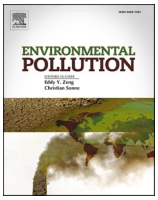
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Pollution patterns and their effects on biota within lotic and lentic freshwater ecosystems: How well contamination and response indicators correspond?☆

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ABSTRACT

Aquatic environments are often severely polluted with chemical substances of anthropogenic origin, which can pose a potential threat to aquatic organisms and human health. In this study, patterns and sources of heavy metals (HMs, 6 metals) and polycyclic aromatic hydrocarbons (PAHs, 16 hydrocarbons), contamination indicators, environmental genotoxicity measures and metrics of ecological status in lotic and lentic ecosystems were collated for the first time. Chemical analysis has confirmed previously reported long-term contamination at certain study sites. The sediments of Lake Talkša, located in a city and characterized by exclusive anthropogenic pressure, exhibited the highest levels of contamination by both HMs and PAHs. Through positive matrix factorization (PMF) analysis, vehicle and industrial emissions were identified as the primary sources of HMs and PAHs. Our results revealed that frequencies of genotoxic aberrations were higher in river sites compared to lakes, with the highest genotoxic risk observed in the Nemunas River below industrial cities Alytus and Kaunas. Surprisingly, even the severely contaminated Lake Talkša showed only a "moderate" grade of genotoxic risk, highlighting the potential for adaptation of biota to long-term contamination especially in lentic ecosystems. The ecological quality status assessed by macroinvertebrate metrics, which may be sensitive to observed high biological contamination, appeared to be unrelated to contamination patterns. Consequently, to obtain the robust information on anthropogenic contamination and its effects, a combination of various assessment methods and metrics should be employed.

1. Introduction

Due to rapid urbanization and industrial development, anthropogenic pressure on the environment, particularly surface waters, is increasing enormously (Ranjbar Jafarabadi et al., 2021a; Webb et al., 2020). Human impact alters biodiversity, community organization, disturbs the complex environmental balance, and induces harmful, often irreversible changes at various levels of biological organization (Webb et al., 2020; Liu et al., 2019). Contamination of surface waters with toxic substances, such as heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs), is particularly concerning as these elements are highly toxic, persistent, non-biodegradable, and bioaccumulative (Webb et al., 2020; Kostka and Lesniak, 2020; Bao et al., 2019; Ranjbar Jafarabadi et al., 2020; Ranjbar Jafarabadi et al., 2021a). Both HMs and PAHs can interact with DNA or enzymatic complexes causing formation of genetic disturbances (Lienesch et al., 2000; Cetin et al., 2018). Permanent, sublethal DNA lesions might induce abnormal physiological responses, increase the risk of developmental and degenerative diseases and formation of reproductive disorders. This might affect the survival of the species and biodiversity (Bistodeau et al., 2006; Vörösmarty et al.,

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2010; Ginebreda et al., 2014). Assessing genotoxic damage in sentinel bioindicator organisms provides relevant information on the status of aquatic ecosystems and enables the identification of early responses caused by adverse changes in environmental conditions (Jha, 2008; Bolognesi and Hayashi, 2011).

Genotoxicity endpoints – micronuclei (MN), nuclear buds (NBs), and nucleoplasmic bridges (NBr) (by their mode of formation partially overlapping aberrations) are widely applied genotoxicity biomarkers (Lindberg et al., 2007; Rao et al., 2008; Dutra et al., 2010; Shimizu, 2011; Fenech, 2011; Utani et al., 2011) and their total frequency serves as a joint parameter of total genotoxicity (GT). Such cumulative assessment of all genotoxicity biomarkers provides more accurate and comprehensive information, since they complement each other and at the same time cover a wider range of DNA damaging events. As cytotoxicity endpoints frequencies of fragmented–apoptotic (FA) and binucleated (BN) cells use to be assessed (Mičić et al., 2001; Baršienė et al., 2006a; Rybakovas et al., 2009).

Lotic systems receive particular attention regarding toxic pollution due to their direct impact on human health and their role in pollutant transport (Taylor and Owens, 2009). In a meantime, lentic waters, serving as sinks for various pollutants, are also heavily affected by human activities (Meena et al., 2018). Unfortunately, in contrast to lotic systems, the toxic contamination in lentic systems remains insufficiently studied, and this knowledge gap hampers our understanding about patterns and environmental impact of pollutants on biota in lentic ecosystems. Additional research on lentic ecosystems is therefore needed and it must include development of appropriate management and remediation strategies for their protection. The prevention and mitigation of undesirable impacts in aquatic ecosystems require robust information on pressure patterns and response indicators that may enable adequate assessment of ecosystem status and potential threats. Equally important is the identification of potential pollution sources.

The previous large-scale study of Lithuanian rivers has demonstrated that levels of environmental genotoxicity were associated with PAH and, to a lesser extent, with HM concentrations (Rybakovas et al., 2019). In this study, for the first time, we set to investigate both lentic and lotic systems with different levels of anthropogenic pressure and collate information on contamination, environmental genotoxicity and ecological quality status of study sites. The purposes of this research were (1) to examine the levels of PAHs and HMs in different lotic and lentic ecosystems, with a focus on determining the patterns and potential sources of contamination; (2) to evaluate the genotoxic and cytotoxic response of biota and the ecological quality status of study sites and reveal patterns of these metrics over study sites in water bodies of different hydrological regimes. Finally, we set to test how well contamination levels and different potential response to pollution indicators correspond in lotic and lentic ecosystems.

2. Materials and methods

2.1. Sampling

Study sites were selected in lotic (flowing water) and lentic (still water) systems based on potentially contrasting contamination loads, expectedly different anthropogenic loadings in rivers, and prior knowledge of pollution in lakes. Eight sites were selected in the largest Lithuanian rivers, Nemunas and Neris rivers in particular, 5 (NE1-NE5) and 3 (NR1-NR3) study sites, correspondingly. Five sites were in the lentic waters, lakes Dusia (DUS), Luokesai (LUO), Simnas (SIM) and Talkša (TAL), and lake-type Antalieptė Water Reservoir (ANT). Detailed description of the study sites and their geographic location are provided in the Supplementary information (Paragraph S1.1, Table S1.1).

HMs and PAHs were measured in sediments, unionid mussels *Unio pictorum* and *Anodonta anatina*, and European perch *Perca fluviatilis*.

