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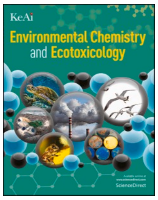

Research Paper

The problem of diffuse pollution of a major river running through predominantly rural area: the case of potentially detrimental levels of tungsten, silver, and manganese in the Mura River

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ABSTRACT

Our aim was to investigate the diffuse pollution impact on large lowland river, namely the Mura River (Croatia), at nine sites in contrasting periods of high/low water discharges, and to characterize potential pollution sources. Physico-chemical parameters and nutrients were analysed in the water, metals/metalloids (44 elements, including rare earths) in water/sediment, and specific microbial genes in sediments. At several sites, the dissolved Ag (0.200–0.220 µg L^{-1}), Mn (133 µg L^{-1}), and W (0.800 µg L^{-1}) surpassed suggested protective concentrations for aquatic biota ($MAC_{Ag} = 0.022$ µg L^{-1}, in new EU WFD proposal; $PNEC_{Mn} = 75$ µg L^{-1}, established for freshwaters; $MAC_W = 0.800$ µg L^{-1}, established in Russia for aquatic systems intended for fishing), whereas high nitrogen (up to 5.8 mg L^{-1}) and phosphorus (up to 1.7 mg L^{-1}) in water were found across all sites. Sediment enrichment factors were especially high for Mn (up to 3.5), Mo (up to 4.3) and W (up to 4.1). Increased microbial Pb-resistance (high *pbrT* gene copies, $\sim 10^{-2}$ gene copies/*rrn* copies) was found in the vicinity of the abandoned coal mines, and elevated *intI1* gene copies ($\sim 10^{-2}$ gene copies/*rrn* copies) throughout the studied river section. The agricultural runoff and the abandoned coal mine drainage were indicated as major contamination sources. Despite the high dilution capacity of the Mura River, the pollution level was detected that can be hazardous for some aquatic organisms. To preserve the rich biodiversity of this UNESCO Biosphere Reserve, further assessment of pollution consequences is highly recommended.

1. Introduction

Water pollution, originating from point and diffuse sources, presents a serious problem, affecting at least half the world's population with its consequences [1,2]. Today, point sources of pollution are mainly under control, since they are relatively easy to identify, quantify, and manage by policy approaches [2–5], whereas it is more difficult to master over the diffuse sources. The diffuse pollutants arise from multitude of diverse urban/rural land use activities that are indirectly discharged to receiving water bodies (through subsurface flow, atmospheric deposition, leaching through the soils to groundwater). The impact is commonly most visible during periods of rainfall and irrigation, and most severely during storm periods [2,4,6,7]. The pollution from diffuse sources is currently the leading cause of water quality impairment, with negative impact on ecosystem health [3,4]. It is usually associated with the following occurrences: nitrogen/phosphorus pollution, pesticide/pharmaceutical pollution, and metal/metalloid pollution, originating from fertilizers/manure applied in agriculture or domestic plant/animal protection, urban storm-water runoff, and leaching of mining/industrial wastes [2,4]. Consequently, diffuse pollution can cause damage to aquatic ecosystems, especially freshwater, including various detrimental effects in aquatic organisms, even loss of biodiversity [2,4,8]. Thus, a policy framework of Organisation for Economic Cooperation and Development (OECD) and European Water Framework Directive (EU WFD [9]), recommend the identification of pollutants (by monitoring soils/sediments/waters in the impacted areas [10]), their sources, pathways, timing, and the sensitivity of the receiving environment, as an initial step in the management of the diffuse pollution [2].

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The rivers and streams with smaller water discharges, and consequently lower dilution capacity, are usually more prone to increased water contamination, even under moderate anthropogenic influence, whereas high water discharge can mitigate even rather high waste inputs, as seen from the comparative examples of the rivers Sava (~80–2000 $m^3 s^{-1}$) and Sutla (~1–70 $m^3 s^{-1}$) in Croatia [11,12]. It is generally not expected to detect high contamination in the major river flowing through an area not heavily burdened by pollution sources, such as the Mura River within Croatia. It is a large lowland river, with water discharge in the range comparable to the Sava River (~50–1300 $m^3 s^{-1}$), that rises in the western part of Austria. It has a total length of 465 km [13], and it flows through four countries: Austria, Slovenia, Hungary and Croatia, encompassing two biogeographical regions: Alpine and Pannonian. It forms a part of world's first five country Transboundary UNESCO Biosphere Reserve "Mura-Drava-Danube", the so-called "Amazon of Europe", which hosts an amazing biological diversity and is a hotspot of rare natural habitats [14]. It flows through Croatia in a length of 83 km, of which 79 km represents the state border between Croatia and Hungary, and forms a part of a protected area of Regional park Mura-Drava. Nevertheless, considering geographical, economic and land use features of its basin within Croatia, the certain influence of diffuse pollution can be expected, mainly from traditional agricultural activities along its entire length, and from historical coal mining along a part of its upstream course [15,16]. In catchments that are dominated by arable fields, the impact of agriculture on water contamination is still considered a major problem, which is very difficult to eliminate [17,18]. On the other hand, several studies have demonstrated lasting detrimental impacts of abandoned coal mines on water quality, emphasizing that it is a major environmental issue [19,20]. In the upper section of the Mura River in Croatia, there are well known thermal baths that discharge their spent waters in the nearby stream, whereas in Slovenia, across from Mursko Središće, there are oil deposits and pharmaceutical facility. In the downstream section, however, there are several additional potential sources of pollution in Hungary, mainly referring to municipal sewage treatment plants, intensively farmed areas and livestock farms. Across from Goričan (Croatia), there are thermal baths Letenye in Hungary that discharge their spent waters into Béci stream, tributary to the Mura River [21]. Thus, additional impact of washout from these sources could also be presumed.

As water/sediment pollution in such ecosystem of high biological diversity can eventually cause a serious damage, the aim of this study was to establish to what degree typical diffuse sources have contaminated this large lowland riverine ecosystem. As metal pollution in surface water is a global environmental problem [22], the main focus of our study was on metal/metalloid contamination. We have conducted comprehensive spatial and temporal investigation of physico-chemical disturbances (including nutrients) and main inorganic pollutants (44 trace and major elements) in the Mura River water and/or sediment. Emerging contaminants, such as technology critical elements (e.g., tungsten; rare earth elements, REEs), that can potentially cause toxicity to biota and that are still not thoroughly investigated, were encompassed by the study. For example, it was reported that tungsten causes toxicity to plants (e.g., inducing programmed cell death), to bacteria, mice, and aquatic organisms (e.g., to some fishes through epithelial damage) [23]. REEs, on the other hand, were reported to affect various aquatic species at different trophic levels (e.g., algae, crustaceans, fishes), causing toxicty from cellular levels (interferring with biological processes) to behavioural and reproductive alterations, and even having synergistic effects [24]. Moreover, altough REEs are often connected to point sources of pollution (such as municipal sewage outlets), they were reported to origin from diffuse sources, such as natural processes as erosion, as well as agricultural runoff, where the latter results from direct REEs supplementation of fertilizers or from indirect fertilizer contamination through phosphate rocks [25]. Taking in consideration the nature of diffuse water contamination, an additional aim was to define the dependence of contaminants concentrations on hydrological regime, i.e. the comparison was done between two opposite periods, the period of extremely high water discharge in the late spring and of low water discharge in the late summer. Moreover, our goal was to associate potential sources of diffuse pollution with the particular aspects of the water/sediment contamination of the Mura River, by analysing potential source materials, such as coal samples from abandoned coal mine pits and water/sediment from the stream receiving spent geothermal waters. Our final aim was to obtain the first information on the effect of the diffuse pollution in the Mura River on aquatic biota. As some bacteria have adapted to the conditions of high metal contamination, either natural or anthropogenic (e.g., in mining areas), by acquiring metal resistance genes [26], our aim was to perform genetic analyses of total bacteria and several selected bacterial metal-resistance genes. In addition, class 1 integrase intI1 gene was applied as a marker for anthropogenic pollution, as it was regularly linked to genes conferring resistance to antibiotics, disinfectants and metals in a wide variety of pathogenic and nonpathogenic bacteria, and its abundance can change rapidly [27,28].

2. Materials and methods

2.1. The sampling of the river water and sediment, and procuration of the coal samples

The sampling of water and sediment of the Mura River was carried out in two campaigns in 2023, in June (13th to 15th) and October (3rd to 4th). The samplings were performed at nine sites along the river-section flowing through Croatia, and largely forming the border with Hungary (Fig. 1). The sites were the following: 1) MI (46.52511° N, 16.31714° E) – initial sampling point, just downstream from the border of Croatia and Slovenia; 2) MB (46.53553° N, 16.36064° E) – a sampling point near the bridge on the Mura River, in the municipality of Sveti Martin na Muri (4 km downstream of MI); 3) HL (46.52913° N, 16.41316° E) – village of Hlapičina (4 km downstream of MB); 4) MS (46.51481° N, 16.43879° E) – town of Mursko Središće (2.5 km downstream of HL); 5) KR (46.49446° N, 16.49873° E) – village of Križovec (6 km downstream of MS); 6) PT (46.47187° N, 16.55041° E) – village of Podturen (7 km downstream of KR); 7) DK (46.46596° N, 16.61440° E) – village of Dekanovec (7.5 km downstream of PT); 8) GR (46.38956° N, 16.71828° E) – town of Goričan (17 km downstream of DK); and 9) KT (46.33695° N, 16.87039° E) – town of Kotoriba, few kilometres upstream of the mouth of the Mura River into Drava (27 km downstream of GR). As in the upstream section of the studied area there is a thermal resort Sveti Martin, with its wastewaters being poured out in the stream Gradiščak (SG), we have additionally analysed the water and sediment of this stream (46.49701° N, 16.33671° E), which flows towards the Mura River, but inflows into the floodplain, on the right bank of the Mura River (Fig. 1). There are also several other potential sources of diffuse pollution along the studied river-section. On the Croatian side of the Mura River, there is a stretch of abandoned coal mine pits along the river-flow, from the village Hlapičina to the village Križovec (~9–10 km long), while just across the river from Mursko Sredisće, on the Slovenian side, there are oil deposits and pharmaceutical facility producing antibacterial drugs. Further downstream, in Croatia, close to the mouth of the Mura River and the town Kotoriba, there are large facilities for production of plastic and metal products. On the Hungarian side, there are municipal sewage treatment plants and livestock farm on the river stretch from Goričan to Kotoriba, as well as thermal baths across the river from Goričan (downstream from our sampling point). Also, along the entire studied section of the Mura River, at both banks, there is a stretch of arable fields.

For analyses of dissolved metals/metalloids, the surface river water was collected in triplicates in polyethylene plastic bottles (0.25 L), rinsed with HNO_3 (*v*/v 10 %, *p.a.*, Kemika, Croatia) and Milli-Q water prior to sampling. The aliquots of sampled water were immediately filtered through FilterBio® CA syringe filters (0.45 μm pore diameter,

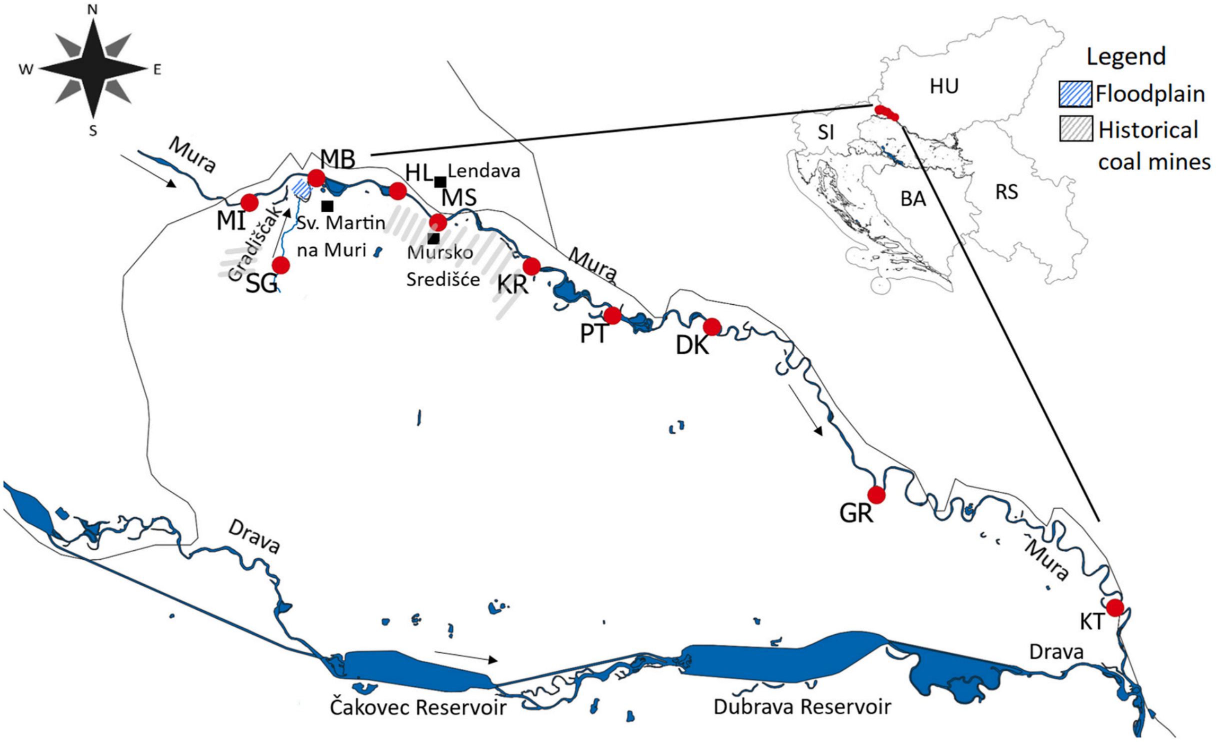

Fig. 1. The map of study area, i.e. the Mura River section that runs through Croatia or forms the state border between Croatia and Slovenia/Hungary, with marked sampling sites, floodplain, and historical coal mines. The site legend: (1) the Mura River sites: MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba; (2) the stream Gradiščak site: SG.

Labex Ltd., Germany) and acidified with HNO$_3$ (v/v 2 %; Normatom, 67–69 %; VWR Chemicals, USA) to obtain the dissolved fraction. The river-water was separately collected for the laboratory measurements of several physico-chemical parameters and nutrients (total phosphorus, TP, and nitrogen, TN; nitrates and ammonia; turbidity and chemical oxygen demand, COD – in the white plastic bottles of 1 L; for chlorophyll-a, Chl_a – in dark plastic bottles of 1 L), and stored at +4 °C for the subsequent analyses.

At each site, upper 10 cm of river sediment were collected with a spatula in triplicates from the lateral parts of the riverbed (right riverbank, on Croatian side), put in plastic bags, and stored under ice-cold conditions. By limiting sampling to the top 10 cm, the study focuses on recent contamination inputs rather than historical contamination from deeper sediment layers, providing a more accurate assessment of current sediment quality and recent anthropogenic impacts. As the water-level was lower in October sampling, the sediment samples collected then were closer to the middle point of the riverbed compared to those collected in June, as presented in the Fig. ESM.1. Subsamples of fresh sediments were put aside for genetic analyses of bacterial community and granulometric analysis.

The samples of brown coal from abandoned coal mine pits in the area of town Mursko Središće were procured from *Center for culture "Rudar"* (Mursko Središće).

2.2. Determination of physico-chemical characteristics of the river water

Several physico-chemical parameters were determined *in situ* in the river water at respective sampling sites using the portable field probes, calibrated prior to each sampling. The water temperature (t_{water}), pH, dissolved oxygen (DOX) concentrations, and conductivity were measured using portable digital meters SevenGopro SG7 (Mettler Toledo, Switzerland-USA). The remaining physico-chemical parameters were measured in the laboratory applying the methods from Water Analysis Handbook [29] and using UV/VIS spectrophotometer DR/6000 (HachLange, USA); or applying the standard titration methods [30]. TP was measured according to Hach method LCK348; ammonium, according to Hach method 8155; nitrates, according to Hach method 8192; TN, according to oxidative digestion method (ISO 11905-1:1997); turbidity, according to Hach method 8237; COD, according to Mn III method (ISO8467:1993); and Chl_a, according to ISO 10260:1992 method after water filtration through GF/F filter paper (the filtered sample was stored at −24 °C prior to analysis). Moreover, dissolved CO$_2$ was measured by phenolphthalein titration method, and total and carbonate hardness (TH and CH, respectively) by EDTA titration method. The results are presented in Table 1 and Fig. 2, whereas the legally recommended values for selected physico-chemical parameters are given in Table ESM.1.

