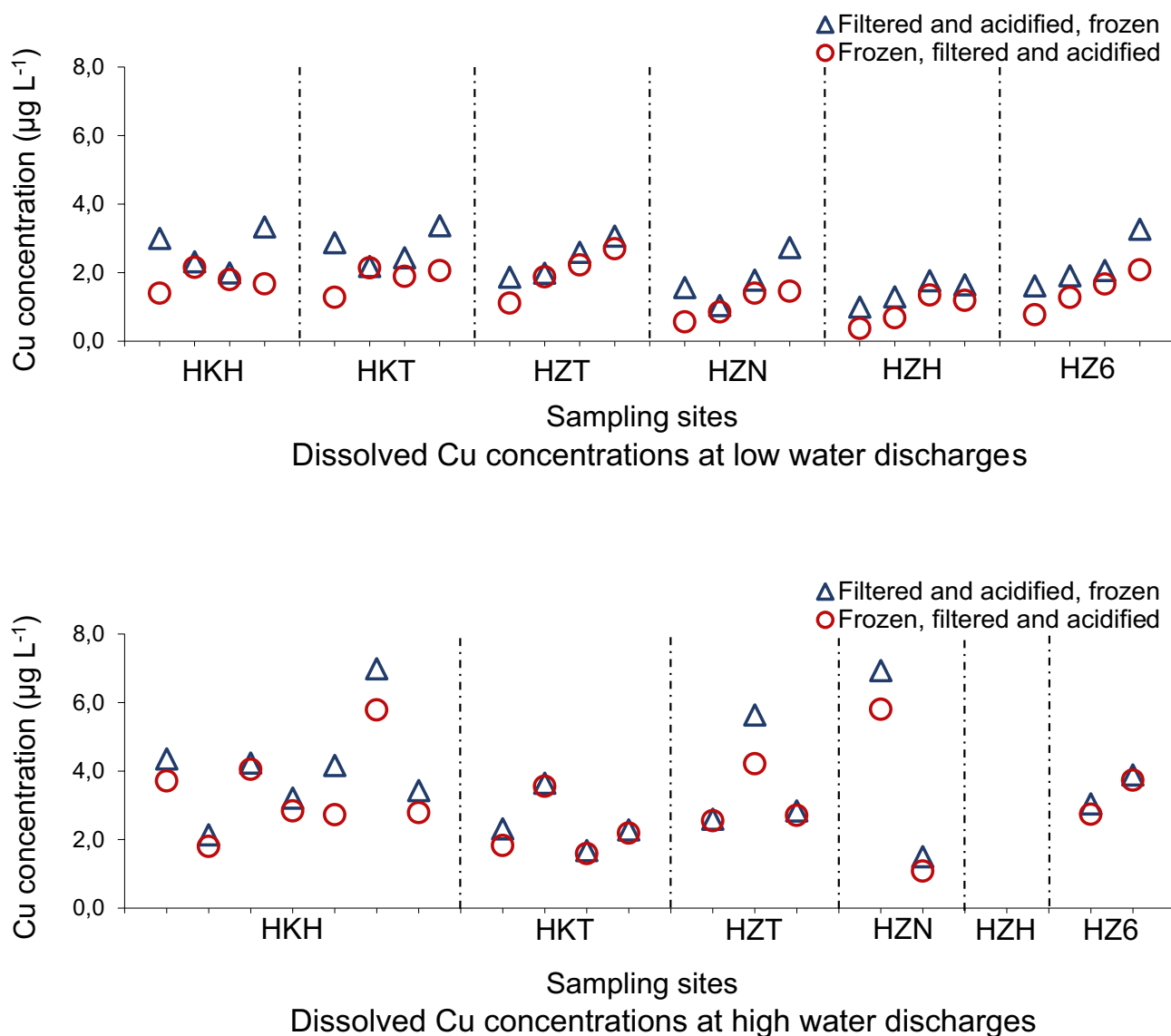
Fig. 7 Dissolved Cu concentrations in river water samples from the Koppány and Zagyva catchments at low and high water discharges, using two sample preservation procedures. Cu concentrations were determined by ICP-MS. Measurement uncertainty is better than $\pm 3\%$