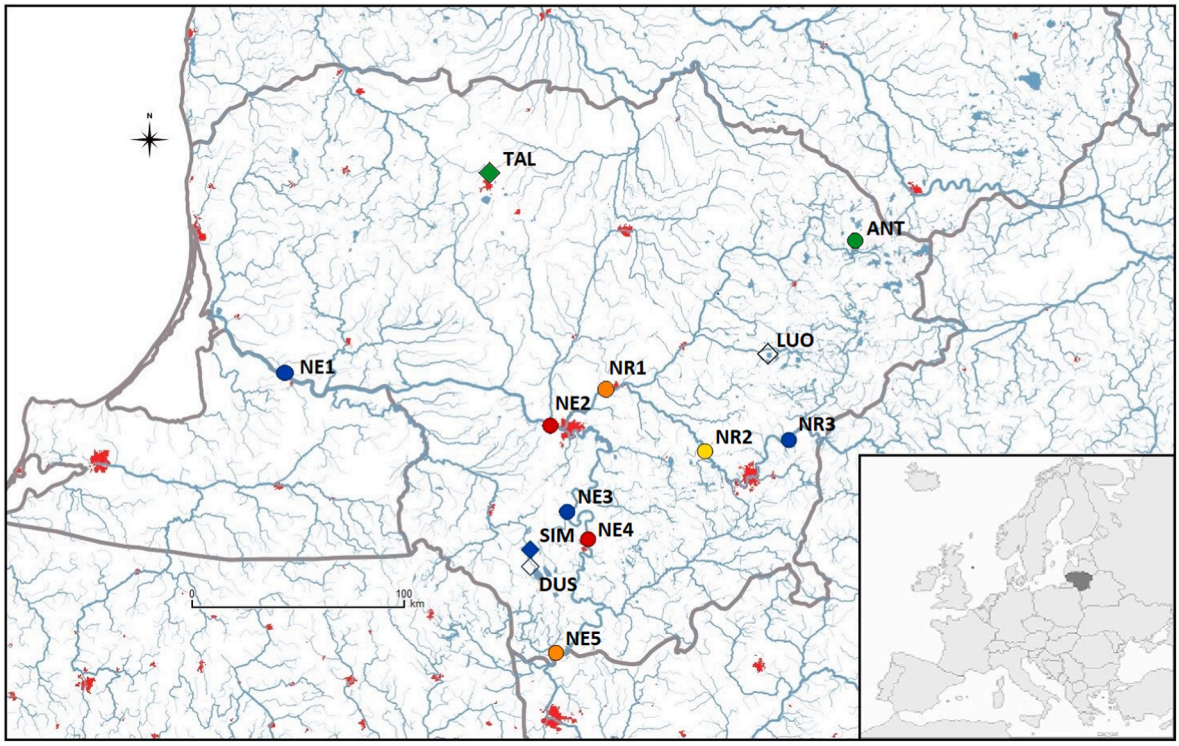


Fig. 1. Location of study sites in rivers – Nemunas (NE1, NE2, NE3, NE4, and NE5) and Neris (NR1, NR2, and NR3), Antalepte reservoir (ANT), and lakes – Simnas (SIM), Dusia (DUS), Luokesai (LUO), Talkša (TAL). Classification of sites into 5 categories according to the level of genetic risk is based on the proportion of bivalves with higher than the background level (6‰) of total genotoxicity effects. Risk categories: ≥20%, low (blue); 21–40%, moderate (green); 41–60%, increased (yellow); 61–80%, high (orange); and >80%, massive (red). The level of genetic risk was not determined in Dusia and Luokesai Lakes since studied unionids were not collected there. Areas of fishing are marked with diamonds.

Perch was selected for study in lentic systems as in a few lakes, lakes Dusia and Luokesai, unionid mussels (which were needed for genotoxicity assessment) were not available. In lakes devoid of unionid mussels, zebra mussels (*Dreissena polymorpha*) were used for chemical analysis.

Sediment and mussel samples were simultaneously collected in wadable depths (~0.8–1.2 m depth) during September–October, while fishing using gill nets (mesh size – 70 mm) was performed during October–November in 2016. For each study site, three surface sediment (0–5 cm) subsamples were collected using a Van Veen grab following recommendations of US U.S. EPA (2001) and EU guidance (2010). A subset of at least 20 mussels were used for homogenization and preparation of three subsamples for chemical analysis at each study site. In lentic sites, fish tissue (muscle and liver separately) samples were taken immediately after fishing from viable perch specimens. Dissection of muscle samples was performed with ceramic knives. Three subsamples of each fish tissue were prepared for chemical analysis. Samples of sediments as well as mussel and fish samples were kept frozen at −20 °C until analysis. For the genotoxicity and cytotoxicity assessment, 10 mussels were used as specified in Table S1.1.

2.2. HM analysis

HM analysis in sediments followed methodology described by Oliver (1973). After drying in a Petri dish for several days, analiquot of the sample (~2 g) was ground. 1 g of sample was heated for 2 h in 60 ml of aqua regia (concentrated HNO_3/HCl 1:1 v/v); cooled extracts were filtered through Whatman filters and concentrated down to a volume of ~2 ml. The as-prepared extracts were then transferred to 100 ml volumetric flasks and diluted with deionized water.

Mussel and fish tissues for metal determination were prepared following a modified procedure by Sen et al. (2011): 1–2 g w.w. of the sample were digested in 10 ml of ultra-pure nitric acid at 95 °C until the solution became clear (~90 min); after adding 3 ml H_2O_2, the solution was kept at 100 °C for 120 min; Afterwards, the extract was concentrated down to a volume of 2 ml, cooled, and filtered through a Whatman filter into a volumetric flask; and finally, the extract was diluted up to 100 ml.

The as-prepared sediment, mussel and fish samples were analyzed for lead (Pb), nickel (Ni), copper (Cu), chromium (Cr), cadmium (Cd), aluminum (Al), and zinc (Zn) with an inductively coupled argon plasma spectrometer PerkinElmer Optima 1000 DV ICP-OES. The NIST standard reference material 2702 was used for quality control. For each sample, three replicates were run. The analytical procedure precision, expressed as a standard deviation, was better than 10%. The accuracies were within 15% of the certified values for Cr and better than 10% for the other elements. The precision, expressed as RSD, was always better than 7% for the measured elements. All concentrations were calculated on a dry weight basis. Water content was measured by drying known amounts of sediment at 105 °C. Limit of detection (LOD) ranged from 0.006 to 0.03 mg kg^{-1}d.w., Limit of quantification (LOQ) ranged from 0.02 to 0.1 mg kg^{-1}d.w.

