Assessment of freshwater mussel Unio tumidus and Unio crassus as biomonitors for microplastic contamination and physico-chemical characterization of their habitats in the Tisza River (Hungary)

Thesis of Ph.D. Dissertation

Wael Almeshal

Supervisor: Dr. Gyula Záray Professor Emeritus

Co-operative Research Centre of Environmental Science

Eötvös Loránd University

Doctoral School of Environmental Science

Head of the School: Prof. Dr. Tamás Turányi

Environmental Chemistry Doctoral Program

Head of the Program: Prof. Dr. Tamás Turányi

DOI: 10.15476/ELTE.2024.028

Budapest

2024

1. Introduction

Plastic pollutants, comprising a substantial proportion of marine litter, stand out as one of the most consequential contaminants within the aquatic environment. Their ubiquitous presence has garnered significant attention within the scientific community, prompting heightened concerns from decision-makers and non-governmental organizations (NGOs) on a global scale in recent decades.

Plastics are synthesized from a variety of distinct monomers. Notably, a handful of prevalent polymer types - PE, PP, PET, PVC, and PEU - constitute approximately 75% of the overall plastic demand (Bellasi et al., 2020). In the contemporary milieu, humanity's reliance on plastics is profound, with their pervasive application spanning virtually all sectors (Plastics Europe, 2022).

Plastic pollution has emerged as an unparalleled and persistent threat to aquatic ecosystems, encompassing both marine and freshwater environments. This crisis inflicts detrimental ecological and biological repercussions, making it one of the most daunting challenges to tackle (Andrady, 2015) (Huerta Lwanga et al., 2016); (Xu et al., 2020). As per the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP), microplastic encompass synthetic solid particles exhibiting regular or irregular shapes (GESAMP, 2015). The emergence of MPs as a distinctive contaminant has elevated global concerns over the past decade. However, the absence of a comprehensive consensus on an overarching definition due to the immense diversity characterizing their presence in the environment persists as a challenge.

Mussels are valuable bioindicators for monitoring microplastic pollution in aquatic environment, due to their role as suspension feeders. Freshwater mussels, e.g., members of the family Unionidae, are partly embedded into the bottom sediment, and as suspension-feeding organisms, they ingest living (bacteria, algae, and protozoans) and non-living (amorphous organic matter, detritus, and inorganic mineral) particles, and simultaneously, the MPs from the suspended sediment streaming above the bottom of the riverbed.

Mussels-mediated nutrient dynamics, biodeposition, and bioturbation can alter the chemical composition and properties of sediments (Haag, 2012; Hoellein et al., 2017; Vaughn & Hakenkamp, 2001). Particulate nutrients (organic C, N, and P) and other key elements (Fe, Mn, and Si) have a substantial influence on the ecology and biochemistry of aquatic environments by regulating the availability of dissolved nutrients, affecting light availability, influencing phytoplankton stocks, growth, grazing rates and community structure, as well as affecting food webs (Beusen et al., 2005; Bilotta & Brazier, 2008; Hickey et al., 2010; Turner & Millward, 2002).

2. Objectives

The aim of my research was to clarify the following questions:

1) Which freshwater mussel species existing in the Central European rivers would be an efficient biomonitor to evaluate the microplastic pollution of the Tisza River?
2) What kind of physico-chemical parameters are characteristic for the habitats of Unionade mussels along the Hungarian section of Tisza River with special attention on the particle-based nutrients and metal contaminants?
3) Is there potential risk of metal contaminants for the mussels as bottom-dwelling animals considering the Consensus-Based Sediment Quality Guidelines?

3. Materials and Methods

Wasserlab Automatic unit (Labsystem Ltd. Budapest, Hungary) was used for the production of ultrapure water (resistivity 18.2 MΩ cm−1). To prepare a 10% KOH solution, appropriate amounts of solid KOH granulate (VWR International Ltd.) were dissolved in ultrapure water. A particle-free $ZnCl_2$ solution with density of 1.5 g cm−3 was prepared by dissolving analytic grade solid $ZnCl_2$ (VWR International, Ltd) in ultrapure water, which was then filtered in a laminar box by applying a Whatman GF/C glass fiber filter with a diameter of 47 mm and pore size of 1.2 μm and a LABORPORT vacuum pump unit (KNF Lab, Freiburg, Germany).

Nitric, hydrochloric, and sulfuric acids, hydrogen peroxide, and solid natrium hydroxide of analytical grade were purchased from VWR International Ltd., while the internal and multi-elemental standard solutions for ICP-MS measurements were purchased from Sigma-Aldrich Ltd. (Missouri, United States).