2.3. Determination of dissolved trace and major elements concentrations in the river water and calculation of Gd-anomalies

Forty trace elements, including lanthanides, were measured directly in filtered and acidified river-water samples, whereas the samples were ten times diluted for the measurement of the four major elements (Ca, K, Mg, Na). Element concentrations were measured in helium collision mode using ICP-MS (Agilent 7900, Agilent Technologies, USA). Calibration straight lines (based on the following trace element concentrations: 0, 1, and 10 μg L^{-1}; and additional concentration for Mn and Fe, 100 μg L^{-1}; and for major elements: 0, 1, and 5 mg L^{-1}) were created for each element using adequate dilutions of multielement standard solutions for trace and major elements (CPAChem, Bulgaria) prepared in 2 % (vol.) HNO$_3$ (Normatom® 67–69 % for trace element analysis, VWR Chemicals, UK). Standard solution mixture, containing Sc, Ge, Y, In, and Tb (CPAChem, Bulgaria), was used as an internal standard (10 μg L^{-1}). Measurements were further performed in adequately prepared blank samples, and the measured concentrations in water were corrected for

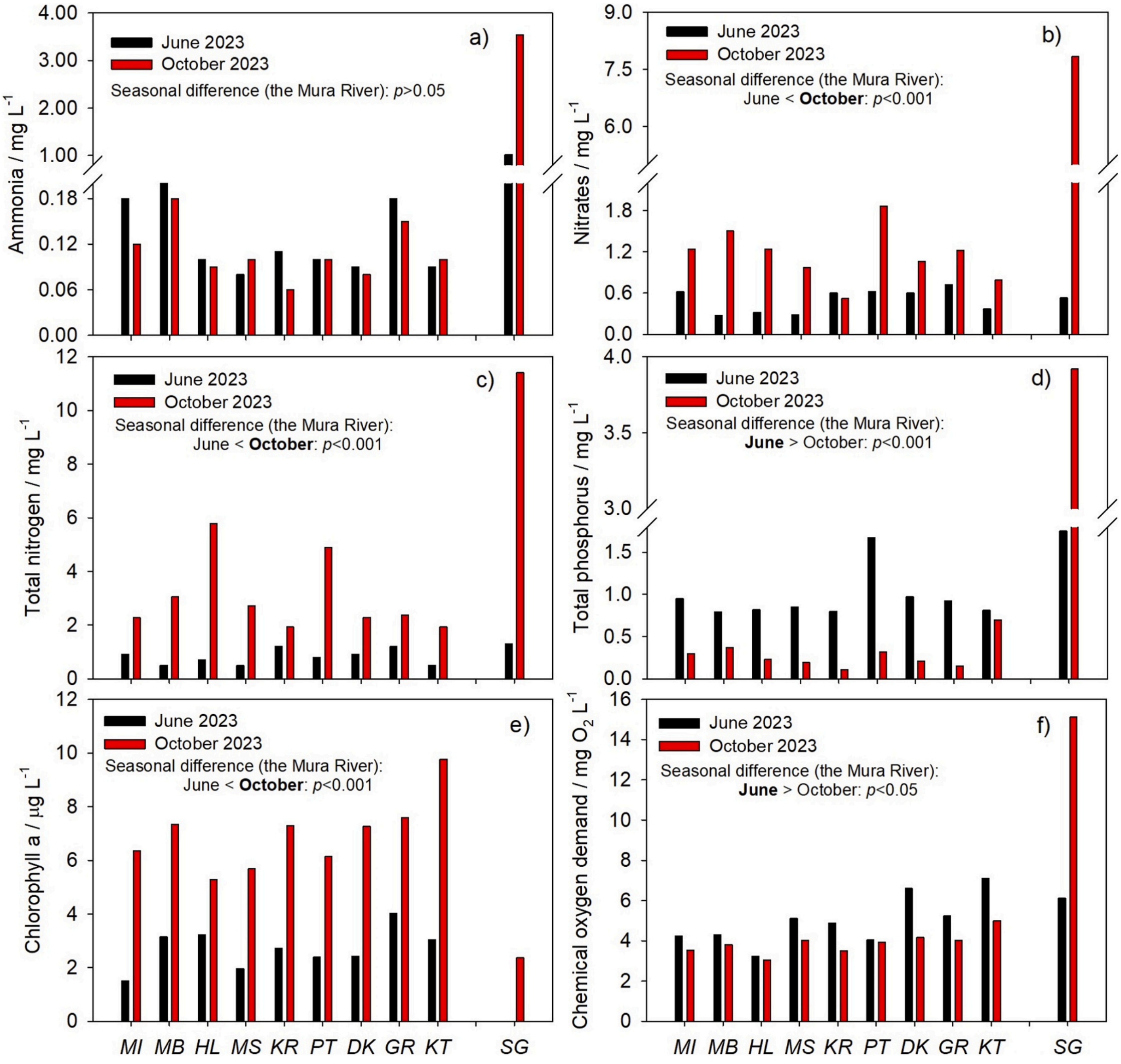

Fig. 2. The values of nutrients (ammonia (a), nitrates (b), total nitrogen (c) and total phosphorus (d)), chlorophyll *a* (e), and chemical oxygen demand (f), measured in June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and in the stream Gradiščak (SG). The results were presented as vertical bars (n = 1). Statistical comparison was made between two samplings (for all nine Mura-sites together), with the *p*-values stated within the figure.

the blanks, if it was needed. The limits of detection (LODs) were calculated as three standard deviations of trace and major element concentrations measured ten times consecutively in the blank samples, applying the same procedure as for the actual samples (Table ESM.2). If any of the obtained concentrations was below LOD, LOD value was applied instead for the purpose of statistical analyses. The accuracy control of the measurement in the water was performed using certified control samples (CPAChem, Bulgaria), and deviations from certified values were generally within ±10 % (Table ESM.2). The results on dissolved trace and major element concentrations in the river-water are presented in Table 2, Figs. 3–5, and Tables ESM.3–4.

Gadolinium anomalies and anthropogenic Gd were calculated according to adapted Hissler et al. [31] equations:

$$\mathrm{Gd-anomaly}: \mathrm{Gd}/\mathrm{Gd}^{*} = \mathrm{Gd}_{\mathrm{EUS}} / (0.4 \times \mathrm{Nd}_{\mathrm{EUS}} + 0.6 \times \mathrm{Dy}_{\mathrm{EUS}})$$

$$\mathrm{Gd}_{\mathrm{ant}} = \mathrm{Gd}\text{–}\mathrm{Gd}^{*}$$

where Gd refers to measured concentrations in the samples; Gd*, to natural concentrations estimated using other REEs; whereas $\mathrm{Gd}_{\mathrm{EUS}}$, $\mathrm{Nd}_{\mathrm{EUS}}$, and $\mathrm{Dy}_{\mathrm{EUS}}$ refer to respective REE concentration normalised on European Shale, a reference material [32]. Hissler et al. [31] have used normalisation on Post Archean Australian Shale (PAAS), whereas Bau et al. [32] recommended rare earths normalisation on European Shale for samples originating from Europe. The results on Gd-anomalies are presented in Fig. 6.

2.4. *Determination of trace and major elements concentrations in the river sediment*

The sediment samples were air-dried, sieved through a 2 mm sieve, homogenised using an agate mill, and stored until further analysis. Prior to analysis, sediment sub-samples (0.05 g) were subjected to total digestion in the microwave oven (Multiwave ECO, Anton Paar, Austria) using a two-step procedure consisting of digestion with a mixture of 4 mL HNO_3 (65 %, *p.a.*, Kemika, Croatia), 1 mL HCl (36 %, *p.a.*, Kemika,

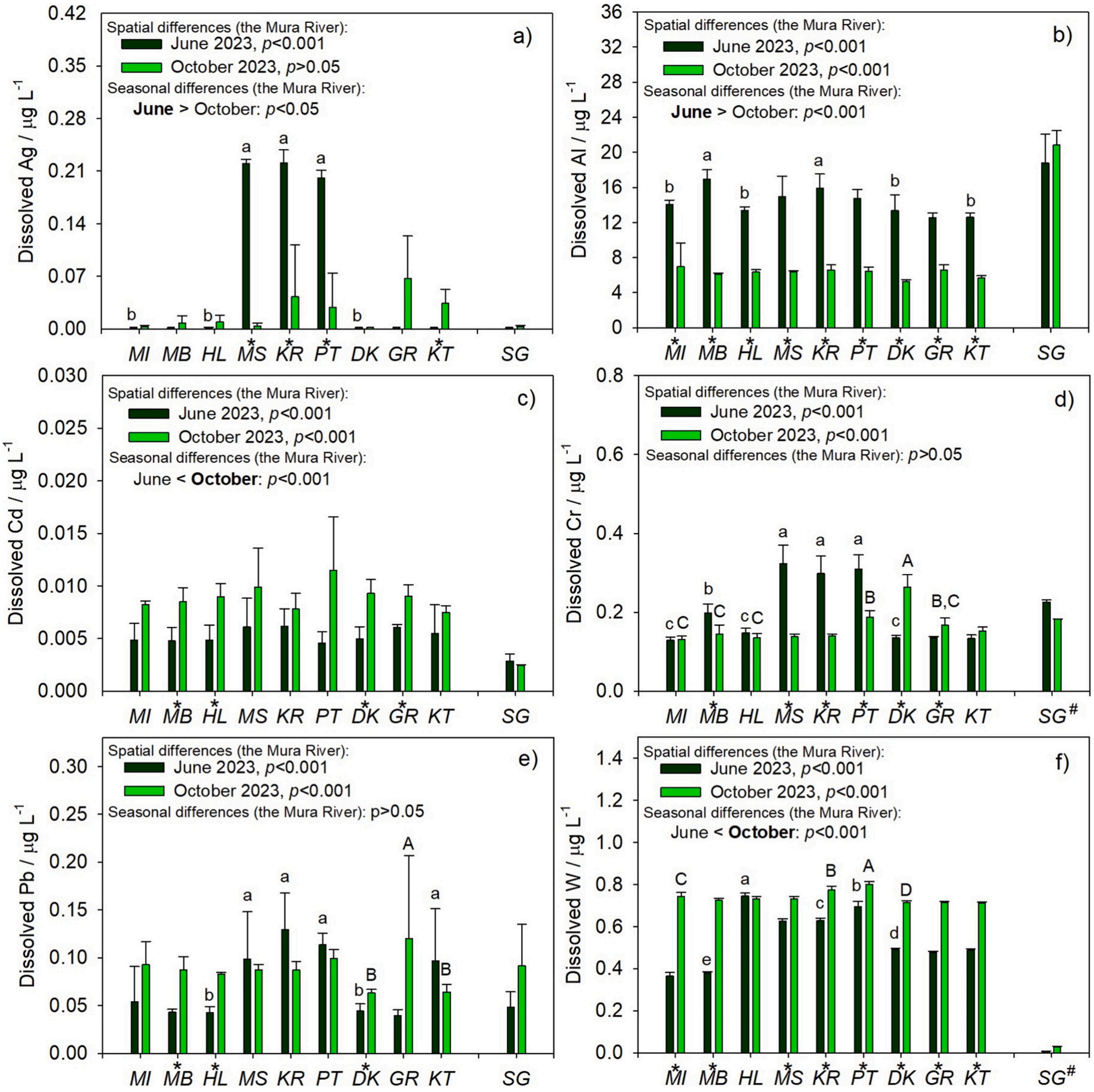

Fig. 3. The concentrations of dissolved metals (Ag (a), Al (b), Cd (c), Cr (d), Pb (e), and W (f)) measured in June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial location in Croatia; MB –Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and in the stream Gradiščak (SG). The results were presented as vertical bars (mean) with assigned standard deviations ($n = 3$). Statistical comparisons were made among nine Mura-sampling sites for each sampling, and between two samplings (for all Mura-sites together), with the p-values stated within the figure (SG was excluded from these comparisons). The selected *post-hoc* comparisons between sites were performed (taking in consideration the number of degrees of freedom); statistically significant differences ($p < 0.05$) between sites were designated with different letters (lower case for June sampling, and upper case for October sampling). Statistically significant differences ($p < 0.05$) between two seasons at each site were designated with asterisks (*).

Croatia), and 1 mL HF (40 %, *s.p.*, Merck, Germany), followed by the addition of 6 mL H_3BO_3 (Fluka, Switzerland). The solution was then transferred to a volumetric flask, filled up to 100 mL, and then diluted tenfold and acidified with 2 % (v/v) HNO_3 (65 %, TraceSELECT, Fluka Germany).

Trace and major elements (total of 47, including lanthanides) were measured using triple quadrupole ICP-MS (Agilent 8900, Agilent Technologies, USA). Calibration curves were generated by external standardization with a series of standard solutions (0, 0.1, 1, and 10 µg L^{-1} for trace elements; 0, 1, and 5 mg L^{-1} for major elements (Na, K, Mg, Ca); and 0, 100, 1000 µg L^{-1} for P and S). These solutions were prepared by appropriate dilutions of multi- and single element standard solutions for trace (100 ± 0.2 mg L^{-1}, Analytika, Czech Republic) and major elements (1000 ± 0.2 mg L^{-1}, Analytika, Czech Republic). Indium (50 µg L^{-1}) was used as an internal standard. The limits of detection (LODs) were calculated and applied on the same principle as in the case of the

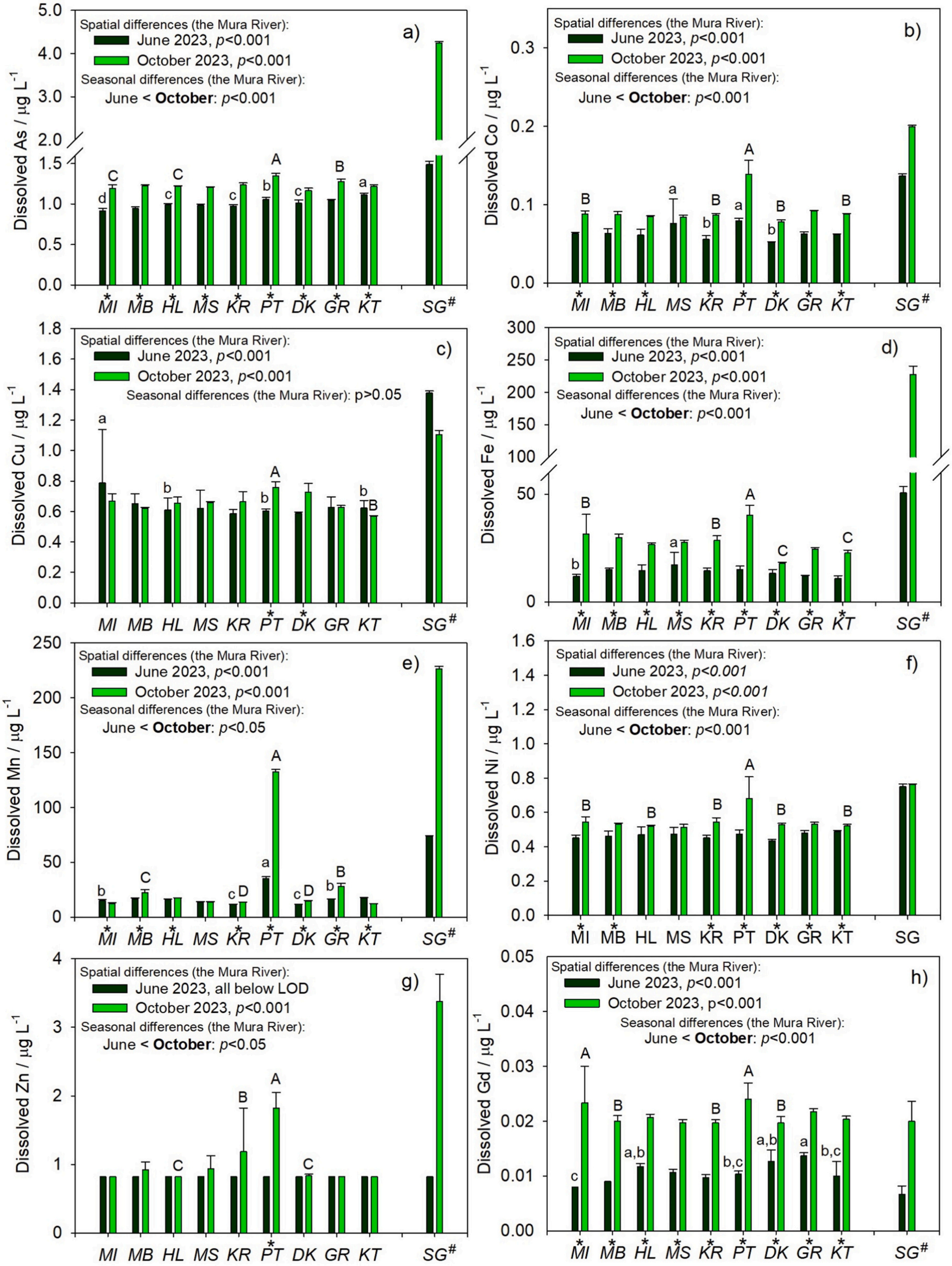

Fig. 4. The concentrations of dissolved metals/metalloids (As (a), Co (b), Cu (c), Fe (d), Mn (e), Ni (f), Zn (g), and Gd (h)) measured in June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and in the stream Gradiščak (SG). The results and statistical comparisons were presented as in the Fig. 3.