2.3. PAH analysis

Sediment samples for GC-MS analysis were prepared following the method that is described in details in our previous study (Raudonytė-Svirbutavičienė et al., 2022). Shortly, the method includes ultrasonic extraction with n-hexane (Sigma-Aldrich, HPLC grade) and methylene chloride (1:1) (Sigma-Aldrich, HPLC grade), filtering, purification using florisil cartridges (Chromabond 6 ml/500 mg), and elution with dichloromethane (DCM, Sigma-Aldrich, HPLC grade). For each sample, three replicates were run. LOD was in the range of 0.0000015–0.00002 mg kg^{-1} for each individual PAH while LOQ varied from 0.00005 to 0.0007 mg kg^{-1}. The details on the method are provided in the supplementary information file (S2).

Biota samples were prepared following a complex method based on Kalachova et al. (2011) and Guillen (1994). The method included saponification with methanolic KOH, extraction with DCM; cleaning with silica SPE cartridges, and elution with DCM.

Samples were analyzed for 16 PAHs: naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acy), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(123-cd)pyrene (Inp), dibenzo(ah)anthracene (DbA) and benzo(ghi)perylene (BgP). The sample preparation and GC-MS analysis methods and quality control are in details described in the supplementary info (S1.3). Supplementary info also contains descriptions of total organic carbon (TOC) and grain size analysis (S1.4), sediment pollution and potential toxicity assessment (S1.5), pollution source identification using EPA PMF method (S1.6), genotoxicity and cytotoxicity assessment, background levels and grades of genotoxic risk (S1.7), and determination of ecological quality status (S).

2.4. Genotoxicity, cytotoxicity and ecological quality status assessment

Level of cytogenetic damage was assessed in epithelial gill cells of mussels *U. pictorum* and *A. anatina* and peripheral blood erythrocytes of the European perch (in lentic waters), the organisms which are widely used as bioindicator organisms for *in situ* studies (Štambuk et al., 2009; Guidi et al., 2010, 2017; Rybakovas et al., 2019). Only *A. anatina* was detected in Lake Talkša, while the unionid mussels were not available in lakes Luokesai and Dusia even by scuba diving. Therefore, European perch was assessed for environmental genotoxicity in lentic water bodies. If available, at least 20 mussels of the similar body size and age were collected at each study site. They were transported to the laboratory in tanks filled with aerated water from the sampling location. Ten mussels at each site were biometrically characterized (shell length measured) and their sample preparation for genotoxicity was carried out on the same sampling day within a one to 3-h period (see Table S1.1). For fish samples, viable and active after removal from nets perches were measured and weighed (scales taken for aging), their blood was taken with heparinized syringe from the caudal vessel, and blood smears were prepared (Table S1.1). Further details on genotoxicity and cytotoxicity assessment, background levels and grades of genotoxic risk are provided in Supplement S1.7.

For the assessment of ecological quality status, a survey of eulittoral macroinvertebrates was performed both in lotic and lentic ecosystems following the procedure described by O'Hare et al., 2007. Two semi-quantitative 3-min samples, a stony/pebbly bottom kick sample and a submerged vegetation sweep sample, were taken by a standard dip net at each study site. Collected samples were used to evaluate the ecological quality status and biological contamination of study sites by using multimetric macroinvertebrate indices developed for lotic and lentic Lithuanian waters, and fauna autochtony assessment method, correspondingly (Šidagytė et al., 2013a,b, for details see Supplement S1.8).

3. Results and discussion

3.1. HMs and PAHs in sediments

In most of the sediment samples, HM concentrations were close to the local background levels for rivers and lakes (Fig. 2, Table S2.1). The highest HM loadings were detected in Lake Talkša (TAL) and the uppermost study site in the Nemunas River (NE5). Cr concentration exceeded the probable effect concentration (PEC) at two sites: TAL (81.5 mg kg^{-1} d.w.) and NE5 (101.9 mg kg^{-1} d.w.). Lake Talkša also exhibited Threshold Effects Levels (TEL) (MacDonald et al., 2000) exceeding Cu concentration (47.2 mg kg^{-1} d.w.). Pb value was slightly higher than the estimated TEL in Lake Luokesai (LUO2; 30.4 mg kg^{-1} d.w.).

The levels of PAHs varied among the studied river sites, ranging from 0.114 ± 0.012 to 1.32 ± 0.094 mg kg^{-1}, d.w (Fig. 2, Table S2.2). The Neris River showed moderate levels of PAH contamination, with slightly higher concentrations observed only in the vicinity of Vilnius City (NR2). In contrast, the Nemunas River exhibited varying levels of PAH contamination. One relatively unpolluted site downstream from Alytus City (NE4) had lower tPAH concentrations (0.325 mg kg^{-1} d.w.), while elevated concentrations were found at other sites (0.648–1.320 mg kg^{-1} d.w., Fig. 2, Table S2.2). At the Nemunas River site NE3, close to Birštonas town, almost half of the individual PAHs (45%) exceeded the Threshold Effect Level (TEL). Some PAHs such as BaA, Ant, Phe, and Acy exceeded TEL only occasionally. In a meanwhile, Nap exceeded the Threshold Effect Concentration (TEC) in more than half of the sampling sites, including both lotic and lentic systems (NE3, NE4, NE5, NR1, NR3, TAL, LUO). The PAH levels recorded in the studied Lithuanian river were found to be similar to those in the Narew (Poland), Danube (Hungary), and Tiber (Italy) rivers (Table S2.3; Baran et al., 2002; Škrbić et al., 2005; Montuori et al., 2016). However, the PAH concentrations were significantly lower than those detected in the Vistula, Odra (Poland) or Seine (France) rivers (Table S2.3; Dmitruk et al., 2008a,b; Kannan et al., 2003; Motelay-Massei et al., 2004). These findings highlight the varying levels of PAH contamination in Lithuanian rivers, with potential implications for the ecological health of these water bodies. Additionally, it is crucial to consider the pollution sources and the influence of the water body's hydrology on the pollution patterns in the rivers.