(Eggelton and Thomas 2004; Zuliani et al. 2022). Data from Figs. 2, 3, 4, 5, 6, 7, and 8 further show that dissolved concentrations of PTEs determined in samples that were filtered and acidified and then frozen were generally higher than in samples that were first frozen and filtered and acidified after thawing. The difference in PTE concentrations between the two sample preservation procedures indicates the extent of adsorption onto the $CaCO_3$ precipitate and subsequent co-precipitation of PTEs when the samples are not acidified before freezing. The degree to which PTEs co-precipitate with $CaCO_3$ also depends on the concentration of elements in the water samples and the tendency of each individual element for adsorption onto $CaCO_3$. The influence of water hardness on the co-precipitation of PTEs with $CaCO_3$ is also evident from

the significant statistical differences (p value $< 5.0 \times 10^{-2}$) observed between the two sample preservation procedures (Table 1). Due to the generally lower dissolved concentrations of PTEs, the p values for samples at high water discharges are higher (they varied less) than at low water discharges. The most distinctive PTE at low water discharges was Zn (p value 3.6×10^{-8}), followed by Cu, Ni, and Cr (corresponding p values 6.2×10^{-7}, 6.7×10^{-6}, and 7.7×10^{-5}, respectively), while As, Cd, and Pb differed less (corresponding p values 3.8×10^{-4}, 9.2×10^{-3}, and 2.2×10^{-2}, respectively). For example, Zn concentrations determined at low water discharges were reduced by about 85 to 95% of their true value due to co-precipitation with $CaCO_3$ when samples were not acidified before freezing. For the same reason, Cu, Cr,

Fig. 8 Dissolved Zn concentrations in river water samples from the Koppány and Zagyva catchments at low and high water discharges, using two sample preservation procedures. Zn concentrations were determined by ICP-MS. Measurement uncertainty is better than ±3%
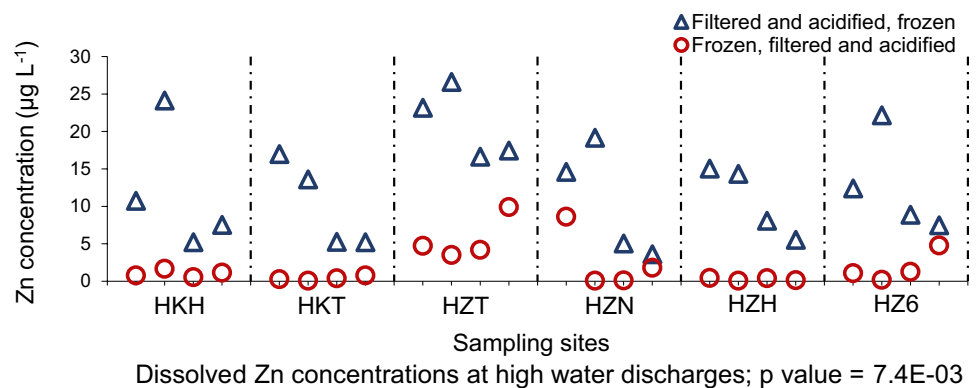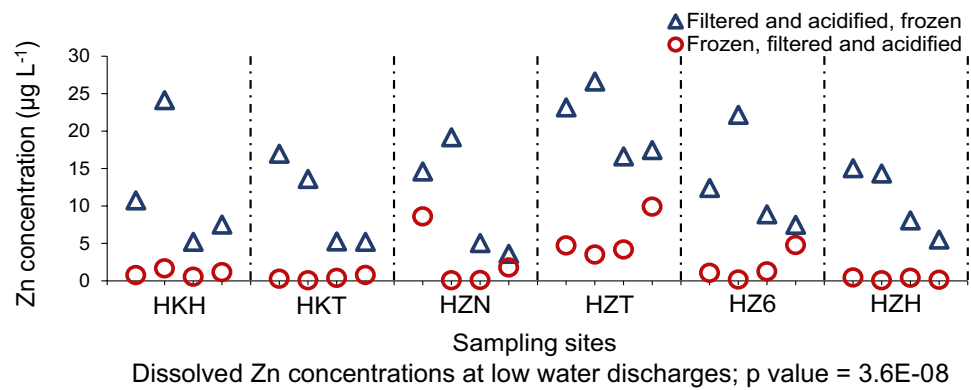
and Ni concentrations can be reduced by up to 50%, 95%, or 60%, respectively. In the river waters investigated, there is no known source of hexavalent Cr (Cr(VI)). Cr(VI) is an oxyanion (Ščančar and Milačič 2014; Milačič and Ščančar 2020). Due to its negative charge, it is not expected that it will adsorb to the surface of the $CaCO_3$ precipitate. Experimental data show that a considerable fraction of Cr is adsorbed, since after thawing, its dissolved concentration is much lower than the concentration of the dissolved Cr before freezing. These data indicate that Cr in the investigated river waters is most likely present in the trivalent oxidation state. In river waters, As is mostly present as the oxyanion of pentavalent arsenate or trivalent arsenite species, among which arsenate predominates (Baeyens et al. 2007). Due to the negative charge of arsenate, the extent of adsorption and co-precipitation of As with $CaCO_3$ is much smaller than with Zn, which means that the concentrations of As with the two sample preservation procedures differ less. This is also evident from *p* values, which are 3.6×10^{-8} for Zn and 3.8×10^{-4} for As.