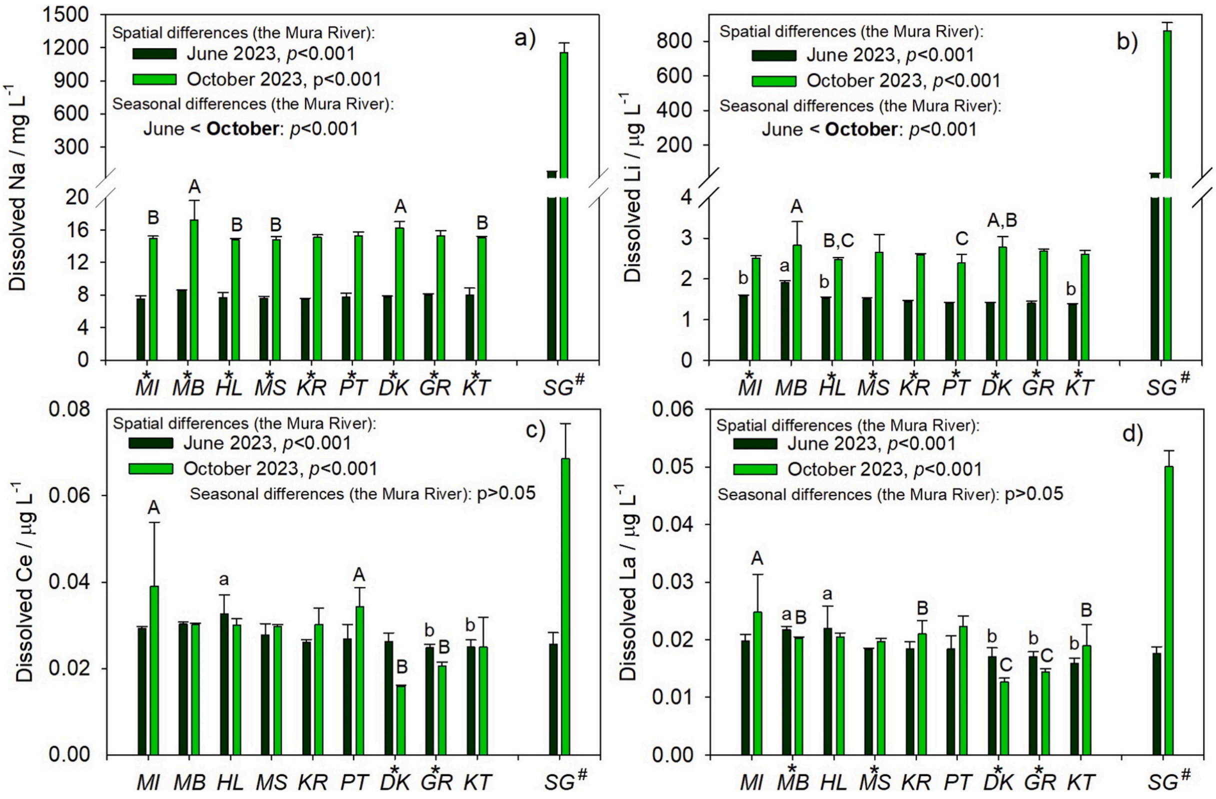

Fig. 5. The concentrations of dissolved metals (Na (a), Li (b), Ce (c), and La (d)) measured in June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and in the stream Gradiščak (SG). The results and statistical comparisons were presented as in the Fig. 3.

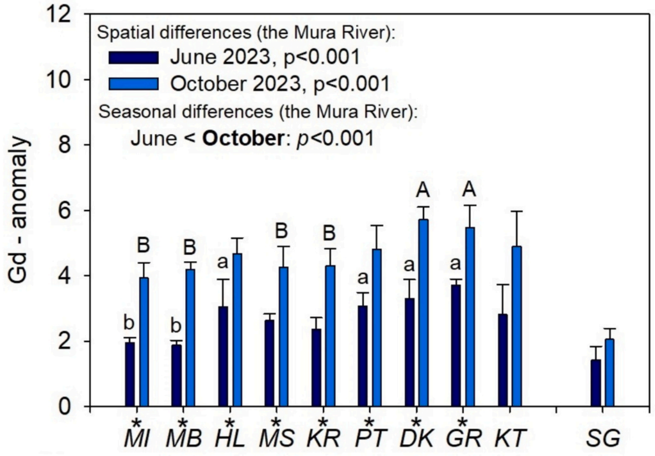

Fig. 6. The Gd-anomalies calculated for June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and at the stream Gradiščak (SG). The results and statistical comparisons were presented as in the Fig. 3.

dissolved elements (Table ESM.2). Analytical quality control of measurements in sediments was performed by simultaneous analysis of procedural blanks and certified reference materials of stream sediment NCS DC 73015, and deviations from the certified values were generally within ±15 % (Table ESM.2). The results on trace and major elements concentrations in the sediment are presented in Table 3, Figs. 7–9, and Tables ESM.5–6.

Enrichment factors (EF) were calculated for all elements analysed in sediments. The obtained concentrations of all elements were first normalised on Al concentrations, as a reference element. EFs were calculated according to the following equation:

$$\mathrm{EF} = (c_M/c_{Al})/(F_M/F_{Al})$$

where c_M refers to measured concentration of the studied element in the sediment; c_{Al}, measured concentrations of the Al in the sediment; F_M and F_{Al}, background concentrations of the studied element and Al, respectively, in the sediment based on upper category values for the study area from the Geochemical Atlas of Europe [33]. The results for the EFs are given in Table 3 and Tables ESM.7–8.

2.5. Determination of grain size distribution in the samples of river sediment

The granulometric analysis of the sediment was carried out for samples collected at the Mura River sampling sites in June 2023 using a laser diffraction particle size analyser (LS 13320, Beckman Coulter, Brea, CA, USA). Sediments were classified according to Shepard [34], and ternary diagram of sediment grain size distribution is presented in Fig. ESM.2.

2.6. Determination of trace and major elements concentrations in coals and coal water eluates

The coal samples (two pieces) were homogenised using an agate mill. Prior to analysis, homogenised coal sub-samples (0.05 g; three sub-samples from each piece) were subjected to total digestion in the microwave oven (Multiwave ECO, Anton Paar, Austria) using the same

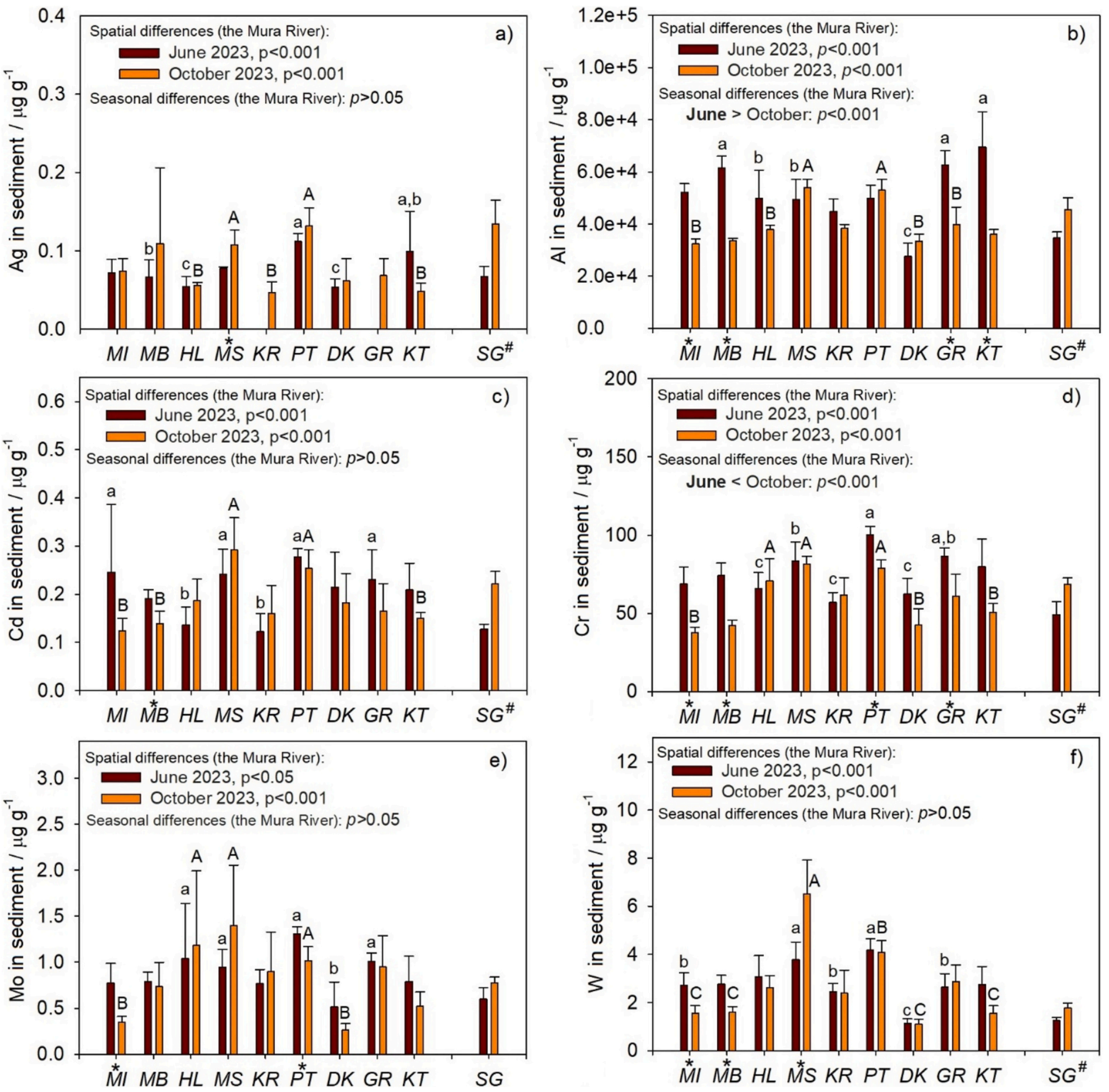

Fig. 7. The concentrations of metals in sediments, Ag (a), Al (b), Cd (c), Cr (d), Mo (e), and W (f), measured in June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and in the stream Gradiščak (SG). The results and statistical comparisons were presented as in the Fig. 3.

procedure as applied for sediments. Additionally, coal water eluates were prepared by water extraction using the European Standard Batch Leaching Test (EN 12457–2, CEN, 2002). Milli-Q (MQ) water with a liquid-to-solid ratio of 10:1 (10 mL MQ, 1 g sample) served as the extraction medium. The prepared samples were shaken on a horizontal shaker for 24 h. After completion of the extraction time, the leachates were separated from the solids by filtration through 0.45-μm filters and acidified with 1 % (*v*/*v*) HNO_3 (TraceSELECT, Fluka Germany).

Prior to analysis, eluates were diluted 100-fold and acidified with 2 % (v/v) HNO_3 (TraceSELECT, Fluka Germany).

Trace and major element measurements in digested coal samples and coal water eluates were performed applying the same instrumentation and procedure as for the analyses in digested sediments. LODs for coals are the same as for the sediments, whereas LODs for water eluates are given separately in Table ESM.2 (based on the same principle). Analytical quality control for measurement in water eluates was performed by elemental analysis in certified control sample GEMS/WATER (Canada), and deviations from the certified values were generally within ±15 % (Table ESM.2). The results on trace and major elements concentrations in the coals and coal water eluates are presented in Table 4.

2.7. Extraction of DNA from sediments and quantification of metal resistance and integrase genes

Total community (TC-) DNA was extracted from fresh sediment samples (0.25 g fresh mass) collected in October 2023 at five representative sites along the Mura River (MI, MS, KR, PT, KT) and Gradiščak Stream (SG) using the DNeasy Powersoil Pro Kit (Qiagen, USA) following the manufacturer's protocol. The quality and concentration of the DNA was determined using a Nanodrop (BioSpec Nano, Shimadzu, Japan) and a Qubit (Thermo Fisher Scientific, USA), with all extractions kept at −20 °C until subsequent analyses. Quantitative PCR (qPCR) was performed on the extracted DNA to quantify several target genes: 16S rRNA gene, which serves as a marker for total bacterial quantity, the

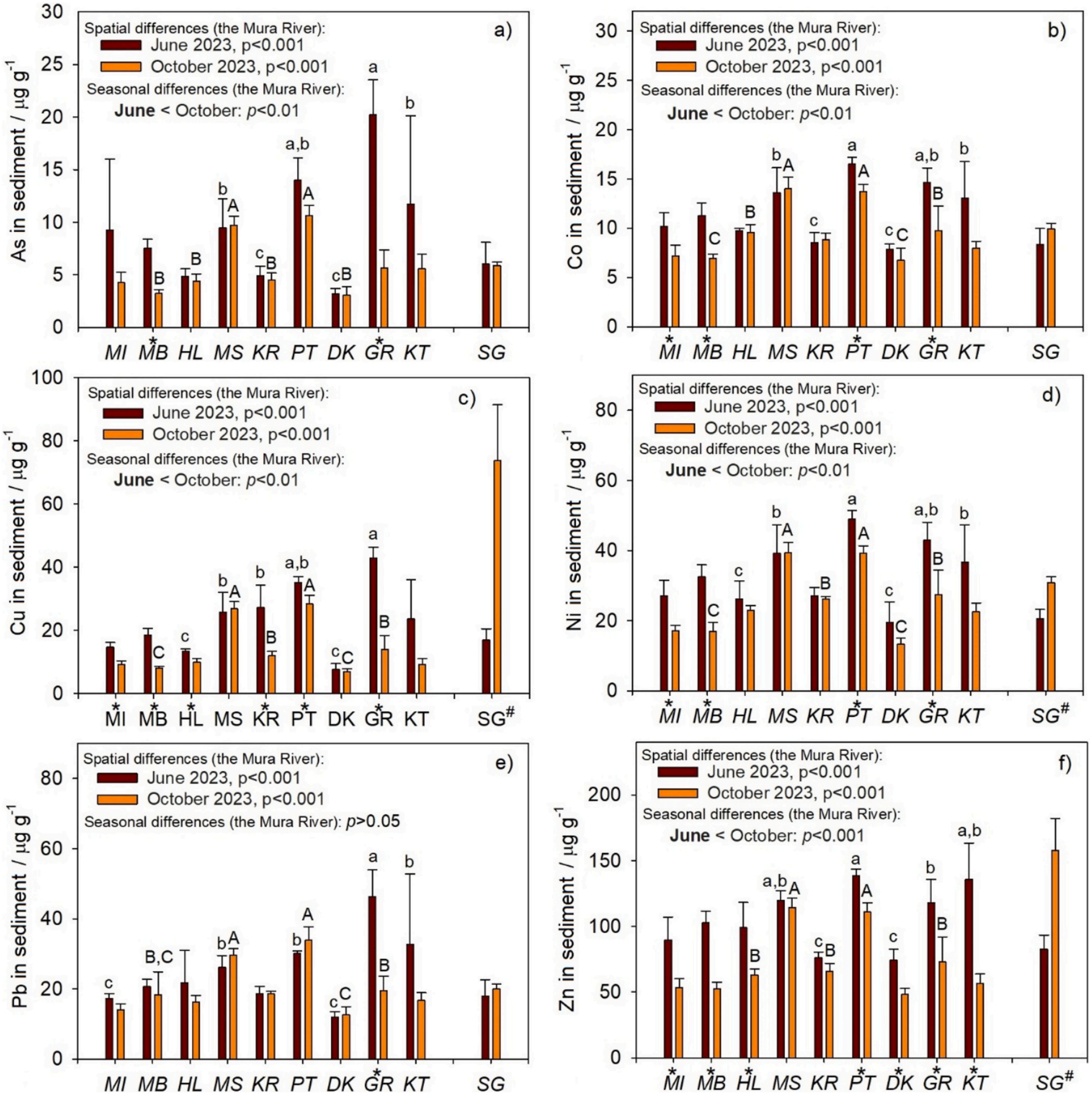

Fig. 8. The concentrations of metals in sediments, As (a), Co (b), Cu (c), Ni (d), Pb (e), and Zn (f), measured in June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and in the stream Gradiščak (SG). The results and statistical comparisons were presented as in the Fig. 3.

class 1 integron-integrase gene *int*I1, which serves as a proxy for anthropogenic pollution [27], and three metal resistance genes – *czc*D (Co, Cd, Zn resistance), *pbr*T (Pb resistance), and *cnr*A (Co, Ni resistance) – which serve as indicators of metal pollution. The primers targeting these genes and the qPCR conditions were as described by Dragun et al. [35]. Gene abundances were calculated per number of copies of the 16S rRNA gene (*rrn*) (Fig. 10) and per gram of dry sediment (Fig. ESM.3).

2.8. Water discharge and water-level

The information on water discharges and water-levels of the Mura River in 2023 (Figs. ESM.4–5), measured at the stations Mursko Središće and Goričan, respectively, were obtained from the Meteorological and Hydrological Service of the Republic of Croatia.