Among lentic systems, Lake Talkša exhibited the highest PAH loading, with each individual PAH exceeding the Effects Range-Low value (ERL). Furthermore, InP and DbA values significantly surpassed the accepted probable effects limit (PEL; MacDonald et al., 2000), indicating potential adverse biological effects on the local fauna (Table S2.2). The total PAH concentrations measured in sediments from Lake Talkša (6.377 ± 0.281 mg kg^{-1}) were comparable to those recorded in the highly urbanized lakes of Poland and Germany (Table S2.4; Popenda and Włodarczyk-Makuła, 2016; Bojakowska et al., 2018; Heimann et al., 2011). The Antaliepte Reservoir also showed considerable PAH contamination, with over 30% of individual PAHs exceeding the Threshold Effects Concentration (TEC). Lakes Simnas and Luokesai were moderately loaded with PAHs, while Lake Dusia was the least contaminated among the studied lentic water bodies (Fig. 2, Table S2.2).

It is worth noting that sediments with high content of fine sediment fraction and rich in total organic carbon (TOC) exhibited higher pollution with both HMs and PAHs. This is related to larger surface to volume ratio and thus greater adsorption capacity of such sediment. Additionally, organic carbon is known to form complexes with metals, which increases the stability of HMs in sediments (Ranjbar Jafarabadi et al., 2020).

To determine the potential toxicity of sediments, the potential ecological risk index (RI) for HMs and the total benzo(a)pyrene equivalent (TEQcarc) were calculated (S1.5). The highest RI value of 44.5 was recorded for Lake Talkša. Lake Luokesai and the Nemunas River sites NE1, NE3, and NE5 showed RI values between 10 and 22 while those in the other sites were below 10 (Table S2.1; Fig. S2.1). Following classification by Hakanson (1980), low potential ecological risk was posed by heavy metals even in the most polluted areas of the study area. In this study, copper was found to play the major role in sediment contamination with HMs (Fig. S2.1).

TEQcarc varied between 1.2 and 210.6 ng TEQcarc g^{-1} in the rivers (Table S2.1; Fig. S2.1): its value did not exceed 15 ng TEQcarc g^{-1} in the Neris and Nemunas River close to Alytus (NE4) while significantly higher TEQcarc (67.2–210.6 ng TEQcarc g^{-1}) were observed in other sampling stations across the Nemunas River. TEQcarc in the main Lithuanian rivers were comparable to those observed in the Tiber River (Table S2.4; Montuori et al., 2016), but were significantly lower than those recorded for highly contaminated urban rivers, such as Loire River in France (Table S2.4; Guo et al., 2011; Dong et al., 2014; Bertrand et al., 2015). The TEQcarc values of Lithuanian rivers were found to be relatively low, suggesting a minor ecological risk associated with PAH contamination.

Different TEQcarc values were obtained for the lakes (Table S2.1; Fig. S2.1). TEQcarc for Lake Dusia did not reach 1 ng TEQcarc g^{-1} but was significantly higher in lakes Luokesai (17.3 ng TEQcarc g^{-1}) and Simnas (36.7 ng TEQcarc g^{-1}). Elevated TEQcarc value was detected in Lake Antalieptė (157.5 ng TEQcarc g^{-1}) while the Lake Talkša exhibited the highest potential sediment toxicity: its TEQcarc reached 1196 ng TEQcarc g^{-1} (Fig. S2.1, a). While BaP played the dominant role for total TEQcarc in most of the samples, BaA was the absolute leader in the locations with relatively low pollution: it accounts for more than 90% of the total TEQcarc in Lake Dusia and in the Nemunas Station NE10 (Fig. S2.1, c). The Neris River demonstrated TEQcarc of mixed origin: BaA was responsible for 24 %–46% of the of the toxicity while InP and BbF made up to 52% (Fig. S2.).

3.2. Potential pollution sources

For the identification of PAH and HM emission sources, a PMF analysis was applied (Fig. 3).

In the HM-PMF model, F1 (45.27%) was loaded with Pb, Cu, and Cr. Cd was also attributed to this factor as Cd loading on F1 was higher than on the other factors. All these metals are widely used in industry but might also originate from the car tires (Degaffe and Turner, 2011; Lai et al., 2017; Lee, 2011; Mehr et al., 2019; Ranjbar Jafarabadi et al., 2020; Salim et al., 2019; Raudonytė-Svirbutavičienė et al., 2022). F1 played the most important role in the Lake Talkša. F1 was attributed to the mixed industrial/vehicle emission source.

F2 (34.16%) was defined by high loadings of Zn Cu, and Ni. Ni is a known component of automotive lubricants and car-tires, while Zn and Cu are also used in the production of car tires (Degaffe and Turner, 2011; Lai et al., 2017; Lee, 2011; Salim et al., 2019). The presence of these metals in the sediment could be attributed to the vehicle brake emissions (Salim et al., 2019; Hwang et al., 2016). F2 was an important source in most of the study area. It played the most prominent role in the sites NR1, NR2, NE1. Albeit not the dominant source, F2 was also important in the site NE5, lakes Talkša and Luokesa.

F3 (20.57%) was characterised by extremely high loadings of Cr and was important in the site NE5 and in Lake Talkša. Metal manufacturing, cleaning, chrome plating, textile and leather industries are among the main sources of chromium (Aquim et al., 2019; Cao et al., 2018; Cunningham et al., 2019; Hausladen et al., 2018; Hu et al., 2019). Lake Talkša has been known to be heavily affected by the previous industrial activities. In the 20th century, the Lake had been constantly loaded with an untreated sewage from a large leather tannery and a shoe factory situated nearby (Kozlovska et al., 2015). The site NE5 is located downstream from industrial Grodno City in Belarus. Hence, it might have been affected by uncontrolled industrial emissions as well.

In the PAH-PMF model, the first factor (F1, 52.03%) was defined by high loadings of BkF and BaP and moderate loadings of Ace, DbA, Acy, Flu, Ant, Flu, Pyr, BaA, and Chr. In general, the profile was characteristic of pyrogenic sources (Wang et al., 1999). Ace and Acy are well-known tracers of industrial activity (Kong et al., 2013; Wu et al., 2014). DbA, Flu, Pyr, BaA, BaP, might also originate from industrial emissions (Mougin, 2002). On the other hand, high-ring-number PAHs BghiP, BkF, and BaP are markers of emissions from diesel and gasoline vehicles (Caricchia et al., 1999; Lang et al., 2015; Miguel and Pereira, 1989; Wang et al., 2020; Wu et al., 2014), while Pyr and Chr are often present in diesel vehicle emissions (Jamhari et al., 2014; Fang et al., 2019; Wang et al., 2020). All this suggest that F1 might be a complex industrial/vehicle emission source. It could be seen from Fig. 3 that F1 had a negligible impact on most of the study sites and played a major role in Lake Talkša which is situated in an urbanized and industrialised territory in the eastern part of Šiauliai City (Fig. 1; Kozlovska et al., 2015; Raudonytė-Svirbutavičienė et al., 2021). Strict environmental