Experiment 1

The mussels were collected from the Tisza River at depth of 0.8-1.2 m and within a 5-10 m coastal strip at four sampling sites along the Tisza River. The defrosted mussels underwent meticulous processing, including measuring shell length and removing soft tissues, which were subsequently weighed to calculate wet mass. The soft tissues were separately digested in 10% KOH solution at 40 C for 24 hours and incubated at room temperature for another 24 hours. To increase the efficiency of digestion procedure as a first step an ultrasonic treatment was applied at frequency of 37 kHz for 30 minutes. Microplastics (MPs) were separated from potential residues by adding a particle- free $ZnCl_2$ solution with a density of 1.5 g cm−3. The ensuing 48-hour density separation procedure was followed by pressure filtration of a 200 mL supernatant, using high-purity synthetic air to minimize the riskof laboratory air contamination. The concentrated particles and fibers were then collected on a Whatman GF/C glass fiber filter, dried, and stored under controlled conditions until analysis. Notably, blank samples underwent the same rigorous procedures to account for potential contamination. This systematic approach ensures the reliability and accuracy of the obtained data, providing a robust foundation for subsequent analysis and interpretation of microplastic presence in mussel soft tissues.

The loaded filters were visually inspected under a Nikon SMZ1000 stereomicroscope and a Nikon ECLIPSE LV100 POL (Nikon, Tokyo, Japan) polarization microscope with maximum magnifications of 80× and 1000×, respectively. For the chemical identification of particles and fibers, a Horiba Jobin Yvon (JY) LabRAM HR 800 Raman spectrometer equipped with a frequency-doubled Nd-YAG green laser with a 532 nm excitation wavelength was applied, displaying 120 mW at the source and 23 mW on the sample surface. An OLYMPUS 100 × (N.A. = 0.9) objective was used to focus the laser beam on the

analyzed sites. For the spot Raman analysis, a 100 μm confocal hole, with 600 grooves/mm optical grating and a cumulative 60 s exposition time were selected. The spectral resolution of measurements varied from 2.4 to 3.0 cm–1. The spot Raman data were processed through LabSpec 6 software 6.5.1.24 (Horiba Scientific, Paris, France).

Experiment 2

The sediment samples were collected in the time period of August 2–4, when the algal population was relatively high and defined by relatively high chlorophyll-a levels (20 μg/L). At all sampling sites, three sampling points located about 6–8 m from the river bank and spaced roughly 4–5 m apart were selected. At these sampling points, 5 L of suspension was pumped into amber bottles from the 10–15 cm thick suspension layer streaming over the bottom of the riverbed using a portable, pressure-difference "SEDIMONER" sampler developed by Aqua-Terra Lab Ltd. (Veszprém, Hungary). The suspended sediment samples were homogenized and three samples of 200 mL each were separately filtered using pre-combusted glass fiber filters with a pore size of 0.7 μm. The empty filters were weighed before the filtration. The loaded filters were dried at 80°C for 12 h and re-weighed to determine the dry mass of solid particles for calculation of the mass concentration of TSS.

The bottom sediments were wet-sieved over a 2 mm mesh to eliminate large detritus and benthic organisms and dried at 80°C for 12 h in a laboratory oven together with the suspended sediments. Three replicates of both sediment samples were individually homogenized. About 30–90 mg of these samples were resuspended in 200 cm3 ultrapure water and subjected to ultrasonic treatment at frequency of 37 kHz for 5 minutes. The cumulative and differential size distribution functions were determined with the use of a Shimadzu SALD-2300 laser diffraction particle size analyzer (Shimadzu, Kyoto, Japan).

For measuring the TOC contents, 300-500 mg dried and homogenized sediment samples were analyzed using a multi N/C 3100 analyzer (Analytik Jena, Jena, Germany). The total Kjeldahl nitrogen (TN) of dried sediments was measured by automated colorimetry with preliminary distillation/digestion on the basis of Standard Method 1,687 (EPA-821-R-01-004, January 2001).

To determine the pseudo-total concentration of phosphorus (TP), arsenic, and different metals, 500 mg dried sediments were treated with 8 cm3 aqua regia at a temperature of 200°C for 20 min in a TopWave microwave-assisted digestion system (Analytik Jena, Jena, Germany). Three replicates were prepared for all sediment samples. Following the sedimentation of solid particles (predominantly silicates), 2 cm3 of

the clear solution was removed by a pipette and diluted 25-fold with ultrapure water. After the addition of internal standards (Sc, Y, In) in concentration of 20 µg/L, the main (Al, Fe, Mn, P) and trace elements(As, Cd, Co, Cr, Cu, Hg, Li, Ni, Pb, Sn, Zn) were determined using a Plasma Quant Elite inductively coupled plasma mass spectrometer (Analytik Jena, Jena, Germany). To characterize the reliability of thisanalytical procedure a recovery test was carried out for 8 elements (Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn) analyzing the BCR- 146R (Sigma Aldrich, Missouri, United States) certified reference material. The recovery values changed in the range of 82% and 114%.