2.9. Calculations, statistical analyses, and graphical data presentation

All calculations were done in Microsoft Office Excel (version 16). Risk quotients (RQ), as a tool of ecological risk assessment, were calculated according to the following equation: RQ = MEC/PNEC, where MEC is maximum measured environmental concentration, and PNEC predicted no effect concentration [36].

The map of sampling area was created using QGIS (version 3.28.3.). The bar-charts were created in statistical program SigmaPlot 11.0 for Windows, except for Figs. 10 and ESM.3 where GraphPad Prism version 10.4.1 for Windows (GraphPad Software, USA) was used.

The statistical data analyses were performed by SAS®OnDemand for Academics software (SAS Studio 3.8 on SAS 9.4). The comparisons between different sites and periods (Fig. 2–9) were analysed as repeated measures analysis of variance by PROC MIXED procedure with a

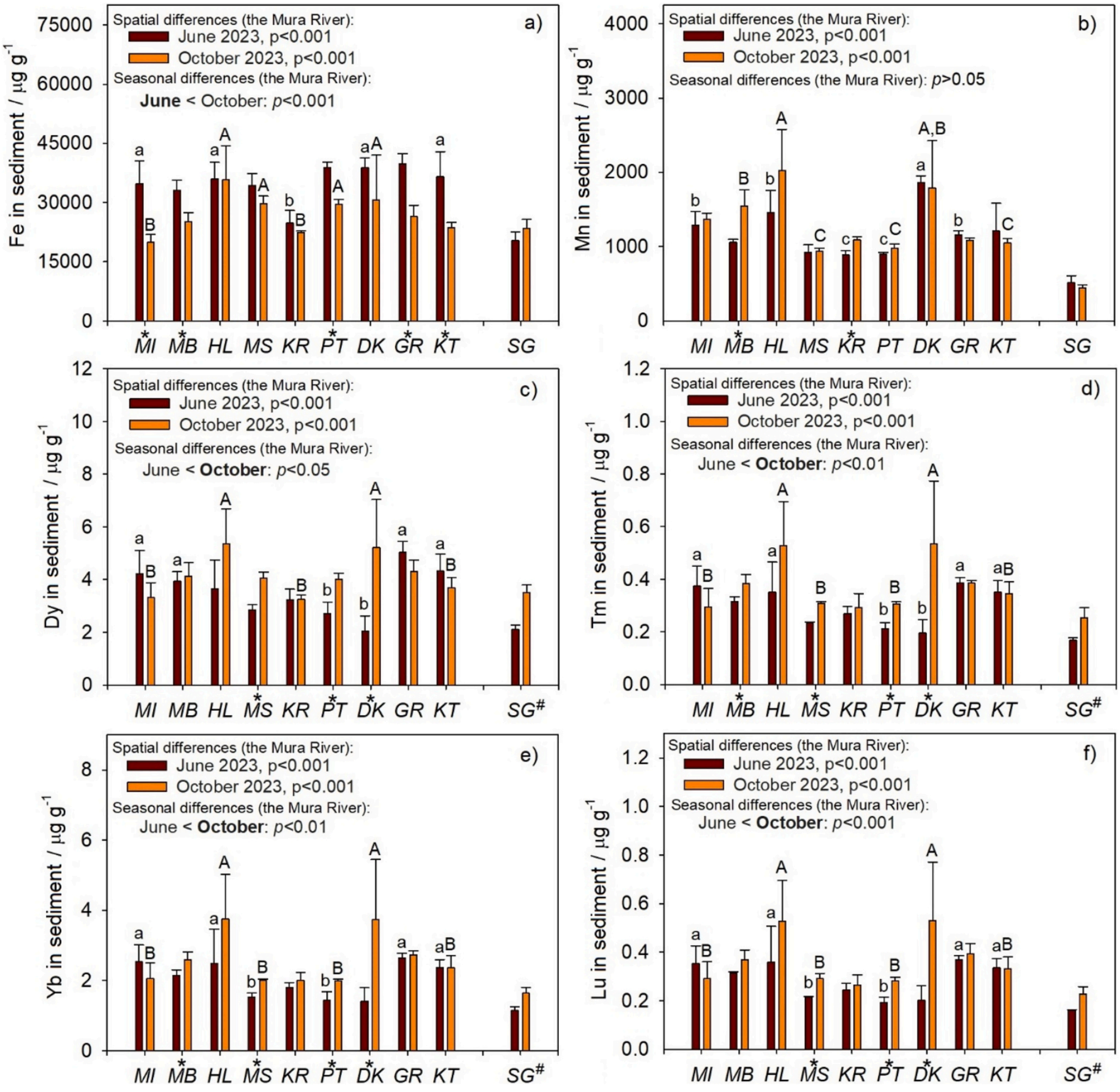

Fig. 9. The concentrations of metals in sediments, Fe (a), Mn (b), Dy (c), Tm (d), Yb (e), and Lu (f), measured in June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and in the stream Gradiščak (SG). The results and statistical comparisons were presented as in the Fig. 3.

significance level set at $\alpha = 0.05$. Data normality and variance homogeneity were achieved by using logarithmic transformation (ln(x)) where it was necessary.

Multidimensional preference analyses, which resulted in biplots (Fig. ESM.6) with the scores (parameter concentrations) represented as points and the structure (variables) represented as vectors, were performed by PROC PRINQUAL.

For the qPCR data analysis, the absolute and relative concentrations were first log10-transformed and tested for normality using the Shapiro-Wilk test. If the data had a normal distribution, a one-way analysis of variance (ANOVA) or *t*-test was performed to compare the mean gene concentrations between sites. For non-normally distributed data, the Kruskal-Wallis test was applied. All these analyses were performed using GraphPad Prism v10.4.1.

3. Results

3.1. Assessment of the river water/sediment quality

3.1.1. Physico-chemical water characteristics

In total 14 physico-chemical parameters were measured in the water at nine sites of the Mura River in two sampling campaigns: at high water discharge (June) and prolonged low water discharge (October) (Table 1, Fig. 2). Six parameters did not show differences between two samplings, namely turbidity, DOX, dissolved CO_2, CH, ammonia, and water temperature. Moderate water temperature reflected the periods of sampling, late spring and early autumn, thus being comparable in both sampling campaigns, namely ranging from 16.1 to 17.7 °C in June, and 16.5–17.5 °C in October.

Only two measured parameters showed significantly higher values in June, i.e. in rainy period, namely up to seven times higher for TP and

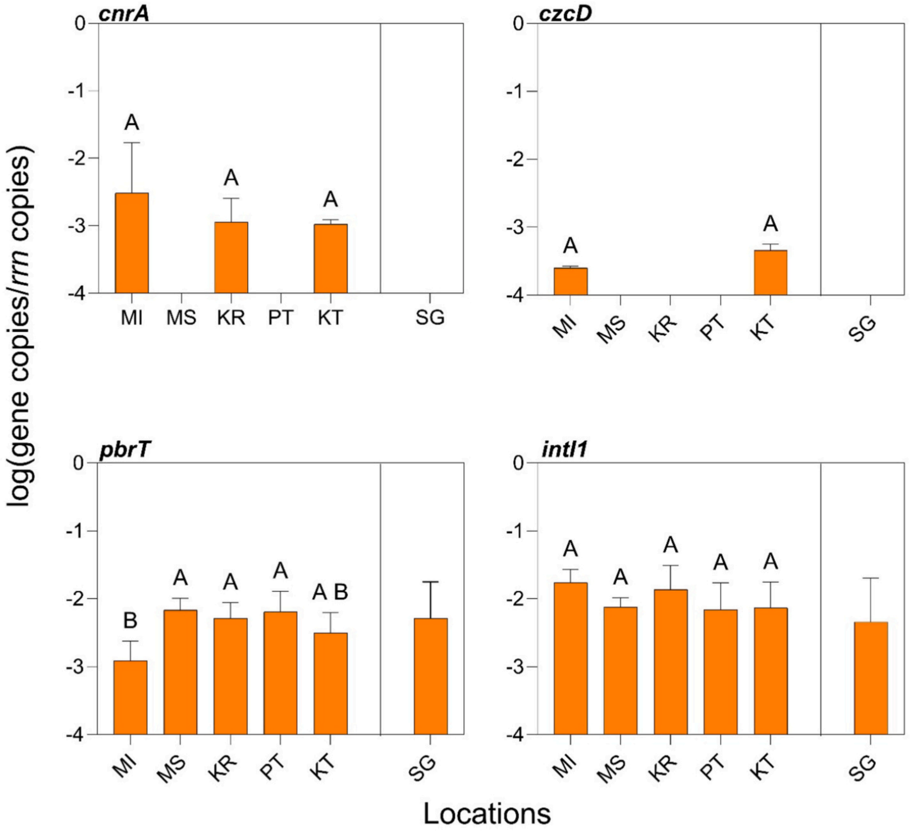

Fig. 10. Relative abundance of metal resistance genes (*cnrA, czcD,* and *pbrT*) and class 1 integron-integrase (*intl1*) in sediments from five Mura River locations (MI – the initial, most upstream location in Croatia; MS – Mursko Središće; KR – Križovec; PT – Podturen; KT - Kotoriba) and stream Gradiščak (SG). Each value represents the mean ± standard deviation of three replicates. Different letters indicate significant differences between locations ($p < 0.05$; comparison was made among five Mura River locations).

Table 1

The values of eight physico-chemical parameters measured in June and October 2023 at nine sampling sites along the section of the Mura River flowing through Croatia (MI – the initial, most upstream location in Croatia; MB – location near bridge, in the municipality of Sveti Martin na Muri; HL – Hlapičina; MS – Mursko Središće; KR – Križovec; PT – Podturen; DK – Dekanovec; GR – Goričan; KT – Kotoriba), and at the stream Gradiščak (SG) ($n = 1$). Asterisks (*) denote statistically significant differences between two sampling campaigns, for all Mura River sites considered together; higher value in all three cases was obtained in October (SG is not included in these comparisons).

		MI	MB	HL	MS	KR	PT	DK	GR	KT	SG
Water temperature	*June*	17.2	16.5	16.1	16.4	16.6	17.2	16.3	17.0	17.7	19.3
/ °C	*October*	16.5	16.9	16.9	17.1	17.5	17.3	17.1	17.1	17.4	22.0
Turbidity	*June*	12	17	20	15	10	19	16	8	21	5
/ FAU	*October*	9	8	14	17	12	14	12	9	10	31
Conductivity	*June**	262	269	206	265	264	265	263	273	281	976
/ µS cm^{-1}	*October*	380	380	370	376	376	381	373	384	365	5820
pH	*June**	8.07	8.04	7.94	8.11	8.11	8.13	8.01	8.11	8.07	8.22
	October	8.42	8.44	8.42	7.88	8.60	8.58	8.27	8.44	7.65	8.09
Dissolved oxygen	*June*	8.98	9.60	9.44	9.49	9.45	9.41	9.61	9.51	9.17	7.52
/ mg O L^{-1}	*October*	9.73	10.2	10.4	10.1	10.5	10.2	8.96	9.06	9.11	0.78
CO_2	*June*	1.25	1.30	2.03	1.52	1.33	1.40	1.35	1.37	1.24	10.8
/ mg L^{-1}	*October*	1.30	1.29	1.53	1.56	1.41	1.42	1.38	1.40	1.43	21.6
Carbonate hardness	*June*	4.35	6.10	5.20	5.21	5.85	7.00	5.01	7.73	7.45	14.7
/ °dH	*October*	5.74	6.24	5.65	6.64	6.10	7.11	6.72	7.42	7.48	17.9
Total hardness	*June**	7.21	7.34	7.61	7.70	8.04	8.71	7.16	8.92	9.32	15.1
/ °dH	*October*	8.72	8.51	8.44	8.82	8.83	8.92	8.27	9.02	9.43	17.9

only slightly higher (up to 60 %) for COD.

Large portion of physico-chemical parameters has showed significantly higher values during prolonged low water-level, in October, namely conductivity (up to 80 %), pH (up to 10 %), TH (up to 20 %), and especially nitrates (up to ~6 times), TN (up to ~8 times), and Chl_a (up to ~4 times).

Visual comparison among sites revealed that several parameters did not exhibit high spatial variability, namely water temperature, conductivity, pH, and DOX. However, among studied sampling sites, one site, namely PT, could be singled out due to the most pronounced increase in several physico-chemical parameters, including carbonate and total hardness, and the concentrations of nitrates, TN and TP, depending on the season. In addition, increase of three parameters was observed at HL site (turbidity, CO_2, and TN), CO_2 at MS, and ammonia at MB and

Table 2

Comparison of dissolved major and trace elements concentrations in the river-water of the Croatian section of the Mura River (min-max of station averages), in two sampling campaigns (June 2023, rainy period; October 2023, dry period), to several other Croatian rivers (ind&agr refer to impact of industry and agriculture; ind&urb refer to impact of industry and urbanization), as well as world average values and background values (upper category values) according to Geochemical Atlas of Europe [33] (http://weppi.gtk.fi/publ/foregsatlas/). Moreover, ratios of concentrations measured in the Mura River water to world average and FOREGS values are provided, and recommended concentrations by Croatian and European legislation for several elements.

	Mura (min-max) (this study, June)	**Mura (min-max)** (this study, Oct)	**Mrežnica (agr)** [35]	**Mrežnica (ind&agr)** [35]	**Ilova (ind&agr)** [37]	**Sava-Zagreb (ind&urb)** [38]	**World average (WA)* / FOREGS$_{max}$**	**Ratio: water$_{min-max}$ to WA**	**Ratio: water$_{min-max}$ to FOREGS$_{max}$**	**Legal limits**
Elements in mg L^{-1}										
Na	7.48-8.58	14.8-17.2	1.50±0.06	2.11±0.17	26.5±0.2	-	- / 13	-	0.6-1.3	-
K	1.73-1.99	2.70-2.91	0.442±0.043	0.553±0.066	4.58±0.07	-	- / 2.5	-	0.7-1.2	-
Mg	6.67-7.57	9.56-10.3	14.0±0.5	13.4±1.5	16.9±0.2	-	- / 38	-	0.2-0.3	-
Ca	32.1-35.1	46.0-49.6	56.3±2.2	51.8±6.4	57.0±0.8	-	- / 110	-	0.3-0.5	-
Elements in µg L^{-1}										
Ag	0.002-0.221	0.002-0.067	<0.001	<0.001	-	-	-	-	-	0.010^{a}/0.022^{b}
Al	12.6-16.9	5.30-6.98	1.91±0.74	2.32±0.08	6.8±0.6	5.23	- / 9.50	-	0.6-1.8	-
As	0.914-1.11	1.17-1.35	0.251±0.017	0.255±0.095	4.5±0.7	0.521	0.620 / 4.06	1.5-2.2	0.2-0.3	7.5^{c}
Ba	17.8-20.3	27.8-30.1	6.20±0.17	6.48±0.49	29.1±1.7	-	23/35.7	0.8-1.3	0.5-0.8	-
Bi	<LOD	<LOD	<0.001	0.003±0.003	<0.005	-	- / 0.003	-	<0.3	-
Cd	0.005-0.006	0.008-0.012	0.006±0.001	0.007±0.003	0.053±0.003	0.012	0.080 / 0.016	0.1-0.2	0.3-0.8	0.09^{d}/0.6^{e}
Co	0.052-0.079	0.078-0.139	0.028±0.001	0.035±0.002	0.121±0.011	0.100	0.148 / 0.220	0.4-0.9	0.2-0.6	-
Cr	0.129-0.323	0.131-0.263	0.168±0.006	0.147±0.013	<0.06	0.320	0.700 / 0.380	0.2-0.5	0.3-0.9	9^{c}
Cs	0.011-0.018	0.042-0.060	0.001±0.000	0.004±0.000	0.090±0.007	-	0.011 / 0.012	1.0-5.5	0.9-5.0	-
Cu	0.586-0.788	0.569-0.757	0.067±0.019	0.143±0.040	0.72±0.03	0.815	1.48 / 1.45	0.4-0.5	0.4-0.5	4.8^{c}
Fe	10.9-17.1	17.8-40.3	8.00±0.25	17.1±1.5	21.6±1.5	13.4	66.0 / 67.0	0.2-0.6	0.2-0.6	16.0^{f}
Li	1.38-1.92	2.39-2.83	0.061±0.002	0.096±0.008	-	1.66	1.84 / 4.60	0.8-1.5	0.3-0.6	-
Mn	11.7-35.1	12.0-133	1.89±0.03	4.46±0.40	18.4±0.9	2.96	34 / 220	0.3-3.9	0.1-0.6	-
Mo	0.595-0.754	1.32-1.46	0.732±0.004	0.711±0.071	0.98±0.06	-	0.420 / 0.530	1.4-3.5	1.1-2.8	-
Ni	0.435-0.489	0.514-0.681	0.050±0.003	0.137±0.074	1.80±0.08	0.653	0.801 / 3.00	0.5-0.9	0.1-0.2	$(2^{a})4^{d}$/$(8.2^{b})34^{e}$
Pb	0.039-0.129	0.063-0.120	<0.060	<0.060	<0.07	0.065	0.079 / 0.093	0.5-1.6	0.4-1.4	1.2^{d}/14^{e}
Rb	1.79-1.94	2.51-2.86	0.335±0.003	0.450±0.042	3.74±0.25	-	1.63 / 1.30	1.1-1.8	1.4-2.2	-
Sb	0.140-0.155	0.187-0.206	0.031±0.000	0.046±0.005	0.301±0.017	-	0.070 / 0.130	2.0-2.9	1.1-1.6	-
Se	0.085-0.148	0.093-0.139	0.099±0.012	0.135±0.059	1.01±0.11	-	0.070 / 0.240	1.2-2.1	0.4-0.6	-
Sn	0.012-0.061	0.008-0.080	<0.003	<0.003	-	-	-	-	-	-
Sr	105-116	169-179	83.4±1.1	84.2±7.5	150±12	129	60.0 / 260	1.8-3.0	0.4-0.7	-
Ti	0.044-0.111	0.058-0.152	<0.140	<0.140	-	-	0.489 / 1.80	0.1-0.3	≤0.1	-
Tl	0.007-0.008	0.009-0.011	0.005±0.000	0.005±0.000	0.013±0.002	-	- / 0.003	-	2.3-3.7	-
U	0.507-0.562	0.669-0.757	0.630±0.004	0.643±0.047	1.57±0.11	-	0.372 / 1.70	1.4-2.0	0.3-0.4	-
V	0.396-0.516	0.524-0.653	0.385±0.006	0.442±0.045	4.1±0.3	0.496	0 710 / 0.880	0.6-0.9	0.5-0.7	-
W	0.364-0.745	0.712-0.801	-	-	-	-	0.100 / 0.015	3.6-8.0	24.3-53.4	-
Zn	<LOD	<LOD-1.82	<0.519	<0.519	<7	2.24	0.600 / 4.00	<1.4-3.0	<0.2-0.5	35^{c}
Lanthanides in µg L^{-1}										
La	0.016-0.022	0.013-0.025	-	-	-	-	0.120 / 0.034	0.1-0.2	0.4-0.7	-
Ce	0.025-0.033	0.016-0.039	-	-	-	-	0.262 / 0.120	0.1	0.1-0.3	-
Pr	0.004-0.005	0.003-0.007	-	-	-	-	0.040 / 0.009	0.1-0.2	0.3-0.8	-
Nd	0.017-0.022	0.013-0.028	-	-	-	-	0.152 / 0.040	0.1-0.2	0.3-0.7	-
Sm	0.003-0.005	0.003-0.007	-	-	-	-	0.036 / 0.009	0.1-0.2	0.3-0.8	-
Eu	0.002-0.003	0.002-0.003	-	-	-	-	0.010 / 0.005	0.2-0.3	0.4-0.6	-
Gd	0.008-0.014	0.020-0.024	-	-	-	-	0.040 / 0.010	0.2-0.6	0.8-2.4	-
Dy	0.003-0.005	0.004-0.007	-	-	-	-	0.030 / 0.005	0.1-0.2	0.6-1.4	-
Ho	0.001	0.001	-	-	-	-	0.007 / 0.002	0.1	0.5	-
Er	0.003-0.004	0.004-0.005	-	-	-	-	0.020 / 0.006	0.2-0.3	0.5-0.8	-
Tm	0.001	0.001	-	-	-	-	0.003 / 0.002	0.3	0.5	-
Yb	0.003-0.004	0.004-0.005	-	-	-	-	0.017 / 0.006	0.2-0.3	0.5-0.8	-
Lu	0.001	0.001-0.002	-	-	-	-	0.002 / 0.002	0.5-1.0	0.5-1.0	-