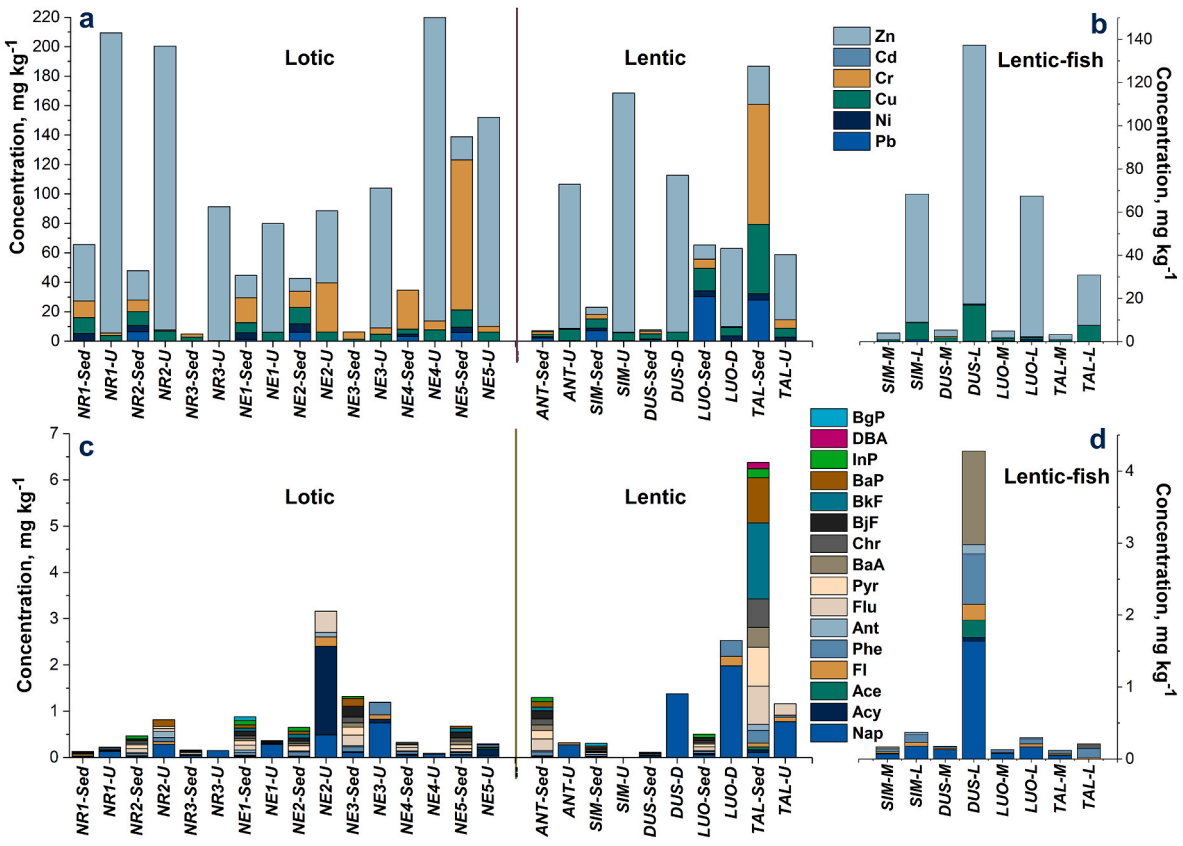


Fig. 2. Concentrations of metals (mg kg^{-1}d.w.) and PAHs (mg kg^{-1}d.w.) in the study area: (a) concentrations of metals in sediment (S) and mussels (U – unionids, D – dreissenids); (b) concentrations of metals in fish collected in lentic ecosystems (M – muscles, L-liver); (c) concentrations of PAHs in sediment (S) and mussels (U – unionids, D – dreissenids); (d) concentrations of PAHs in fish collected in lentic ecosystems (M – muscles, L-liver).

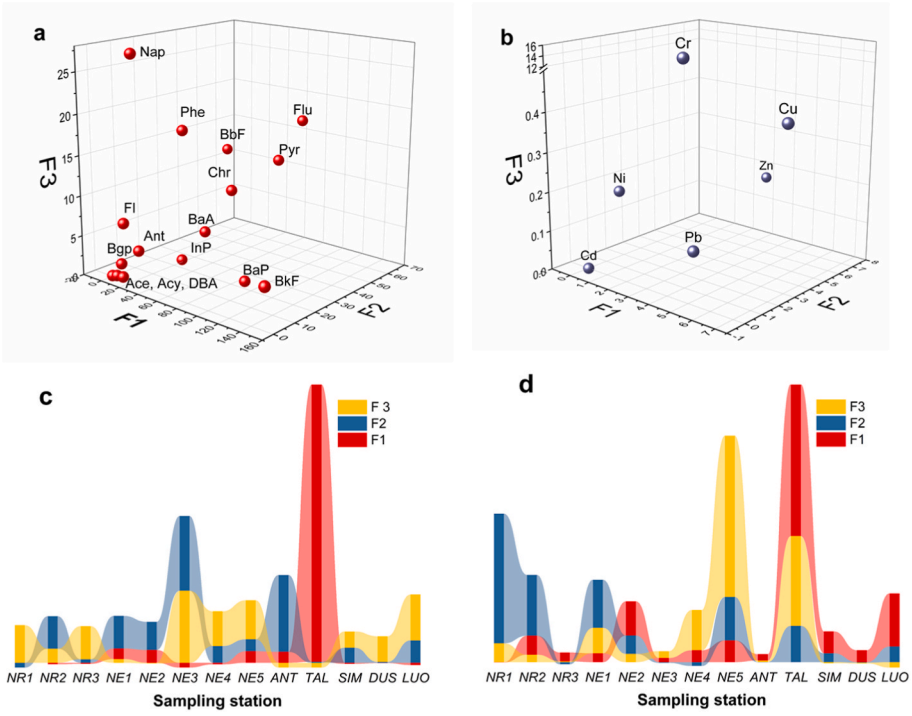


Fig. 3. Results of the PMF analysis: F1, F2, F3 factor loadings on individual PAHs (a) and metals (b); factor contribution on the sampling stations: PAHs (c), metals (d).

requirements have minimised the current impact of industrial objects in the area, but a couple of decades ago, wastewater was constantly discharged from several large industrial objects into the lake (Kozlovska et al., 2015; Paliulis, 2014). The impact of vehicle emissions and the influence of the surrounding city is also obvious as surface runoff gets to the lake from the busiest street of the city. These facts support the statement that F1 is a complex industrial/vehicle emission source.