The results of this study show that PTEs can co-precipitate with $CaCO_3$ to a large extent if the samples are not acidified before freezing. The extent of co-precipitation with $CaCO_3$ depends on PTE concentrations, their solubility in river water and tendency to adsorb onto surface of negatively charge $CaCO_3$ precipitate. The data demonstrated that when preparing samples for the analysis of dissolved PTE concentrations, individual aliquots of river water must be filtered and acidified before freezing. Acidification before freezing prevents the precipitation of $CaCO_3$ after thawing and the subsequent co-precipitation of PTEs with $CaCO_3$. Although a larger number of filters is required, which also means higher costs when analyzing a large number of composite samples, only filtering and acidifying the samples before freezing enables accurate and reliable determination of dissolved concentrations of PTEs in river water samples. Though the experiments were performed on hard or very hard water samples, similar effect of co-precipitation of PTEs with $CaCO_3$ during freezing, but to a lesser extent, can be expected for moderately hard water samples. For soft water samples, this phenomenon is not expected.

Table 1 Mean concentrations with standard deviations of the dissolved contents of PTEs in river water samples from the Koppány and Zagyva catchments at low (n=24)* and high water discharges (n=18)*, using two sample preservation procedures. PTE concentrations were determined by ICP-MS. p values were calculated using Student's t-test at a 0.05 level of significance

Element	Procedure	Low water discharges Mean concentration ($\mu g\ L^{-1}$)	p value	High water discharges Mean concentration ($\mu g\ L^{-1}$)	p value
Pb	Filtered and acidified, frozen	0.158 ± 0.222	2.2×10^{-2}	0.151 ± 0.204	5.2×10^{-2}
	Frozen, filtered and acidified	0.050 ± 0.040		0.037 ± 0.021	
Cd	Filtered and acidified, frozen	0.013 ± 0.007	9.2×10^{-3}	0.016 ± 0.008	3.1×10^{-3}
	Frozen, filtered and acidified	0.007 ± 0.003		0.013 ± 0.007	
As	Filtered and acidified, frozen	4.12 ± 2.19	3.8×10^{-4}	3.20 ± 4.40	4.4×10^{-4}
	Frozen, filtered and acidified	3.37 ± 2.05		2.85 ± 1.32	
Cr	Filtered and acidified, frozen	1.33 ± 1.21	7.7×10^{-5}	0.915 ± 1.15	1.0×10^{-2}
	Frozen, filtered and acidified	0.095 ± 0.059		0.111 ± 0.040	
Ni	Filtered and acidified, frozen	2.05 ± 0.455	6.7×10^{-6}	2.02 ± 0.812	7.4×10^{-3}
	Frozen, filtered and acidified	1.63 ± 0.482		1.78 ± 0.600	
Cu	Filtered and acidified, frozen	2.20 ± 0.700	6.2×10^{-7}	3.60 ± 1.56	2.7×10^{-4}
	Frozen, filtered and acidified	1.50 ± 0.587		3.09 ± 1.27	
Zn	Filtered and acidified, frozen	12.9 ± 6.58	3.6×10^{-8}	4.15 ± 4.07	7.4×10^{-3}
	Frozen, filtered and acidified	1.95 ± 2.75		1.95 ± 2.37	

*For Cd and Pb, samples with concentrations below the limit of quantification were excluded from t-test calculations

4 Conclusions

When analyzing a large number of river water samples, the costs of analysis can be reduced, and the analysis time shortened if composite samples are used. Storing samples at -20 °C prevents PTE adsorption, but sample storage at low temperatures can cause precipitation of $CaCO_3$ and subsequent co-precipitation of dissolved amounts of PTEs. This phenomenon is especially pronounced in samples with high water hardness. PTEs can co-precipitate to a large extent. Experimental results from this study showed that co-precipitation can cause up to 95% loss of dissolved Zn and Cr concentrations, 60% of Ni concentration, and 50% of Cu concentrations from the analyzed sample. Since As in river water is mainly present as negatively charged arsenate, its adsorption capacity on $CaCO_3$ precipitate is lower. Consequently, the extent of co-precipitation is also smaller. For Pb and Cd, which are generally present in river water in very low concentrations, which are close to LOD values, the loss is not detectable, but may be significant if lower LODs are reached. To prevent co-precipitation of PTEs with $CaCO_3$, samples should be filtered and acidified before freezing. The results of the present research significantly contribute to the use of appropriate sample preservation procedures in the analysis of dissolved contents of PTEs in composite samples of hard river water and to the validity of analytical data.

Data availability Data from this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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