*world average for dissolved metal/metalloid concentrations based on water filtration using 0.2 µm-pore-diameter filter [39];
[a]annual average (AA) environmental quality standard (EQS) for inland surace waters according to EC [40]; [b]maximum allowable concentration (MAC) EQS for inland surface waters according to EC [40]; [c]AA according to Official Gazette [41]; [d]AA EQS for inland surface waters according to EPCEU [9]; [e]MAC EQS for inland surface waters according to EPCEU [9]; [f]proposal for AA by Crane et al. [42]; Legend: ratio >1 to <2; ratio ≥2 to <3; ratio ≥3 to <4; ratio ≥4.

GR. Finally, a trend of increasing values towards downstream sites was observed for CH and TH in both seasons.

3.1.2. Dissolved metals/metalloids in the river water

Comparison between data for entire studied river section in two seasons revealed statistically significantly higher concentrations of majority of studied elements in October, namely of As, Ba, Ca, Cd, Co, Cs, Dy, Er, Fe, Gd, K, Li, Mg, Mn, Mo, Na, Ni, Pr, Rb, Sb, Sm, Sr, Tl, U, V, W, Yb, and Zn (Figs. 3–5, Table 2, Tables ESM.3–4), during lower water discharge (Figs. ESM.4–5). The concentrations were on average up to ~3.5 times higher in October, but the intensity of difference depended on the element and the sampling site. This was especially evident for Mn, which was approximately four times higher in October than in June at PT site, whereas at all the other sites seasonal differences for Mn were much lower, even negligible. Only two metals were present in water in significantly higher concentrations in June, during rainy high water-level period, namely Ag and Al (Fig. 3a–b; Table 2, Table ESM.3). In case of Al, seasonal difference was more or less uniform for the entire studied river-section, with on average two times higher concentrations in June; contrary, extreme increase of Ag was recorded at only three sites, namely MS, KR, and PT, and the average concentrations were up to 50 times higher in June than in October. Several elements have not presented significant seasonal variability, namely Bi, Ce, Cr, Cu, Eu, Ho, La, Lu, Nd, Pb, Se, Sn, Ti, and Tm (Figs. 3–5, Table 2, Tables ESM.3–4). Multidimensional preference analysis confirmed these observations, showing clear separation of two sampling seasons regarding the metal/metalloid concentrations (Fig. ESM.6a), with Ag and Al vectors being directed towards June sampling, and opposite to the elements with higher concentrations measured in October; lanthanides and Ti were placed separately, between the seasons, confirming the absence of their seasonal variability. It is interesting to notice Gd deviation from the other lanthanides, it being higher in October, as majority of the trace elements.

The elements with the most prominent spatial variability are presented in Figs. 3–5, and include 18 metals/metalloids. At Fig. 3, six elements (Ag, Al, Cd, Cr, Pb, and W) were presented that had somewhat higher concentrations in the river-water in the vicinity of abandoned coal mines and urban centres Mursko Središće at Croatian site and Lendava at Slovenian, covering river section from HL to PT (Fig. 1). This increase was most obvious for Ag, and especially in June, with dissolved Ag concentrations reaching as much as 0.221 µg L^{-1} at three sites (MS, KR, and PT). In October, Ag was much lower, and only sporadically increased concentrations were detected at downstream section of the river (Fig. 3a). In June, Cr, Pb, and W had similar spatial distribution to Ag, with obvious concentration increase at the same sites, and in the case of W extended to HL (Fig. 3d–f). Only slight Al and Cd increases were observed at MS, KR and/or PT site (Fig. 3b,c).

At Fig. 4, eight elements (As, Co, Cu, Fe, Mn, Ni, Zn, and Gd) were presented that had the highest concentrations at the sampling site PT. This was especially prominent in the case of Mn (Fig. 4e), with 2–3 times higher Mn concentrations at PT compared to the other sites in June, and as much as 5–10 times in October. The differences among sites were less pronounced for the other seven elements, but statistically significant for all in October.

Third group of elements presented in Fig. 5 (Na, Li, Ce, and La) showed higher concentrations at the most upstream sites. Specifically, both Na and Li (Fig. 5a–b) were slightly but significantly higher at MB site, immediately downstream from floodplain receiving water from stream Gradiščak (recipient of thermal baths' wastewaters), compared to the neighbouring sites in at least one sampling season. Cerium and La (Fig. 5c–d), on the other hand, in general showed mutually comparable spatial distributions, with slight decrease towards downstream river section.

For Gd (Fig. 4h), on other hand, the downstream decreasing trend was not observed. Moreover, average Gd-anomalies ranged from ~2–4 in June and from ~4–6 in October, depending on the site; higher values were observed at downstream sites, pointing to anthropogenic contribution (Fig. 6). Calculated anthropogenic Gd amounted from 0.003 µg L^{-1} at upstream site to 0.010 µg L^{-1} at downstream sites in June (~40–75 % of measured Gd), whereas in October that quantity reached 0.022 µg L^{-1} (~80 % of measured Gd).

Additionally, ratios of measured dissolved metal/metalloid concentrations to predicted background levels (Table 2) were calculated, and revealed several elements that highly surpassed even the upper levels of applied background categories. Ratios ≥4 were obtained for Cs and W; ratios >3 for Tl; and ratios >2 for Gd, Mo and Rb, at the entire studied river-section in October, and for W in both seasons.

3.1.3. Metals/metalloids in the river sediments

According to grain size distribution analysis performed only for June samples, sediment at majority of sites had clay content below 10 % (~4–7 %), high content of sand (46–82 %), and medium content of silt (15–47 %). The exceptions were sampling sites PT and GR, with 12 % and 17 % of clay, respectively, somewhat higher content of sand (26–29 %), and silt as a predominant component (54–62 %). Accordingly, the samples at DK were classified as sand, majority of samples from the remaining sites as silty sand, except for PT and GR, where samples were classified as sandy silt and sandy/clayey silt, respectively (Fig. ESM.2).

The measured concentrations of metals/metalloids in sediments (Figs. 7–9, Table 3, and Tables ESM.5–6) were compared between two sampling campaigns, when sediments were collected at different distances from the centre of the riverbed (Fig. ESM.1), to determine whether they were influenced by the sampling spot within the riverbed. About half of the elements analysed showed no statistically significant differences, indicating a generally even distribution within the riverbed (Table ESM.5–6). However, the majority of the remaining elements were observed in higher concentrations in June when sampling was further away from the centre of the river (As, Al, Co, Cr, Cu, Fe, K, Li, Na, Ni, P, Sn, Ti, U, V, and Zn), while only heavier members of the lanthanides (Dy to Lu) were more enriched in October when sampling was closer to the centre of the river. This separation of heavy lanthanides from the remaining elements was confirmed by multidimensional preference analysis, presented in Fig. ESM.6b.

To ensure reliable spatial analysis, we have combined the cognitions from both campaigns, but put greater emphasis on the October dataset, when sediment was sampled closer to the centre of the river and less susceptible to additional influences (e.g., deposits from other sources). In October, many elements showed spatial distributions that were mutually similar to a certain point, namely the highest (or some of the highest) concentrations were observed at two sampling sites: MS and PT. This referred to majority of analysed elements, namely Ag, Al, As, Ba, Bi, Cd, Co, Cr, Cs, Cu, K, Li, Mg, Ni, P, Pb, Rb, S, Sb, Se, Sn, Th, Ti, Tl, U, V, W, and Zn, as well as lighter lanthanides (La to Gd) (Table ESM.5–6). However, certain specificities could be observed that enabled a separation of elements in several groups according to their spatial distributions.

At Fig. 7, six elements are presented with higher concentrations generally inclining towards upstream river section, namely Ag, Al, Cd, Cr, Mo, and W. In this group of elements, we can especially point to higher concentrations of Cd, Mo and W at MS site (Fig. 7c,e,f), i.e. in the

Table 3

Comparison of major and trace elements concentrations in the sediments (fraction <2 mm) of the Croatian section of the Mura River (min-max of station averages), in two sampling campaigns (June 2023, rainy period; October 2023, dry period), to several other Croatian rivers (ind&agr refer to impact of industry and agriculture; mww&agr refer to impact of municipal wastewaters and agriculture), as well as enrichment factors (EF; min-max of station averages) calculated based on values from Geochemical Atlas of Europe [33] (http://weppi.gtk.fi/publ/foregsatlas/). Moreover, recommended limit concentrations by USA and Australian authorities are provided.

	Mura (min-max) (this study, June)	**Mura (min-max)** (this study, Oct)	**Mrežnica (agr)** [35]	**Mrežnica (ind&agr)** [35]	**Ilova (ind&agr)** [37]	**Sava-Medno (dam)** [43]	**Sava-Podsused (mww&agr)** [43]	**EF-Mura (min-max)** (this study)	**Legal limits**
Elements in mg g^{-1}									
Na	10.7-14.5	8.46-10.7	0.326±0.016	1.70±0.13	7.7±1.2	-	-	1.2-2.8	-
K	7.28-19.0	6.36-15.3	0.823±0.032	3.58±0.26	18.8±1.5	27.7	14.8	0.8-1.5	-
Mg	3.39-14.4	7.15-13.7	2.36±0.13	5.45±0.42	14.8±1.6	28.0	26.3	0.8-1.9	-
Ca	8.71-22.7	13.9-25.1	368±21	263±9	-	80.6	113	1.1-2.7	-
Al	27.6-69.6	32.4-53.9	5.14±0.35	28.0±4.1	84±2	87.1	54.1	-	-
Fe	24.8-39.8	19.9-35.8	7.22±0.62	23.2±2.0	35.8±1.8	42.9	28.6	1.0-2.7	-
Mn	0.884-1.86	0.936-2.03	0.405±0.024	0.458±0.010	0.960±0.140	0.844	0.690	0.9-3.5	-
P	0.392-1.15	0.348-0.809	0.350±0.023	0.526±0.035	-	-	-	0.8-2.0	-
S	0.272-0.783	0.262-0.969	-	-	-	-	-	-	-
Ti	2.97-5.04	1.98-4.07	0.337±0.020	1.33±0.03	-	4.54	2.86	0.5-1.1	-
Elements in μg g^{-1}									
Ag	0.054-0.112	0.047-0.132	-	-	-	-	-	-	1.0[a]/3.7[b]
As	3.21-20.2	3.05-10.6	4.81±0.40	5.25±0.53	12±4	17.2	12.0	0.6-2.0	9.79[c]/33.0[d]
Ba	174-469	206-467	43.4±4.6	141±16	500±50	-	-	0.8-1.3	-
Bi	0.139-0.468	0.142-0.523	0.044±0.003	0.047±0.004	0.45±0.06	-	-	-	-
Cd	0.122-0.277	0.124-0.292	0.710±0.074	0.483±0.022	3.8±0.7	0.62	0.77	0.6-1.8	0.99[c]/4.98[d]
Co	7.86-16.5	6.76-14.0	3.68±0.22	8.67±0.75	14.8±0.7	-	-	1.1-2.0	-
Cr	57.1-100	37.6-81.5	11.4±0.9	65.6±9.2	96±5	192	91.4	1.0-2.0	43.4[c]/111[d]
Cs	0.971-5.73	1.53-5.52	0.549±0.038	5.50±0.48	6.8±0.5	-	-	0.5-1.4	-
Cu	7.67-42.9	6.96-28.4	7.16±1.73	20.6±2.0	32±2	47	92.4	0.6-2.2	31.6[c]/149[d]
Li	16.6-43.0	18.1-41.9	4.66±0.34	31.0±3.0	-	-	-	1.0-1.9	-
Mo	0.515-1.31	0.261-1.40	0.253±0.047	4.25±0.73	0.65±0.12	-	-	1.1-4.3	-
Ni	19.5-49.0	13.3-39.3	9.34±0.86	51.0±8.9	43±3	-	-	0.9-2.3	22.7[c]/48.6[d]
Pb	12.0-46.4	12.6-33.9	17.7±1.3	15.3±2.2	45±7	69.3	141	1.1-2.5	35.8[c]/128[d]
Rb	19.6-84.0	31.9-88.4	6.71±0.41	26.8±0.9	101±3	-	-	0.5-1.1	-
Sb	0.585-1.62	0.553-1.52	0.283±0.008	0.456±0.057	1.27±0.07	2.6	10.0	0.6-1.4	2[a]/25[b]
Se	0.079-0.357	0.055-0.353	-	-	-	-	-	-	-
Sn	2.27-4.52	1.45-5.26	1.29±0.18	1.23±0.28	-	10.7	15.8	0.7-2.5	-
Sr	79.2-148	101-148	81.6±7.0	217±22	147±5	164	234	1.1-2.0	-
Th	1.95-10.9	4.41-8.58	-	-	-	16.2	8.6	0.4-1.0	-
Tl	0.226-0.747	0.256-0.599	0.191±0.010	0.258±0.007	0.67±0.01	-	-	0.7-1.6	-
U	1.18-2.38	1.18-2.17	0.444±0.050	8.92±1.75	4.6±0.2	-	-	0.9-1.6	-
V	53.9-117	43.6-93.2	17.3±1.2	84.1±12.8	103±3	-	-	0.8-1.5	-
W	1.12-4.17	1.10-6.51	-	-	-	-	-	1.1-4.1	-
Zn	74.2-139	48.2-114	46.0±13.7	40.1±5.2	162±10	786	364	1.4-2.7	121[c]/459[d]
Lanthanides in μg g^{-1}									
La	6.75-34.3	14.8-29.3	-	-	-	-	-	0.4-1.1	-
Ce	13.8-67.8	29.6-58.0	-	-	-	-	-	0.4-1.1	-
Pr	1.76-7.97	3.56-7.00	-	-	-	-	-	0.4-1.0	-
Nd	7.10-31.7	14.4-27.6	-	-	-	-	-	0.4-1.0	-
Sm	1.52-6.37	3.04-5.44	-	-	-	-	-	0.5-1.0	-
Eu	0.406-1.41	0.724-1.22	-	-	-	-	-	0.7-1.3	-
Gd	1.39-5.48	2.76-4.52	-	-	-	-	-	0.5-0.9	-
Dy	2.04-5.03	3.25-5.37	-	-	-	-	-	0.6-1.7	-
Ho	0.438-0.978	0.688-1.17	-	-	-	-	-	0.6-1.9	-
Er	1.38-2.84	1.98-3.57	-	-	-	-	-	0.6-2.0	-
Tm	0.195-0.386	0.292-0.535	-	-	-	-	-	0.5-2.0	-
Yb	1.41-2.64	1.99-3.75	-	-	-	-	-	0.5-2.1	-
Lu	0.192-0.368	0.265-0.531	-	-	-	-	-	0.5-1.9	-

[a] ISQG-Low – Interim sediment quality guidelines (lower 10th percentile of chemical concentrations associated with adverse biological effects in

field studies and laboratory bioassays) according to Australian Sediment Quality Guideline [44];

[b] ISQG-High – Interim sediment quality guidelines (lower 50th percentile of chemical concentrations associated with adverse biological effects in field studies and laboratory bioassays) according to Australian Sediment Quality Guideline [44];

[c] TEC – Consensus-based Threshold Effect Concentrations – below which harmful effects are unlikely to be observed [45];

[d] PEC – Consensus-based Probable Effect Concentrations – above which harmful effects are likely to be observed [45]; Legend: ratio >1 to <2; ratio ≥2 to <3; ratio ≥3 to <4; ratio ≥4.

surroundings of former coal mining area, in comparison to PT site.