The second factor (F2, 29.00%) was loaded with BbF, Flu, and Pyr. Moderate impact of Chr was also observed. InP was also attributed to this factor since its loadings on F2 were significantly higher (60%) than on the other factors. Pyr and InP are considered tracers of gasoline vehicle emissions (He et al., 2020; Qui et al., 2020). BbF, Flu and Chr are found in emissions from both gasoline and diesel vehicles (Davis et al., 2019; Ravindra et al., 2008; Wu et al., 2014; Wang et al., 2020). Hence, factor 2 was considered a source of vehicular emissions. This factor played the most important role in the sites NE3 and Antalieptė Reservoir and had some significant contribution at riverine sites NR2, NE1, NE2, NE4.

F3 (18.97%) was predominantly loaded with Nap. Some contribution from Phe and Flu was also observed, but the latter compounds were associated with F1 and F2. F3 was the most significant in the sites NE3, DUS, LUO and played an important role in sites NE4, NE5, and in the Neris River (NR1 – NR3). High loadings of LMW PAHs indicate either the existence of a local emission source or a relatively recent pollution. Naphthalene might originate from various human activities: gasoline and oil combustion, biomass burning, fires, the use of mothballs, fumigants (Jia and Batterman, 2010; Raudonytė-Svirbutavičienė et al., 2022). Even such everyday activities as tobacco smoking and food cooking might add some contribution to the Nap pollution (Jia and Batterman, 2010). On the other hand, Nap might also enter rivers and lakes via surface run-off from the nearby peatlands (Stakėnienė et al., 2019; Tsibart et al., 2014; Turetsky et al., 2014).

3.3. HMs and PAHs in biota

Concentrations of HMs and PAHs measured in mussel and fish tissues are provided in Fig. 2 and in Tables S6, S7. Concentrations of the most HMs in mussels were relatively low. Only Cu and Zn concentrations exceeded background assessment concentration (BAC) (Table S2.5). None of the HMs exceeded the estimated Environmental Assessment Criteria (EAC) and thus were not expected to cause adverse biological effects (OSPAR, 2016). PAHs impact on aquatic organisms might have been stronger. Nap exceeded EAC in lakes TAL, DUS, LUO and the Nemunas sites NE2 and NE3. Flu concentration was higher than EAC in Lake Talkša (TAL) and in NE2. The highest tPAH loadings were observed at the same Nemunas site NE2, while the largest PAHs concentrations among lakes was recorded in Lake Luokesai.

To gain insights into bioaccumulation patterns, correlations between pollutant levels in sediments and mussels were analyzed. Pearson's correlations were 0.89, 0.81, and 0.97 ($p < 0.005$) for Cr, Zn, and Ni, respectively. This suggests that mussels accumulate Cr, Zn, and Ni primarily from the surrounding sediment (Replinger et al., 2017).

Among PAHs, only Fl and Phe showed significant correlations (0.66 and 0.52, $p < 0.05$), indicating that sediments may be the main source of these PAHs for biota (Replinger et al., 2017). Although no sediment-biota correlation was observed for Nap, it was the most prevalent compound detected in mussels, even in the least polluted lakes (Fig. 2). As a low-molecular-weight PAH, Nap is expected to have a shorter half-life and be less likely to accumulate in sediments compared to high-molecular-weight PAHs (Ravindra et al., 2008). Therefore, it is more probable that Nap bioaccumulation in mussels is primarily driven by its presence in the water column.

The highest HM and PAH concentrations were detected in perch liver and muscles from Lake Dusia, while the lowest levels were found in perch from Lake Talkša, despite its highly polluted sediments (Table S2.6, Fig. 2). The most significant tissue-specific differences were observed in fishes from Lake Dusia, where total PAH concentration in fish liver was over 22 times higher than that in muscles. It was already reported that HMs accumulate in metabolically active organs rich in metallothioneins (Thomas et al., 1985). Perches from Lake Dusia exhibited intense accumulation of LMW PAHs, such as Nap, Ace, Fl, and Phe. This pattern aligns with the predominance of LMW PAHs of petrogenic origin in the lake sediments, suggesting recent contamination.

Animals located higher in the food chain might be severely affected by bioaccumulated PAHs. However, metabolic reactions involving cytochrome P450 enzymes might reduce or prevent PAH bioaccumulation (Stegeman, 1995). In this study, PAH concentrations measured in soft tissues of unionid mussels from lakes Luokesai, and Talkša were higher than those in perch muscles and liver. The opposite pattern was observed in Lake Dusia, where PAHs were more abundant in perch liver than in mussel tissues.

Bioaccumulation of PAHs in organisms can be influenced by external factors such as exposure level, mobility, feeding behavior, as well as internal factors like tissue specificity, metabolism, and excretion rates (Dwiyitno et al., 2016). Moreover, interaction with PAHs may trigger protective and reparative mechanisms, including detoxification and antioxidant systems (Rocher et al., 2006).

3.4. Genotoxicity (GT), cytotoxicity (CT), and ecological quality status

In the uppermost Nemunas site (NE5, downstream from industrial Grodno City, Belarus) the relatively high grade of GT risk was determined (GT – 7.6‰). This site was characterized by increased contamination with HMs (Table S2.1, Fig. 4). However, the highest GT values and the massive GT risk were detected at sites located downstream from Kaunas (NE2) and Alytus (NE4) cities (8.8 and 10.4‰, respectively) (Fig. 4). Total PAH concentrations measured in *U. pictorum* at NE2 were the highest among mussels from all the study sites (Fig. 2, Table S2.5), with predominant compounds of HMW PAHs (Flu, Ant, InP). Nap dominated at NE4. The sites NE1 and NE3 located at some distance from larger cities, the later one in a forested area of the Nemunas Loop Regional Park, were characterized by the lowest GT risk (below 2.8‰). PAH bioaccumulation in mussels from NE3 was comparatively high, but more than two-fold higher PAH concentrations were detected in mussels from NE2. It should be noted that NE3 mussels were mainly loaded with Nap which is considered as non-genotoxic, though in certain cases cytotoxic (Schreiner, 2003).