4. Thesis points

On basis of my experimental data and observations, my main scientific results are summarized in the following thesis points:

1. The soft tissues of both mussel species contained synthetic and natural fibers as dominant solid particles with length of 20–1000 µm and diameter of 10-75 µm.
2. The amount of accumulated particles in the soft tissues of Unio tumidus was nearly twice higher than in the Unio crassus (5.2- 8.32.vs. 2.7-4.9 items/individual, or 0.72-1.03 vs. 0.25-0.51 items/g soft tissue). Therefore the Unio tumidus can be recommended as ‘living sampling device”for monitoring of microplastic contamination in freshwater environment.
3. The dominant color of fibers was blue and the basic material of all blue fibers was polyethylene terephthalate colored with indigo dye.
4. Cellulose-based fibers were also present, with two distinguishable groups based on diameter (10–25 µm and 30–75 µm), and these are characteristic of polyester/cellulose blends.
5. The number of fibers/g soft tissue values increased by 35% in both mussel species collected along the 392 km section of the Tisza River due to the contaminants transported by the tributaries and increasing number of wastewater treatment plants located in the river bank..

6. The mass concentration of Near-Bottom Suspended (NBS) sediment particles within the 392 km long section of Tisza River ranged between 209 and 274 mg/L.
7. The NBS sediments contained smaller grains than the BS samples, roughly 85- 90% of the particles were less than 60 um. Contrarily, only 2.4- 51.8 % of BS particles were less than 60 um. The characteristic size range for the NBS and BS particles were 2–60 and 60-500µm, respectivel, and different grain size groups were observed in both sediment types, indicating particles from various sources.
8. Relative to the BS samples, the NBS sediments contained 30, 23 and 15% more organic carbon, nitrogen and phosphorous, respectively. The rounded C:N:P molar ratios in the NBS sediments were 60:4:1, whereas they were 45:3:1 in the BS. The C/N molar ratios suggest that soil-derived organics are the dominant sources of organic matter in both sediment types.
9. Various metal contaminants, including Al, Mn, Cr, Sn, Zn, and others, are present in higher concentrations in NBS sediments compared to.the BS samples and their concentrations show positive correlations with total organic carbon (TOC), except for Hg in NBS and Zn in bottom sediments.
10. The concentrations of As, Cd, Cr, Cu, Pb, and Ni are generally below the threshold effect concentrations (TEC) and the probable effect concentrations (PEC) defined by the Consensus-Based Sediment Quality Guidelines developed for evaluation of potential risk of metal contaminants in sediment for the benthic-dwelling organisms., except for Hg, which exceeds the PEC value.

Papers appended to the dissertation

1. **Wael Almeshal**, Anita Takács, László Aradi, Sirat Sandil, Péter Dobosy, and Gyula Záray (2022). Comparison of Freshwater Mussels Unio tumidus and Unio crassus as Biomonitors of Microplastic Contamination of Tisza River (Hungary). *Environments*, 9, 122.
DOI: 10.3390/environments9100122
2. Péter Dobosy, **Wael Almeshal**, Ádám Illés, Davaakhuu Tserendorj, Sirat Sandil, Zsófia Kovács, Anett Endrédi, and Gyula Záray (2023). Particle-based nutrients and metal contaminants in the habitat of Unionidae mussels in the Tisza River (Hungary). *Frontiers in Environmental Science.*11. 1209118. DOI: 10.3389/fenvs.2023.1209118