At Fig. 8, another six elements are presented with higher concentrations generally inclining towards downstream river section, namely As, Co, Cu, Ni, Pb, and Zn, especially in June sampling.

Finally, Fig. 9 presents six elements, which were separated from the majority by the fact that their concentrations were not the highest at MS and PT sites, but at HL and DK sites. These elements include Fe and Mn, and heavier lanthanides (Dy to Lu), four of which are presented as representative of the group.

Among studied elements, two had enrichment factors (EF) >4 at some sites, namely Mo and W, one >3, namely Mn, and 16 elements ≥ 2 (Na, Ca, Fe, P, As, Co, Cr, Cu, Ni, Pb, Sn, Sr, Zn, and heavier lanthanides (Er, Tm, Yb); Table 3). High EFs of Mo were observed in the sediments of the entire studied Mura river-section, but the highest values of both Mo and W were observed in the area of former coal mining, from HL to PT (Table ESM.7). Manganese, Fe, Er, Tm, and Yb showed enrichment in accordance with their spatial distributions, especially strong at DK site, whereas enrichment of Co, Cu, Ni, P, and Pb was observed at PT, and Zn at both MS and PT sites (Table ESM.7–8).

3.1.4. *Total bacteria, metal resistance genes and class 1 integrons in the river sediments*

Analyses of bacterial 16S rRNA, metal resistance genes and class 1 integrons were only carried out in the river sediments at the sites that were estimated to be more heavily anthropogenically contaminated based on the chemical analyses (MS, KR, PT and KT), and at the first, presumably least contaminated, site (MI), only for the sampling in October.

The average 16S rRNA gene copy number in the sediment samples from the five sites was between 8.2 and 9.3 log gene copies per gram with the lowest bacterial abundance observed at the MI site and the highest at the most downstream site (KT). Because of variations in bacterial abundances among sites (Fig. ESM.3), the relative gene abundances (gene copies per *rrn* copies) were calculated (Fig. 10).

The *cnrA* gene was only detected at three sites: MI, KR and KT, with relative abundances ranging from −2.98 log gene copies/*rrn* copies (site KT) to −2.51 log gene copies/*rrn* copies (site MI). The *czcD* gene was only detected at the MI and KT sites, with relative abundances of −3.60 and −3.34 log gene copies/*rrn* copies, respectively (Fig. 10). In contrast, the *pbrT* gene was detected at all studied sites. However, its relative abundance at three sites (MS, KR and PT) was significantly higher (−2.29 to −2.17 log gene copies/*rrn* copies*)* than at the MI site (−2.91 log gene copies/*rrn* copies*)*. The relative abundance of this gene at the KT site was similar to that at the MI site (Fig. 10). The *intl1* gene was also detected in the sediments of all five sites, with similar relative abundances across sites, ranging from −2.12 to −1.76 log gene copies/*rrn* copies (Fig. 10).

3.2. *Assessment of the potential pollution sources*

3.2.1. *Stream Gradiščak – recipient of thermal baths' wastewaters*

In the water of stream Gradiščak, several physico-chemical parameters showed much higher levels in October sampling, when water-level was extremely low (Fig. 2; Table 1). Six times higher turbidity and conductivity, and two times higher CO$_2$ and COD were recorded. Nutrient increase was even higher, with 15 times higher nitrates concentrations, nine times higher TN, three times higher ammonia, and two times higher TP. As stream Gradiščak inflows in floodplain between MI and MB site (Fig. 1), possibly affecting the latter through washout and leakage, the comparison of measured values in SG was made with upstream MI site. In June, conductivity was higher four times, CO$_2$ nine times, and ammonia six times in SG compared to MI. In October, difference was even more pronounced with almost all measured parameters higher in SG (mostly NH$_3$ – 30 times; conductivity, CO$_2$, and TP – 13–15 times; and nitrates and TN – 5–6 times).

The dissolved metal/metalloid concentrations in SG (Table ESM.3–4; Figs. 3–5) were generally higher in October, concentrated during low water-level. The highest increase in October compared to June was observed for Li (25 times), Na, K, Ba, Cs, Rb, Ti, and lanthanide Eu (9–15 times), whereas several other elements, namely As, Co, Fe, Mn, Mo, Sb, Sn, Sr, V, W, Zn, and the remaining lanthanides were increased 50 % to five times. Increased concentrations of four elements, namely Mg, Ca, Cr, and Cu were observed in June, probably due to runoff from nearby agricultural fields, but although significant, this increase was rather small (i.e., 20–40 %). Comparison with MI site in June revealed higher concentrations of several elements in SG: Cs (40 times), Ba, Li (20–25 times), Eu, Na (9–11 times), and Mn, Fe, and Rb (5 times). In October, even higher differences between two watercourses were observed, with concentrations higher in SG compared to MI found for Li (>300 times), Ba, Cs (160–170 times), Eu, Na (70–85 times), Rb (50 times), Mn (19 times), and Fe, K, Sn, Sr, and Ti (7–12 times). Gd-anomalies were calculated also for SG, and the obtained value was much lower than in the Mura River (≤ 2).

Sediment in SG was classified as silty sand (Fig. ESM.2). Although statistically significantly higher concentrations of several elements were found in sediment of SG in October than in June, the differences were much less pronounced than for dissolved elements, and generally did not surpass factor of 2 (Table ESM.5–6; Figs. 7–9). However, that was still enough to lead to observable difference in metal content in SG sediment compared to MI sediment in October, with higher concentrations of Cu and S (8–9 times), Ba, Cs, P, and Sn (3–4 times), and Li, Mo, Se, Ti, and Zn (2–3 times). Differences in June were less pronounced. The enrichment factors (Table ESM.7) in October confirmed the contamination of SG sediment with several elements above the upper values of predicted background levels, namely Cu (EF = 4.9), Zn (EF = 3.3), Ba (EF = 3.0), Cs, Mo, and P (EF = 2.3–2.5).

The *cnr*A gene and the *czc*D gene were not detected in the sediment of SG (Fig. 10). In contrast, the *pbr*T gene was detected with a relative abundance of −2.28 log gene copies/*rrn* copies (Fig. 10), which was comparable to the MS–PT sites. The *int*I1-gene was detected with a relative abundance of −2.33 log copies/*rrn* copies, which was slightly lower than the relative abundances of this gene at studied sites on the Mura River (Fig. 10).

3.2.2. *Abandoned coal mines*

The analysis of brown coal samples and their water eluates revealed the enrichment of the coal from the abandoned Mursko Središće coal mines with several elements (Table 4). Ratios of metal/metalloid concentrations measured in the coal samples to concentrations in the river sediment at the MS site, and to Clarke values, pointed to highest enrichment with As (ratios ~70–90 times), S (ratios ~40–60 times), Mo and W (ratios ~10–20 times), Se (ratios up to 6 times), and Sb and U (~2–4 times). The measurement of these elements in coal water eluates revealed that their leachability from intact coal was rather low (As, 0.9 %; S, 8.5 %; Mo, 0.1 %; W, <0.1 %; Se, 2.5 %; Sb, 0.3 %; and U, 0.2 %).

Table 4

Major and trace element concentrations in coal samples from Mursko Središće coal mines and in coal water eluates ($n = 6$; expressed as mean ± standard deviation for totals, and mean concentration/mean relative leached mass for eluates). Additionally, literature reports are given for coals, and ratios of measured elemental concentrations in coal from Mursko Središće mines to Clarke values for brown coals and to sediment concentrations at Mursko Središće (MS) site. The ratios above 1 (indicating metal enrichment in coals) are presented in bold numbers.

	Concentrations				**Ratios**	
	Coal - total (this study)	**Coal - eluates** (this study) concentration/%	**Coal - total** Clarke values* [46]	**Coal - total** Raša mine [47]	**Coal$_{MS}$ to Clarkes**	**Coal$_{MS}$ to sediment$_{MS}$** (June/October)
Elements in mg g^{-1}						
Na	≤0.005	-	-	-	-	<0.01
Mg	1.38±0.25	0.720/52.2	-	-	-	0.13/0.11
Ca	9.62±1.34	3.09/31.0	-	-	-	0.54/0.47
Al	1.03±0.52	0.016/2.3	-	-	-	0.02/0.02
Fe	26.9±15.1	0.887/2.3	-	-	-	0.79/0.91
S	45.2±20.7	3.99/8.5	-	-	-	**62.0/46.6**
Elements in μg g^{-1}						
Ag	0.011±0.007	<0.001/<0.7	0.090±0.020	-	0.12	0.14/0.10
As	694±749	6.82/0.9	7.6±1.3	2.68	**91.3**	**73.3/71.5**
Ba	26.9±5.0	0.353/1.4	150±20	17.7	0.18	0.07/0.06
Bi	0.032±0.017	<0.001/<0.2	0.84±0.09	0.04	0.04	0.08/0.07
Cd	0.083±0.108	0.003/11.5	0.24±0.04	0.20	0.35	0.34/0.28
Co	3.37±1.52	0.388/9.2	4.2±0.3	1.62	0.80	0.25/0.24
Cr	9.74±2.81	0.035/0.4	15±1	14.1	0.65	0.12/0.12
Cs	0.122±0.116	0.002/2.8	0.98±0.10	0.42	0.12	0.03/0.02
Cu	7.56±4.53	0.045/0.7	15±1	8.93	0.50	0.29/0.28
K	96.2±84.0	<1.03/<1.2	-	-	-	0.01/0.01
Li	0.602±0.610	-	10±1.0	10.5	0.06	0.02/0.02
Mn	58.4±20.2	15.6/23.0	100±6	-	0.58	0.06/0.06
Mo	24.9±4.8	0.029/0.1	2.2±0.2	2.75	**11.3**	**26.4/17.8**
Ni	13.1±2.7	0.777/5.1	9.0±0.9	8.92	**1.46**	0.33/0.33
P	80.3±41.8	5.09/7.3	200±30	-	0.40	0.08/0.10
Pb	0.683±0.514	0.018/2.3	6.6±0.4	1.82	0.10	0.03/0.02
Rb	1.14±0.92	0.016/1.8	10±0.9	3.05	0.11	0.04/0.01
Sb	2.09±0.88	0.005/0.3	0.84±0.09	0.15	**2.49**	**1.71/1.62**
Se	1.65±0.82	0.035/2.5	1.0±0.15	31.8	**1.65**	**4.77/6.00**
Sn	0.148±0.039	0.009/6.2	0.79±0.09	1.08	0.19	0.04/0.04
Sr	35.7±5.0	8.34/23.0	120±10	199	0.30	0.29/0.24
Th	0.320±0.251	0.001/0.5	3.3±0.2	-	0.10	0.06/0.04
Ti	45.6±32.8	<0.004/<0.1	720±40	-	0.06	0.01/0.01
Tl	0.248±0.078	<0.001/<0.2	0.68±0.07	0.09	0.36	0.49/0.41
U	7.31±5.76	0.008/0.2	2.9±0.3	0.79	**2.52**	**3.70/3.36**
V	4.33±4.15	0.013/0.7	22±2	16.8	0.20	0.05/0.05
W	15.8±13.1	0.004/<0.1	1.2±0.2	-	**13.2**	**4.20/2.43**
Zn	25.4±12.5	2.28/8.6	18±1	17.7	**1.41**	0.21/0.22
Lanthanides (μg g^{-1})						
La	1.79±.0.46	<0.028/<1.7	10±0.5	-	0.18	0.12/0.06
Ce	3.48±0.65	0.044/1.3	22±1	-	0.16	0.12/0.06
Pr	0.395±0.086	<0.004/<1.1	3.5±0.3	-	0.11	0.10/0.06
Nd	1.72±0.31	<0.017/<1.0	11±1	-	0.16	0.11/0.06
Sm	0.410±0.089	<0.004/<0.9	1.9±0.1	-	0.22	0.12/0.08
Eu	0.111±0.026	<0.001/<1.0	0.50±0.02	-	0.22	0.13/0.09
Gd	0.501±0.128	<0.004/<1.0	2.6±0.2	-	0.19	0.17/0.11
Dy	0.569±0.141	<0.005/<1.0	2.0±0.1	-	0.28	0.20/0.14
Ho	0.126±0.038	<0.001/<1.1	0.50±0.05	-	0.25	0.21/0.16
Er	0.377±0.106	<0.003/<0.9	0.85±0.08	-	0.44	0.22/0.17
Tm	0.051±0.021	<0.001/<1.1	0.31±0.02	-	0.16	0.22/0.17
Yb	0.336±0.139	<0.002/<0.9	1.0±0.05	-	0.34	0.22/0.17
Lu	0.051±0.021	<0.001/<1.1	0.19±0.02	-	0.27	0.24/0.18

*Coal Clarke values are the average trace element contents in the World coals (presented here for brown coals) [46]; Legend: ratio >1 to <2; ratio ≥2 to <3; ratio ≥3 to <4; ratio ≥4.

4. Discussion

4.1. Establishing the water/sediment quality status of the studied section of the Mura River

The quality status of the studied section of the Mura River was first established by comparison with legal recommendations at national and international level, as available. Several physico-chemical parameters are regulated within Croatian legislation, including pH values, concentrations of ammonia, nitrates, TN, TP, and Chl_a, and COD, and the recommended levels for the large lowland rivers are presented in Table ESM.1 [41]. Although some spatial and temporal variabilities were recorded, the overall quality of the Mura River water was generally very good or good, considering pH (very good), ammonia (good), nitrates (very good in June, good in October), Chl_a (very good), and COD (good). The critical parameters were, however, the total amounts of nitrogen and phosphorus, especially the latter one. According to TN, the water quality was very good in June at all sites, and good in October at several sites; the exceptions were MB and MS in October with values slightly above the limits for good quality, and HL and PT, with values two times higher (5.8 mg L^{-1} and 4.9 mg L^{-1}, respectively). As for TP, the ecological quality status was in general not good, and especially in June, when the concentrations were about four times higher than limit for good quality (0.79–0.97 mg L^{-1}), and especially high at PT (as much as eight times above limit, namely 1.67 mg L^{-1}).

For comparison, another Croatian lowland river, Mrežnica, flowing through urban area of Karlovac, and additionally influenced by industry and agriculture, had much lower concentrations of both nitrogen and phosphorus (TN: up to 1.75 mg L^{-1}; TP: up to 0.27 mg L^{-1}), despite having significantly lower dilution capacity compared to Mura (maximal discharge seven times lower) [35].

The legal recommendations for dissolved metal/metalloids in surface waters are set for only several elements (Table 2), either by Croatian legislation [41] or by WFD [9]. Comparison with Croatian legislation indicated much lower levels even of the highest measured As, Cr, Cu, and Zn concentrations compared to recommended values (6–30 times, depending on the element), whereas the comparison with EU legislation revealed the same for Cd, Ni, and Pb, namely much lower concentrations than proposed environmental quality standards (EQS), i.e. annual averages (AA) and maximal allowable concentrations (MAC) (6–10 times and 50–100 times, respectively, depending on the element). New WFD proposal [40], which should be put into effect shortly, proposes much stricter EQS for Ni, and introduces Ag as new priority substance. Nickel measured in the Mura River water was still much lower than new AAs and MACs (namely, three and 12 times, respectively), unlike Ag, which was markedly higher at several sites (MS, KR, PT) in the Mura river-water than its respective AA and MAC values (up to 22 and 10 times, respectively). Comparison was also made for Fe, with values proposed by Crane et al. [42], and it was up to 2.5 times higher in the Mura River than proposed value of 16 μg L^{-1}; however, this limit was considered by many as too strict, and it was never accepted as part of official regulations.