Municipal and industrial effluents from Vilnius City were most likely responsible for the significant GT changes observed in the study sites of the Neris River. Upstream of Vilnius (NR3), the comparatively low grade of GT risk was determined, while downstream (NR1 and NR2), GT values were significantly increased, reaching up to 6.7‰ (Fig. 4). Concentrations of PAHs in the NR3 sediment were nearly three times lower than those observed downstream, differences in Pb and Zn concentrations reached 7 and 11 times, respectively. Total cytotoxicity assessed in the Neris River varied between 1.05 and 1.57‰ while that in the Nemunas River changed from 0.30 (NE5) to 5.25‰ (NE3) (Fig. 4). Previous studies have also indicated high contamination of the Nemunas River below the City of Kaunas and the Neris River below Vilnius and increased frequencies of aneuploidy and polyploidy in somatic and gonadal cells of unionid mussels *U. tumidus*, *U. pictorum*, and *A. cygnea* (Baršienė and Bučinskienė, 2001; Baršienė et al., 1994, 2002). That supports an assumption that these river sites are affected by long-term municipal contamination and are under permanent genotoxic pressure. Increased genotoxicity in the areas affected by domestic, industrial wastewater, and rainwater run-off was demonstrated by multiple studies (Lacaze et al., 2013; Vuković-Gačić et al., 2013; Štambuk et al., 2009). Complex mixtures of municipal effluents might also negatively affect development and growth, cause reproductive impairment, have an impact on survival, and eventually may lead to alterations of community structure (Tetreault et al., 2011; Gillis et al., 2013; Nobles and Zhang, 2015).

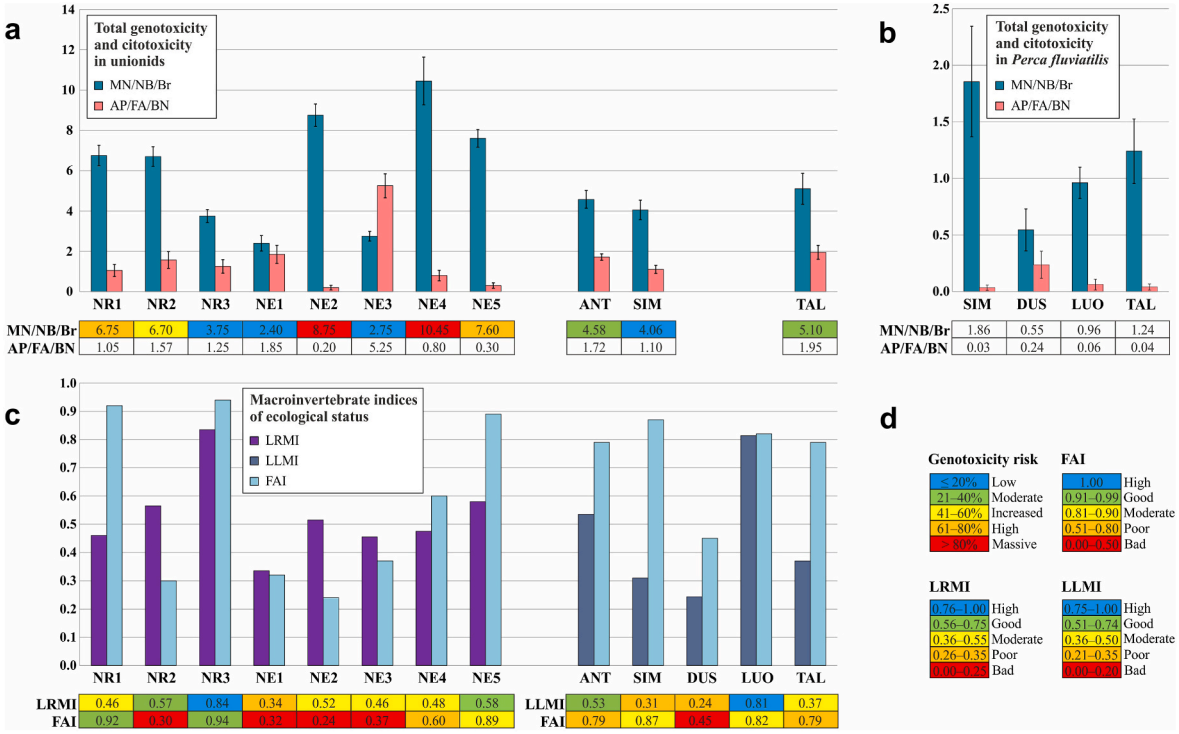


Fig. 4. Geno- and cytotoxicity, macroinvertebrate and species biocontamination indexes in studied lotic and lentic ecosystems. (a) GT and CT (mean ‰ ±SE) assessed in gill cells of unionids and categories of genotoxicity risk; (b) GT and CT (mean ‰ ±SE) in peripheral blood erythrocytes of *P. fluviatilis*; (c) macroinvertebrate indexes and ecological status categories; (d) description of GT categories and percentages used to assign to a certain category, as well as Lithuanian River Macroinvertebrate Index (LRMI), Lake Macroinvertebrate Index (LLMI) and Fauna Autochthony Index (FAI) categories and boundaries of certain category values.

Among the lentic ecosystems, Lake Talkša showed substantial contamination with HMs and PAHs. However, concentrations of these pollutants in mussels were from several to over a hundred times lower than those measured in sediments (Fig. 2). The assessed GT risk grade for *A. anatina* in Lake Talkša was moderate (5.1‰). A similar grade was observed in the Antalieptė Reservoir (4.6‰), the second most contaminated site, despite having almost five times lower PAH concentration in sediments compared to Lake Talkša. Concentrations of PAHs in mussel tissues from Talkša and Antalieptė were more than 5.4 and 2.8 times lower, respectively, than in the sediments (Table S2.1, S3, Fig. 2). The lowest GT risk grade among the studied lentic sites was recorded in unionids from Lake Simnas (2.8‰). The pattern observed in Lakes Talkša and Antalieptė Reservoir could be associated with the elimination of the most sensitive organisms and increased tolerance to long-term contamination (Large et al., 2002; Baršienė et al., 2010). As discussed in section 3.2, leather tannery effluents were identified as sources of pollution in Lake Talkša. In addition, both Talkša and Antaliepte were affected by chronic pollution associated with road runoff, resins, and combustion products.