Conference presentations related to the dissertation

Oral presentations

1. October 2018., Budapest. XVI Hungarian-Italian Symposium on Spectrochemistry: “Microplastic in aquatic environment”, Anita Takacs; **Wael Almeshal**; Gyula Zaray.
2. November 2018., Budapest. VIII. Ökotoxikológia Konferencia: “Felszíni vizek mikroműanyag szennyezése”, Anita Takács; **Wael Almeshal**; Gyula Záray.
3. March 2019., Budapest. Hungarian Hydrology Society: “Mikroműanyagok a vizi környezetben ”, Anita Takacs; **Wael Almeshal**; Gyula Zaray.
4. June 2019., Thessaloniki- Greece. 17th International Conference on Chemistry and the Environment: “Occurrence and concentration level of microplastic in sediments of Danube River, Hungary”, **Wael Almeshal**; Anita Takacs; Gyula Zaray.
5. November 2019., IX. Ökotoxikológia Konferencia, Takács Anita, **Wael Almeshal**, Barta Barbara, Schmera Dénes, Aradi László Előd és Záray Gyula Mikroműanyag-szennyezők kimutatása Chironomidae-, Gammaridea-, Sphaerium- és Dreissena-fajokban.
6. June 2021, XVII Italian-Hungarian Symposium on Spectrochemistry: “Biomonitoring of microplastic pollution by mussels in the Tisza River, Hungary”, **Wael Almeshal**, Anita Takacs, Gyula Zaray.
7. June 2021, XVII Italian-Hungarian Symposium on Spectrochemistry: “Microplastics in freshwater mussels and sediments: review of sources, detection methods and occurrence”, Anita Takacs, **Wael Almeshal**, Gyula Zaray.

Summer school:

1. 21-25 July 2019, Lake Constance and the Danube River, Konstanz, Germany. Summer School Limnology.

References

Andrady, A. L. (2015). Persistence of plastic litter in the oceans. In *Marine Anthropogenic Litter*.https://doi.org/10.1007/978-3-319-16510-3_3

Bellasi, A., Binda, G., Pozzi, A., Galafassi, S., Volta, P., & Bettinetti, R. (2020). Microplastic contaminationin freshwater environments: A review, focusing on interactions with sediments and benthic organisms. *Environments - MDPI*, *7*(4). https://doi.org/10.3390/environments7040030

Beusen, A. H. W., Dekkers, A. L. M., Bouwman, A. F., Ludwig, W., & Harrison, J. (2005). Estimation ofglobal river transport of sediments and associated particulate C, N, and P. *Global Biogeochemical Cycles*, *19*(4). https://doi.org/10.1029/2005GB002453

Bilotta, G. S., & Brazier, R. E. (2008). Understanding the influence of suspended solids on water quality and aquatic biota. In *Water Research* (Vol. 42, Issue 12). https://doi.org/10.1016/j.watres.2008.03.018

GESAMP. (2015). *Sources, fate and effects of MP in the marine environment*.

Haag, W. R. (2012). North American freshwater mussels: Natural history, ecology, and conservation. In *North American Freshwater Mussels: Natural History, Ecology, and Conservation*. https://doi.org/10.1017/CBO9781139048217

Hickey, B. M., Kudela, R. M., Nash, J. D., Bruland, K. W., Peterson, W. T., MacCready, P., Lessard, E. J., Jay, D. A., Banas, N. S., Baptista, A. M., Dever, E. P., Kosro, P. M., Kilcher, L. K., Horner-Devine, A. R., Zaron, E. D., McCabe, R. M., Peterson, J. O., Orton, P. M., Pan, J., & Lohan, M. C. (2010). River Influences on Shelf Ecosystems: Introduction and synthesis. In *Journal of Geophysical Research: Oceans* (Vol. 115, Issue 2). https://doi.org/10.1029/2009JC005452

Hoellein, T. J., Zarnoch, C. B., Bruesewitz, D. A., & DeMartini, J. (2017). Contributions of freshwater mussels (Unionidae) to nutrient cycling in an urban river: filtration, recycling, storage, and removal.*Biogeochemistry*, *135*(3). https://doi.org/10.1007/s10533-017-0376-z

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., Van Der Ploeg, M., Besseling, E., Koelmans, A. A., & Geissen, V. (2016). Microplastics in the Terrestrial Ecosystem: Implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). *Environmental Science and Technology*, *50*(5). https://doi.org/10.1021/acs.est.5b05478

Plastics Europe. (2022). Plastics the fact 2022. *Plastics Europe Market Research Group (PEMRG) andConversio Market &Strategy GmbH.*

Turner, A., & Millward, G. E. (2002). Suspended particles: Their role in Estuarine biogeochemical cycles. *Estuarine, Coastal and Shelf Science*, *55*(6). https://doi.org/10.1006/ecss.2002.1033

Vaughn, C. C., & Hakenkamp, C. C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. In *Freshwater Biology* (Vol. 46, Issue 11). https://doi.org/10.1046/j.1365- 2427.2001.00771.x

Xu, S., Ma, J., Ji, R., Pan, K., & Miao, A. J. (2020). Microplastics in aquatic environments: Occurrence, accumulation, and biological effects. In *Science of the Total Environment* (Vol. 703). https://doi.org/10.1016/j.scitotenv.2019.134699