Comparison with data reported for several other Croatian lowland rivers indicated higher contamination with dissolved elements in the Mura River compared to Mrežnica River (industrial/agricultural contamination [35]), comparable to the Sava River (industrial/urban contamination [38]), and still lower compared to the Ilova River (industrial/agricultural contamination [37]). However, even in the case of the latter two rivers, Mn concentrations at PT site of the Mura River were notably higher, whereas Ag could not be compared due to lack of data for both these rivers. Comparisons were further made with world average (WA) dissolved element concentrations in surface freshwaters [39], as well as with expected geological background values (GBV) for streams in the studied area [33], and several elements could be pointed out as somewhat standing out. The elements that were higher four or more times than WA and/or GBV at some temporal/spatial point in the Mura River were Cs and W; Mn, Mo, Sr, Tl, and Zn were higher three to four times than WA and/or GBV; and As, Gd, Rb, Sb, Se, and U, two to three times. Positive Gd-anomaly at all sites was also detected, ranging from 2 to 4 times in June, and from 3 to 6 times in October. Anomalies >1 are referred to as positive, and refer to measured Gd concentrations that deviate from the smooth line predicted from normalisation of REEs on reference shale, meaning that Gd does not come from the same source as the remaining REEs [48].

Moreover, we have compared metal/metalloid concentrations measured in the Mura River sediments to available low and high Australian Interim Sediment Quality Guideline (ISQG-low and ISQG-high [44]) and USA Consensus-based Threshold/Probable Effect Concentrations (TEC and PEC [45]), as presented in Table 3. Comparison with ISQG-low and ISQG-high revealed generally much lower concentrations of Ag and Sb measured in the Mura River sediments. However, comparison with US guidelines revealed that at least some measured concentrations of As, Cr, Cu, Ni, Pb, and Zn were either equal or even up to twice higher than TEC, whereas Cr and Ni were in some cases even equal to PEC values; contrary, Cd was much lower than both TEC and PEC values.

We have further compared metal/metalloid concentrations measured in sediments with those reported for several Croatian rivers (Table 3), same as we did for their dissolved concentrations in water, and revealed that, regarding the sediments, the Mura River is generally comparable to other agriculturally/industrially contaminated rivers [35,37,43]. However, few elements, namely As, Mn, and Ti, were sometimes present in higher concentrations in the sediments of the Mura River than in the sediments of all the other considered rivers. Enrichment factors, calculated based on expected GBV in sediments of the studied area [33], pointed out several elements with possible anthropogenic origin, with Mn, Mo, and W standing out at several sites, having the highest maximal EFs, above 3. EF between 0.5 and 1.5 is a sign of metal origin entirely from crustal materials or natural processes; EF > 1.5 suggests higher probability of metal originating from anthropogenic sources [49,50].

Thus, as most critical contaminants of the studied section of the Mura River, the following can be singled out: total nitrogen and phosphorus in the river-water, dissolved Ag, Cs, W, Gd, and Mn in the river-water, and Cr, Mn, Mo, Ni, and W in the river sediments.

4.2. Temporal variability of measured parameters

The temporal changes of physico-chemical conditions in water can cause the metal redistribution between dissolved and particulate phase, and consequently variability of dissolved metal concentrations [11]. Since water temperature, pH, and dissolved oxygen, which commonly affect solubility of water contaminants [51,52], were comparable in both sampling campaigns, temporal variability of measured parameters could be mainly attributed to changes in the water discharge, with much higher discharges observed in June (~250 $m^3 s^{-1}$) than in October 2023 (~100 $m^3 s^{-1}$) at two gauging stations (Mursko Središće and Goričan; Figs. ESM.4–5).

As expected, most of the measured parameters, both nutrients and dissolved trace/major elements, were more or less pronouncedly higher during the low water discharge (sampling in October). For example, nitrates, TN, Chl_a, and more than twenty trace elements were observed in higher concentrations in October than in June, including some rare earths, such as Gd which was higher at all sites, leading to approximately 50 % higher Gd-anomaly during low water-level. All four major elements (Ca, Mg, Na, and K) were also increased, and as a result, conductivity and total hardness, too. In previous studies, increased conductivity, as well as the highest concentrations of many elements were associated to the lowest water level in the Krka River, too [52,53]. Moreover, the highest nitrate concentrations in the agricultural stream in Nebraska (USA) was observed during the minimal water discharge [54]. Accordingly, lower levels of majority of analysed parameters in June were probably the result of a dilution caused by increased water discharge due to snow melt in Alps and heavy rains, as high water-levels are commonly recognized as a cause of contaminants dilution [52].

Contrary, multiple higher values of three parameters were measured during the period of high water-discharge, namely TP and dissolved Al in water at all sampling sites and dissolved Ag at the three sites (MS, KR, and PT). Possible cause of these increases could be the resuspension of sediments, since unstable water column could readily mix and resuspend sediments upward from depth (e.g., during storm events) [55,56]. Consequent contaminant desorption and remobilisation from sediments can occur, which was reported by many authors during the periods of high water-level [57,58]. This is a likely cause of increased concentrations of Al and TP in our study. Sediment resuspension as an important source of dissolved Al was previously reported [56], whereas the release of phosphorus from sediment, accelerated by turbulence, is a known and important factor that influences TP content in river water [59,60]. Opposite temporal dynamics of nitrogen and phosphorus was probably a result of high solubility of nitrogen, whereas phosphorus is less mobile and readily binds to particles [18]. However, in the rural agricultural watershed, surface runoff was proven as an additional significant contribution to the dissolved phosphorus during rainfall [59]. The concentration increases of various contaminants during high water-level were commonly attributed to washdown of the waste landfills and/or agricultural soils, and soil erosion during rainfalls [37,57,61]. Thus, the washout from the surrounding soils temporarily enriched with contaminants could be plausible explanation also for Ag enrichment in the river-water, limited to three sites.

4.3. Diffuse pollution of the Mura River – Special points of interest

As a large lowland river running through predominantly rural area, the possible pollution of the Mura River can be mainly associated to several potential diffuse sources, which will be discussed in detail below.

4.3.1. Influence of agriculture

Agriculture is generally known as a major source of diffuse river pollution [10], and it presents predominant activity around the entire studied section of the Mura River in Croatia. Accordingly, we could expect to detect contaminants typical for agricultural pollution at all sampling sites, from the initial one in Croatia (MI), all the way to the mouth of the Mura River into the Drava River (KT). This is exactly what has been recorded for several parameters in the river-water, namely high values were recorded in June for TP (~0.8–1.7 mg L^{-1}) and in October for TN concentrations (~2–6 mg L^{-1}) at all sites.

High nitrogen and phosphorus concentrations in surface waters, comparable to our findings, were detected in the agricultural catchments in Europe (rivers in central Poland, hydro-system Danube–Tisza–Danube in Vojvodina, Bregalnica and Kriva rivers in North Macedonia) (TP: 0.1–1.2 mg L^{-1}; TN: 0.6–10 mg L^{-1}, with seldom higher levels) [18,62,63], whereas even maximal levels previously reported for the agriculturally impacted Sutla River in Croatia were somewhat lower (TP: 0.5 mg L^{-1}; TN: 3.2 mg L^{-1}) [12]. Today, nitrogen and phosphorus contamination of surface waterbodies in areas of intensive agriculture presents one of the most significant water quality problems due to their high amounts applied in fertilizers, even higher than required for plants [18,64,65]. It could potentially lead to eutrophication [66], and concurrent loading of other anthropogenic contaminants (e.g., metals) [64].

Accordingly, in October we have detected especially increased dissolved concentrations of Na (~15–17 mg L^{-1}), K (~2.7–2.9 mg L^{-1}), Cs (0.042–0.060 μg L^{-1}), Fe (~18–40 μg L^{-1}), Mo (~1.3–1.5 μg L^{-1}), Rb (~2.5–2.9 μg L^{-1}), Sb (0.187–0.206 μg L^{-1}), Sr (169–177 μg L^{-1}), Tl (0.009–0.011 μg L^{-1}), and W (~0.700–0.800 μg L^{-1}) along the entire studied river-section; however, some of these parameters additionally stood out at specific sites, which will be discussed later. Among these, Cs, Mo, and Tl, stand out with 3–5 times higher concentrations than expected based on geological background [33], and W as much as 50 times higher. Moreover, in sediment, more or less increased EFs, indicating higher concentrations than geologically expected [33], were observed along the entire studied section of the Mura River for Na (~9–15 mg g^{-1}), Ca (~9–25 mg g^{-1}), Mo (~0.3–1.4 μg g^{-1}), Sr (~80–150 μg g^{-1}), W (~1.1–6.5 μg g^{-1}), and Zn (~50–140 μg g^{-1}), with Mo and W again standing out among them (EFs up to 4).

Thus, in addition to nutrients, W and Mo seemed to be contaminants of primary concern regarding agricultural contamination of the Mura River in Croatia (both group VIB transition elements with similar properties [67]). The obtained dissolved Mo concentrations were similar to those reported for agriculturally impacted section of the Sutla River (Croatia), as well as Bregalnica and Kriva rivers (North Macedonia) (~2 μg L^{-1} and ~ 0.4–1 μg L^{-1}, respectively) [12,62]. Molybdenum is used in agriculture to counteract Mo deficiency in crops, and its increased levels can be associated with use of phosphate fertilizers, which also contain W [23,68,69]. Molybdenum and W enrichment is common and even confirmed in commercial phosphorus fertilizers and the phosphate rocks used in their production [67,70–72]. Additional factor that favours increase of W concentrations is alkaline pH, such as measured in our study, i.e. higher dissolved W levels can be expected at pH values greater than 8.0, at which W exhibits less than 10 % adsorption [67]. Moreover, such slightly alkaline pH values (~8) are characteristic for waters impacted by intense agricultural activity [12,62,73].

Positive Gd-anomalies (3.9–5.7) were also detected at all studied sites, and calculated anthropogenic Gd concentrations amounted to 0.003–0.022 μg L^{-1}. However, the major sources of anthropogenic Gd in aquatic systems are municipal wastewater treatment plants, and it mainly refers to Gd-based contrast agents [74]. As the studied section of the Mura River runs through rural area with no such facility or large cities capable to produce this type of contamination, in further research medical/urban inputs from all three bordering countries should be investigated.

Total bacterial abundance showed patterns typical for agricultural influence throughout the studied river section. The 16S rRNA gene concentrations from sediment TC-DNA increased downstream across the five sites (MI, MS, KR, PT, KT), ranging from 10^8 to 10^9 gene copies/g sediment. These values align with previous reports for river sediments impacted by agricultural activities [75].

The *intI1* gene, a marker for anthropogenic pollution [27], was detected at comparable concentrations (~10^{-2} gene copies/*rrn* copies) across all sampling sites, confirming probable agricultural influence. These values fell within the range reported for agricultural soils (10^{-4} to 10^{-1} copies/*rrn* copies), but closer to the upper reported limit [76–78]. The Mura River sediments showed slightly lower *intI*1 abundance than sediments impacted by pharmaceutical wastewater (~10^{-1} copies/*rrn* copies [79]), but higher than rivers receiving municipal wastewater (~10^{-3} copies/*rrn* copies [80]).

4.3.2. Influence of thermal waters in the upstream river-section

As described in the Materials and methods, thermal baths, situated close to the right bank of the upper section of the Mura river-course in Croatia, discharge their spent thermal waters into the small stream Gradiščak (SG) [81], which flows towards the river, but, just before reaching it, inflows into the floodplain. The discharge of spent geothermal waters presents potential environmental risk and may lead to serious environmental damages [82,83], associated with elevated temperature and high metal and nutrient concentrations, usually above the environmental limits [82,84]. Thus, we have examined the possibility that washout from the floodplain could result with the certain degree of the Mura River water/sediment contamination (at the site just downstream of the said floodplain, namely at MB site) with some of the contaminants that were found in increased concentrations in SG. The results of the measurements performed in the SG water and sediment were compared with those obtained for the Mura River upstream of the floodplain (at MI site) and with the regulations, to define the level of SG contamination and its main contaminants.

High concentrations of ammonia, TP, as well as nitrates and TN (~5–30 times higher compared to MI site, depending on the parameter and season) should be pointed out, as well as high conductivity and water hardness (~1000–6000 μS cm^{-1} and ~ 15–18 °dH respectively), and 3–5 °C higher water temperature, which are typical findings for geothermal waters [83,85]. In comparison, much lower DOX, with concurrent increase of CO_2 was also detected, especially in October (DOX: 0.78 mg L^{-1}; CO_2: ~20 mg L^{-1}), during the low water discharge, when the conditions were almost anoxic; increased CO_2 level is also characteristic for thermal waters [86]. The values similar to those found in the SG stream in the dry period were actually reported for the thermal baths spent water itself in the Heybeli geothermal field (Turkey; conductivity: ~4000 μS cm^{-1}; DOX: 0.9 mg L^{-1}; [82]), indicating negligible dilution in the SG stream.

Nutrient concentrations (ammonia, nitrates, TN and TP) and COD were higher (~3–13 times, depending on the parameter and season) than limits set by Croatian law for good water quality in small lowland streams (Table ESM.1 [41]). Moreover, NH_3 concentrations were comparable to those reported for the stream receiving geothermal waters in Hudai field, Turkey (~1–4 mg L^{-1} [85]), and above proposed ammonia levels for freshwater aquaculture species that should be <1.0 mg L^{-1} at pH below 8.5 [87].

As seen from above discussion on physico-chemical parameters, the SG water contamination was more pronounced during the dry, low water-discharge period in October, similar to the Mura River itself. Such temporal trend could be also observed for several other contaminants that were present in higher concentrations in the SG water in comparison with the MI site. Namely, multifold higher concentrations of many elements were recorded, especially Li (up to >300 times higher, i.e. ~34–860 μg L^{-1}), Ba and Cs (up to 160–170 times higher, i.e. ~0.4–5 mg L^{-1} and ~ 0.6–8.7 μg L^{-1}, respectively), and Na (up to 70–85 times higher, i.e. ~80–1150 mg L^{-1}). Geothermal waters typically contain high levels of alkali metals (Li, Cs, Na) and sulphate [83–86,88]. Unfortunately, we did not have the opportunity to measure sulphur in the water, but Li and Na concentrations during low-flow period were much higher than their concentrations reported for the receiving stream in Hudai geothermal field (Li: ~80–115 μg L^{-1}; Na: ~130–170 mg L^{-1}; [85]) and comparable or even higher than in the Heybeli thermal baths spent water (Li: ~1.1 mg L^{-1}; Na: 500 mg L^{-1}; [82]). For these elements, the legal limits were not as yet established, but some toxicity tests indicated certain levels of toxicity to aquatic organisms already at ~300 μg L^{-1} of Li, but only if Na concentration is low; in the presence of sufficient level of Na (above ~17 mg L^{-1}), such as observed in our study, the organisms should be able to endure much higher Li concentrations [89]. On the other hand, proposed AA-EQS for Ba in surface freshwaters is 93 μg L^{-1} and MAC-EQS. 1.1 mg L^{-1} [90], with measured Ba in SG reaching considerably higher values.

Due to morphological characteristics of the stream, its sediment was less abundant, being constantly carried by the flow and newly replaced; thus, its contamination, although visible, was less obvious than the water contamination. However, the concentrations of sulphur stood out, being up to 10 times higher in SG sediment compared to the MI site, and confirming above mentioned high sulphur input from geothermal waters [83,86]. The contamination of SG sediment was also more obvious in October, probably due to lower flow velocity and consequent slower sediment replacement, with several elements being present in concentrations above TEC-values, but still below PEC-values (namely, Cr, Cu, Ni, and Zn) [45]. Moreover, EFs for several elements (namely, Ba, Cs, Cu, and Zn) in October, which were equal to 2.5–5, confirmed the anthropogenic contamination of SG sediment. Despite that, metal resistance genes in SG sediment were either undetected (*cnr*A gene – for Co/Ni resistance; and *czc*D gene – for Co/Cd/Zn resistance), or detected in lower levels than in the Mura River sediment (*int*I1 gene – indicator of anthropogenic pollution). Only the *pbr*T gene (Pb resistance) showed comparable relative abundance to mining/agriculturally impacted sites (MS–PT).

Due to a high dilution capacity, the thermal wastewaters carried by the stream Gradiščak had, after all, almost negligible effect on the contamination of the Mura River water/sediment, but still observable. Namely, at the site MB in comparison to its two neighbouring sites (MI and HL), concentration increases in water were either visible, or even statistically confirmed, precisely for those contaminants that were multifold increased in SG, i.e. for ammonia, dissolved Na, Al, Ba, Cr, Li, and Mn, being especially evident during the rainy period in June and indicating the washout from the floodplain.