In lentic ecosystems, total genotoxicity (GT) values in *A. anatina* and *U. pictorum* did not differ significantly between study sites (one-way ANOVA, $F = 2.5$, $p = 0.07$ and $F = 1.9$, $p = 0.18$, respectively). However, significant site-specific differences in GT were observed in lotic ecosystems ($F = 17.68$, $p < 0.001$). These results suggest that estimates of environmental GT in heavily contaminated lentic water bodies may be distorted due to adaptation of biomarker organisms to hostile environments. Such adaptation may reduce sensitivity and precision of GT estimates. Various mechanisms, including those responsible for reduced entry or effective removal of contaminants may contribute to mussel protection against genotoxic impacts. Adaptation to elevated contamination likely allowed mussels to survive in Lake Talkša, leading to reduced sensitivity and accuracy of biomarker responses. Similar patterns of reduced sensitivity have been observed in the North Sea, in the area characterized by long-term contamination by copper mining activities (Baršienė et al., 2006b; Zorita et al., 2006).

On the contrary, in lotic ecosystems, translocations of mussel glochidium larvae are much wider due to the water flow and unrestricted fish movement. Therefore, contaminated river stretches are regularly settled by mussels originating from the river sites with varying levels of pollution. Consequently, these new settlers, when surviving in contaminated environments, might be more sensitive to pollution and provide more robust GT assessments. Thus, the hydrology of the water body might have a substantial effect on the sensitivity of biomarkers when assessing the GT risk.

Among the four lentic ecosystems evaluated for genotoxicity (GT) in perch, the highest GT values were observed in Lake Simnas, followed by lakes Talkša and Luokesai (Fig. 4). Lake Dusia had the lowest level of genotoxic aberrations, despite the highest PAH concentrations recorded in perch liver (Fig. 2). The cytotoxic effects in perches from Lake Dusia were the highest among the study sites. Chemical analysis suggested a possible recent spillage of PAH compounds in Lake Dusia, indicated by elevated concentrations of low-molecular-weight (LMW) PAHs in the liver. This spillage event may have led to the formation of cytotoxic aberrations, which can suppress cell division and result in a low genotoxic response. Additionally, as discussed earlier, the genotoxic impact of LMW PAHs is generally lower. Overall, the long-term bioaccumulation of PAHs is better reflected in the muscles of perches than in the liver.

The ecological quality status in the Nemunas River ranged from good in the uppermost site to the poor in the lowest site, and this pattern was unrelated to GT estimates as well as pollution indicators (Fig. 4). The Nemunas River is inhabited by numerous populations of alien crustaceans, as indicated by high biocontamination estimates, and that may contribute to weak response of conventional macroinvertebrate metrics to environmental quality (Arbačiauskas et al., 2011). In the Neris River, the uppermost site (NR3), characterized by the lowest concentrations of HMs and PAHs, was assessed to have high quality status, while the downstream sites were more affected by pollution and demonstrated

lower quality status, good and moderate quality (Fig. 4).

Among the lakes, the high ecological quality status was determined only for Lake Luokesai, while the lowest one was estimated for Lake Dusia even though its sediments showed the lowest concentrations of contaminants. However this lake is severe biocontamination by Ponto-Caspian peracarid crustaceans, and that can impact robustness of macroinvertebrate based estimates of ecological quality. In common to Lake Dusia, the poor status was determined for Lake Simnas. The most polluted Lake Talkša as well as Antalieptė Reservoir exhibited moderate ecological quality (Fig. 4). Obtained results indicate that macroinvertebrate metrics meant for ecological quality status evaluation in lentic ecosystems may not be well responsive to anthropogenic contamination due to adaptation of local biota to the long-term pollution. Generally, ecologically aggressive alien macroinvertebrate species may also distort estimates of environmental quality (Arbačiauskas et al., 2011).

4. Conclusions

In this study, to best of our knowledge, the metrics of contamination, environmental genotoxicity, and ecological quality status were collated for the first time across lotic and lentic systems with varying levels of pollutants of anthropogenic origin.

PAHs were found to be ubiquitous contaminants in studied Lithuanian rivers and lakes while HMs exhibited relatively low concentrations. The studied sites exhibited significant differences in pollution levels, with vehicle and industrial emissions identified as the primary sources of both HMs and PAHs. The Neris River was less polluted in comparison to the Nemunas River which exhibited several sites with elevated pollution levels. The most contaminated lentic ecosystem was Lake Talkša, and the cleanest one was Lake Dusia. The TEQcarc values of Lithuanian rivers were found to be relatively low, suggesting a minor ecological risk associated with PAH contamination. In contrast, high TEQcarc values were detected in lakes Antalieptė and Talkša. Despite that, frequencies of genotoxic aberrations were higher in river sites compared to lakes. The highest levels of genotoxicity risk (massive risk) were determined at the Nemunas River sites located below the larger municipalities, while at the most severely contaminated Lake Talkša site, only a "moderate" grade of genotoxic risk was assessed. Thus, it may be concluded that in environments with long-term contamination, especially in lentic ecosystems, genotoxicity assessment using unionid mussels may not always be sufficiently sensitive. Decreased biomarker sensitivity might be influenced by adaptation processes, which could be caused by various mechanisms, including those related with reduced intake or more efficient removal of contaminants.

Assessments of ecological quality status by macroinvertebrate metrics were not tightly interrelated with contamination estimates. Response of macroinvertebrate metrics to contamination can be distorted by biocontamination with alien, especially ecologically aggressive macroinvertebrate species, and may be also impacted by biota adaptation to long-term toxic pollution. Results of this study suggest that, in order to obtain robust information on anthropogenic contamination, potential threats, and impacts of pollution on biota, complex study methods and assessment metrics should be applied.

CRediT author statement

Eva Raudonytė-Svirbutavičienė: Conceptualization, Idea development, Investigation (PAH sample preparation and analysis), Writing-Original draft preparation, Visualisation, **Kęstutis Jokšas:** Supervision, Investigation (metal analysis), Writing - Review & Editing, **Rimutė Stakėnienė:** Statistical analysis, Writing - Review & Editing, **Aleksandras Rybakovas:** Idea development, Field sampling, Investigation, Methodology, Writing- Original draft preparation, Visualisation, **Reda Nalivaikienė:** Methodology, Investigation, **Giedrė Višinskienė:** Investigation, Visualisation, **Kęstutis Arbačiauskas:** Funding acquisition, Study initiation, Idea formulation and elaboration, Coordination, Field sampling and investigation, Writing - review & editing.

Declaration of competing interest

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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