At the same time, in June, we have found a number of elements slightly increased in the sediment at the MB site (e.g., Al, Ba, Cr, Cs, Li, P, S, Sb, Tl, V), when the sampling was done closer to the bank due to high water-level, indicating certain sediment contamination of the marginal river-bed section. The absence of the same pattern in October, indicated that the contamination effect from the thermal wastewaters was too weak to reach the middle section of the river-bed. Similarly, Zachora-Buławska et al. [83] reported only localized impact of discharged geothermal waters on the recipient river (including a localized impact on macrofaunal assemblages), confined to the immediate vicinity of the outflow and not extending further away, not even 3 m from the bank. Slight or no significant effects in the lake/river that receive the inputs from the thermal bath discharges, directly or indirectly, were also reported, with the water quality being probably maintained by the dilution effect, and dependent on the current water-level [82,91].

Despite the low effect on the river, the strong effect on the receiving stream should be considered, corroborating the previous reports from the other parts of the world (e.g., deterioration of receiving streams water quality in geothermal fields in Turkey [82,85]). As Davraz et al. [85] emphasized, thermal water discharges to the surface freshwaters, especially to small water bodies, have to be monitored in order to prevent serious environmental issues.

4.3.3. *Influence of abandoned coal mines in the area from Hlapičina to Podturen*

The brown coal exploitation in the area of Mursko Središće (mainly from village Hlapičina to village Križovec) was initiated in the 1920s, and ceased to operate in 1970s [15,16]. However, the pits remained and are located in the immediate vicinity and parallel to the Mura River, just below the surface of agricultural land, with occasional reports in daily press on agricultural machines falling through the soil into the underlying coal pits. The washout of coal dust and runoff into the Mura River from these coal pits together with agricultural runoff is thus an actual possibility.

In the vicinity of these abandoned coal mines and covering river section from HL to PT (7 km downstream from the last coal mine pits), additional sediment enrichment with Mo (up to 1.4 μg g^{-1}) and W (up to 6.5 μg g^{-1}) was seen, with EFs mainly equal to ~2–4, in both seasons. Both Mo and W mobilisation from mine wastes was previously described, with mining activities being the major source of environmental contamination with W [23,68]. Tungsten is commonly detected in coals, and W-containing particles were detected in the air near coal-burning units at power plants [72,92].

Accordingly, the production of dust that contains metals and its atmospheric transport away from mining sites should be considered as a possible additional source of contamination of the neighbouring agricultural soils, due to deposition of metal-rich airborne particles [67,71,93]. Such scenario presents credible explanation for observed Mo and W contamination of the Mura River water/sediment that is extended both upstream and downstream from actual mining sites, as additional source to agricultural runoff.

At the sites near abandoned coal mine pits, evident increases in concentrations of dissolved Ag, Cr, Pb, and W were also recorded in the river-water during high water discharge in June, indicating washout as a probable source. In the case of dissolved W, which was present in high concentrations at all sampling sites in October (0.700–0.800 μg L^{-1}), the possible effect of nearby mines was shown in the form of absence of dilution during the increased water discharge at the sites near the mine pits (June: 0.630–0.750 μg L^{-1}), which was otherwise observed at all the other sites (June: 0.360–0.500 μg L^{-1}). On the other hand, although dissolved Mo did not present an increase in water nearby the coal mine pits, it was up to three times higher (June: 0.600–0.750 μg L^{-1}; October: 1.3–1.5 μg L^{-1}) than world average or expected GBV [33,39]. In both cases, the absence of clear dissolved concentration peaks near abandoned coal mines could be possibly associated to mixed influence of agricultural activities and coal mine pits.

To confirm the association of observed water/sediment contamination originating from the former coal mining, we have obtained the samples of the local brown coal from Mursko Središće (MS), and the analysis has revealed more than 10 times higher Mo and W concentrations in the coal compared to the Clarke values (average trace element contents in the world coals [46]), as well as 2–4 times higher W and 20 times higher Mo concentrations compared to their concentrations in the river sediment at the nearby MS site. Comparison was also made with the coal from Raša mine in Istria, Croatia [47], and it was confirmed that coal from Mursko Središće has ~10 times or more higher concentrations of As, Mo, Sb, and U (levels of W were not reported). Tungsten concentrations in Mursko Središće coal were, however, higher than reported by Taranushich [94] for coals of eastern Donbas (9.6 ± 7.9 mg kg^{-1}). Leachability of Mo and W from intact coal was estimated as rather low ($\leq$0.1 %), indicating that it is not expected that their high concentrations in the water/sediment would be found solely as a result of rock weathering. However, the coal dust washout from abandoned mine pits and nearby mine tailings, together with atmospheric transport, could have possibly caused observed Mo and W enrichment around Mursko Središće, and beyond.

PNEC for freshwater biota for Mo is rather high (12.7 mg L^{-1}) [95], indicating that Mo, as an essential element, has low toxicity to freshwater organisms under conditions established in the Mura River. As for W, for a long time it was also viewed as nontoxic and environmentally inert element, and thus not included in the EU and other directives, but due to recent findings it seems there is a need for environmental regulations [72]. In Russia, for example, W is already officially classified as a highly dangerous aquatic contaminant, and MAC equal to 0.800 μg L^{-1} was established for aquatic systems intended for fishing, based on the finding that W in a form of sodium tungstate (Na_2WO_4), when present in concentrations above the suggested MAC, is toxic to fish embryos [96]. Bolan et al. [23] also reported that high W concentrations in soil and aquatic environments in a form of tungstate can cause toxicity in biota, reducing Mo-enzymes activity and causing plant growth inhibition.

Enrichment of coal from Mursko Središće with Cr or Pb, on the other hand, was not confirmed. Thus, mild (twofold) increase of dissolved Cr (up to 0.300–0.320 μg L^{-1}) and Pb (up to 0.100–0.130 μg L^{-1}) in June, or rather absence of dilution in case of Pb, as also observed for W, possibly reflected minor accompanying release of these metals during mining or tailing disposal, as Cr, for example, has similar properties to Mo and W, being a group VIB transition element [67]. Although Pb concentrations were much lower than legal limits (10 times below AA defined by WFD) [9], the relative abundance of the *pbrT* gene, related to Pb resistance in sediment bacteria, was significantly higher at three sites historically associated to coal mining (MS, KR and PT). These sites exhibited values approaching 10^{-2} gene copies/*rrn* copies, approximately ten times higher than levels reported in soils at copper mining sites [97], though still an order of magnitude lower than those observed near e-waste recycling facilities [98]. Such unexpectedly high values likely reflect chronic Pb exposure in sediment, creating selection pressure that favours the proliferation of heavy-metal resistant bacterial communities [99]. This finding indicates that historical contamination continues to influence the adaptation of the microbial community, although current Pb concentrations are well below legal limits.

Based on the coal analyses, neither high dissolved Ag was associated with coal mining. Notable increase of dissolved Ag concentrations in June in the river-water at MS, KR, and PT sites (100 times compared to the other sites; up to 0.200–0.220 μg L^{-1}) was, as already discussed above, much higher than the proposed EQS (AA: 0.010 μg L^{-1}; MAC: 0.022 μg L^{-1}) in new EU WFD proposal [40], as well as compared to threshold level for dissolved Ag of 0.039–0.116 μg L^{-1}, that should be protective for ecologically relevant natural freshwater environments [100]. Moreover, according to new calculations of chronic HC_5 (hazardous concentration to 5 % of species) PNEC values for dissolved Ag in freshwaters (for algae, 0.470 μg L^{-1}; for invertebrates, 0.020 μg L^{-1}; for fishes, 0.220 μg L^{-1}; [101]), RQs for invertebrates and fishes were $\geq$ 1 (11 and 1, respectively), indicating considerable chronic risk for these organisms [36]. Previous studies on rivers in Croatia have never, to our knowledge, reported such high dissolved Ag levels (e.g., Krka: 0.002–0.020 μg L^{-1} [102]; Mrežnica: <0.001 μg L^{-1} [35]). It is possible that increase of Ag contamination of the river-water resulted from washout during rainy period from some other local contamination source. Agricultural fields or pharmaceutical industry were previously reported as the sources of environmental contamination with Ag, since both nanoparticulate (NP) and ionic Ag are commonly used in fertilizers [103] and as antibacterial agents [104,105]. Thus, the potential local sources should be further investigated. AgNPs can induce cell death, DNA damage, and oxidative stress in various cell types [105,106], whereas ionic form (as Ag^+) can also be very toxic, for example to fish, probably due to the disruption of the physiological ion balance [100,107]. Thus, Ag contamination in the Mura River should be carefully monitored during prolonged time period to establish if the observed increase was isolated incident or it occurs periodically, and to examine possible consequences for aquatic biota.

4.3.4. Additional environmental vulnerability due to specific river hydrogeomorphology at Podturen

The sampling site Podturen, as seen from the presented results, was influenced both by agriculture and by coal mining, being the first site downstream from the abandoned coal mine pits. However, it stood out from all the other sampling sites, with numerous elements being noticeably higher in both water and sediment. In addition to high concentrations of dissolved Ag in June, which were comparable to levels measured at two upstream sites (MS and KR), the highest dissolved concentrations of Co, Fe, Mn, Ni, and Zn in water were also detected here, especially during low water discharge in October, and particularly high for Mn (June: 35 μg L^{-1}; October: 133 μg L^{-1}), which is a contaminant typical for the waters running through cultivated land [108]. The detected Mn was well above concentrations proposed in literature as possible future EQS for dissolved Mn in freshwaters (7 μg L^{-1} for long-term, and 24 μg L^{-1} for short-term exposure) [42], and in October even higher than estimated PNEC value for freshwaters (75 μg L^{-1}) [109].

The PT site also had some of the highest concentrations of many elements in sediment, revealing high sediment enrichment (EFs: 2–4) with Co, Cu, Mo, Ni, P, Pb, W, and Zn, especially in June, when sediment sampling was done closer the river-bank due to high water-level. High sulphur was also detected, but enrichment was not calculated due to absence of geological background data.

Despite of sediment enrichment with Co, Ni, and Zn, the metal-resistance genes *cnrA* (Co/Ni), and *czcD* (Co/Cd/Zn) were not even detected in sediments at PT site, and appeared only sporadically at the other sites. The *cnrA* gene was detected at MI, KR, and KT sites ($\sim 10^{-3}$ gene copies/*rrn*), lower than in natural mangrove forest sediments ($\sim 10^{-2}$ gene copies/*rrn* [110]). The *czcD* gene appeared only at the MI and KT sites, with the lowest relative abundance among all studied genes ($\sim 10^{-4}$ gene copies/*rrn*), yet still higher than levels near copper tailing dams (10^{-7}–10^{-5} gene copies/*rrn* [97]).

Higher apparent contamination of water and sediment at PT site compared to the other neighbouring sites, which are exposed to similar sources of pollution, could be associated to specific hydrogeomorphology at Podturen. The sampling was done in a river bend (a lake-like section of the river), which is less influenced by the strong river current of the middle line of the river. Grain size analysis indicated silt as a predominant sediment component at Podturen, i.e. sediment at this site was classified as sandy silt, unlike the majority of remaining sites where it was classified as silty sand. This possibly influenced the enrichment of sediment with metals, as well as increased metal availability to dissolved water fraction. This can be corroborated by the known fact that grain size is significantly influenced by the flow rate/velocity, with finer grain sizes resulting from its decrease [111,112]. And, finer sediment grains (clay and silt), having larger surface area, tend to sequester high quantities of metals and organic matter [113,114]. Similarly to our case, Kim et al. [112] observed that the reduced flow rate/velocity near weirs have resulted with greater proportions of fine sediments and overall increased metal contamination in the sediments of Nakdong River, Korea. Yao et al. [115] also observed that metal concentrations in the suspended sediments of the Yellow River have increased with decrease in particle size, and approximately 80 % of the total metal load was accumulated in the <16 μm fraction.

Moreover, several of elements found in higher concentrations at PT site (e.g., Mn and Fe) are extremely sensitive to oxido-reductive conditions, which can also vary depending on the river current, i.e. between high current and stale waters. The decrease of the dissolved oxygen level in river water, that could occur in the latter, can cause reduction of Fe- and Mn-oxyhydroxides and subsequent release of both these metals into the solution together with the other adsorbed metals (e.g., Co), finally resulting in the increase of their dissolved levels in the river water [11,58]. As dissolved oxygen concentrations did not differ significantly between sites at the times of sampling (DOX: 9.0–10.5 mg L^{-1}), this factor initially did not seem decisive for understanding trace element variability among sites and increased metal concentrations at Podturen. However, the locations of water samplings for metal analyses and of DOX measurement within this same site were some 50 m apart, the former being shallower, and so it is possible that redox conditions differed between them. Thus, further and more thorough investigation of possible contribution of DOX variability to trace element increase at PT site should be performed (e.g., including more frequent measurements, and several spots within this site).

4.3.5. *Possible association of dissolved lanthanides with water carbonate hardness*

Interesting spatial pattern was observed for measured dissolved lanthanides in the water, which showed slight decrease towards downstream river section, and it was especially evident for those present in concentrations above 0.015 μg L^{-1} (La, Ce, Nd). Contrary, carbonate and total hardness increased towards downstream sites, indicating possible negative connection. However, correlation analysis confirmed rather high negative associations between the concentrations of these three metals in both samplings only for carbonate hardness (June: $r = -0.48$ to -0.75; $p = 0.02$–0.20; October: $r = -0.52$ to -0.61; $p = 0.08$–0.16), whereas negative correlation with total hardness was obtained only in June (June: $r = -0.61$ to -0.80; $p = 0.01$–0.08; October: $r = 0.08$ to 0.14; $p = 0.71$–0.83). The concentrations of these three elements also correlated highly among themselves ($r = 0.85$–0.98; $p < 0.01$). This agreed well with previous suggestion of possible decrease of potentially bioavailable La-species (such as La^{3+} or $LaOH^{2+}$) through carbonate complexation [116]. It is a known fact that REE exhibit high affinity for the surfaces of natural carbonates and readily precipitate with them at high pHs, such as measured in the Mura River, leading to their major association with particles, and consequent decrease of dissolved forms [117,118].

For Gd, this trend was not observed ($r = 0.07$ to 0.33; $p = 0.39$–0.86) despite the comparable concentrations to three above mentioned lanthanides, and thus Gd correlation with other lanthanides was also weaker (positive only in October, and not significant; $r = 0.37$–0.54; $p = 0.13$–0.33). As positive Gd-anomalies have been detected (~2–6) with increasing trend towards downstream sites, the reason for this exception can be found in confirmed anthropogenic contribution to dissolved Gd concentrations. Anthropogenic Gd is known for its non-reactivity with sediment particles in river waters, thus being exclusively present in the dissolved REE pool [119,120], which leads to its long environmental half-life in the river systems [31].

5. Conclusions

Our study revealed that large rivers with high dilution capacity can still experience some impairments of water quality despite absence of direct point sources of pollution. The recent concern of European legal authorities about a problem of diffuse river pollution was proven justified, as increased presence of several contaminants, that could have originated only from diffuse sources, has been detected in the Mura River in Croatia – the large lowland watercourse running through rural area that is important and protected at national and international level. Specifically, the nutrient levels exceeded legally recommended limits (TN, TP), whereas several metals surpassed geological background concentrations in water and/or sediments (e.g., Cr, Cs, Gd, Mn, Mo, Ni, W). Most concerning findings were the dissolved Ag, Mn, and W concentrations at certain sites, that surpassed some suggested protective concentrations for aquatic biota, and thus potentially could cause toxicity. Molecular analyses already confirmed increased Pb-resistance (high levels of *pbrT* genes) in the vicinity of the abandoned coal mines, and elevated *intI1* gene copies throughout the studied river section, comparable to agriculturally polluted sediments. The integrated assessment of the obtained data pointed to agricultural runoff (contributing primarily TN, TP, Mo, W, Mn) and the coal mine drainage (contributing Cr, Mo, Pb, W), as contamination sources of primary concern. The cause and frequency of increased Ag presence in water, on the other hand, still remains to be established. Additional influence of water discharge and physico-chemical characteristics, as well as grain size distribution in the sediments, on the metal contamination levels was revealed, and requires the further investigation. Accumulation of tungsten and other metals from coal mine drainage and agricultural runoff through the food chain, as well as their potential chronic toxicity to benthic organisms should also be investigated. As our results refer to a samplings within a single-year, prolonged monitoring of large lowland rivers exposed to diffuse pollution is recommended, as well as careful assessment of effects on aquatic ecosystem, to timely prevent more serious environmental consequences.

CRediT authorship contribution statement

Zrinka Dragun: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Željka Fiket:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Nataša Tepić:** Writing – review & editing, Validation, Software, Investigation. **Zoran Kiralj:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Tomislav Kralj:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis. **Zvjezdana Šoštarić Vulić:** Formal analysis. **Dušica Ivanković:** Writing – review & editing, Investigation. **Mavro Lučić:** Writing – review &

editing, Methodology, Investigation, Formal analysis. **Ana Puljko:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Nikolina Udiković-Kolić:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Damir Valić:** Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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