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)

by

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Nomenclature and Abbreviations

AAS: Atomic Absorption Spectrometry
Al: Aluminum
As: Arsenic
Ar: Argon
BDE-47: Tetrabromodiphenyl ether
BPA: Bisphenol A
BS: Bottom Sediment
CBSQGs: Consensus-Based Sediment Quality Guidelines
Cd: Cadmium
Co: cobalt
Cr: Chromium
Cu: Copper
DDE: dichlorodiphenyldichloroethylene
DDT: Dichlorodiphenyltrichloroethane
EPA: Environmental Protection Agency
EVA: Ethylene-vinyl acetate
FAAS: Flame Atomic Absorption Spectrometry
Fe: Iron
FTIR: Fourier-transform infrared spectroscopy
GF: Glass fiber
H_2: Hydrogen
HBCD: Hexabromocyclododecane
HCHs: Hexachlorocyclohexanes
HCl: Hydrochloric acid
$HClO_4$: Perchloric acid
HDPE: High-Density Polyethylene
He: Helium
HF: Hydrogen fluorideHg: Mercury
HNO_3: Nitric acid
H_2O_2: Hydrogen peroxide
ICP-AES: Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS: Inductively Coupled Plasma Mass Spectrometry
ICP-OES: Inductively Coupled Plasma Optical Emission Spectroscopy
LDPE: High-Density Polyethylene
Li: Lithium
MPs: Microplastics
MPSS: Munich Plastic Sediment Separator
Mn: Manganese
MSFD: Marine Strategy Framework Directive
N: Nitrogen
N/A: Not Applicable
NaCl: Sodium chloride
NBS: Near Bottom Sediment
NGOs: Non-Governmental Organizations
Ni: Nickel
NOAA: National Oceanic and Atmospheric Administration
OH: Hydroxy group
P: Phosphorus
PA: Polyamide

PAHs: Polyaromatic hydrocarbons
Pb: Lead
PBDEs: Polybrominated diphenyl ethers
PCBs: Polychlorinated biphenyls
PE: Polyethylene
PEC: Probable Effect Concentrations
PET: Polyethylene Terephthalate
PEU: Polyurethane
PF: Phenol formaldehyde
PFOS: Perfluorooctanesulfonic acid
POPs: Persistent organic pollutants
POM: polyformaldehyde
PP: Polypropylene
PS: Polystyrene
PS-E: Expanded Polystyrene
PSU: Polyarylsulfone
PU: Polyurethane
PVC: Polyvinylchloride
Py–GC–MS: Pyrolysis gas chromatography-mass spectrometry
SEM-EDX: Scanning Electron Microscopy - Energy Dispersive X-Ray Analysis
Sn: Tin
TEC: Threshold Effect Concentrations
TED–GC–MS: thermal extraction desorption–gas chromatography-mass spectrometry
TN: Total Nitrogen
TOC: Total Organic Carbon
TP: Total Phosphorus
TPE: Thermoplastic Elastomers
TSS: Total Suspended Solids
UF: Urea-formaldehyde
UNEP: United Nations Environmental Program
UV: Ultraviolet
WWTP: Wastewater treatment plant
XRF: X-Ray Fluorescence
Zn: Zin

Contents

Chapter 1. Introduction

Plastic pollutants, comprising a substantial proportion of marine litter, stand out as one of the most consequentialcontaminants 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. The United Nations Environment Programme (UNEP) classifies marine debris as persistent, manufactured, or processed solid materials that are discarded, deposited, orabandoned within the marine and coastal domains (STAP, 2011).

A vivid reflection of this issue can be gleaned from statistical data. In 2021, Europe's plastic production escalated to a staggering 57.2 million tons, a figure that pales in comparison to global production, which soared to an unprecedented 390.7 million tons (Plastics Europe, 2022). Alarmingly, projections suggest that by 2050, the volume of global plastic waste could burgeon to an astounding 12 billion tons, attributing this surge to the parallelgrowth in the global population and corresponding demand (Henry et al., 2019).

Plastics, intricate synthetic organic polymers, are composed of numerous repeating chain monomers synthesized through polymerization reactions (Hammer et al., 2012) These polymers, defined by their substantial molecular mass (exceeding 10,000 mol^{-1}) (Hartmann et al., 2019), lay the foundation for the diverse family of plastics knowntoday. The inception of plastics dates back to 1839, marked by the discovery of vulcanized rubber and polystyrene (PS) (Andrady & Neal, 2009), heralding the beginning of a transformative era. In contemporary times, plastics have permeated every facet of daily life and industrial processes due to their exceptional attributes. Noteworthy among these attributes are flexibility, durability, lightweight nature, inertness, cost-effectiveness, and resistanceto corrosion (Cole et al., 2011; Hammer et al., 2012). This array of distinctive qualities has propelled plastics into a pivotal role, defining our modern existence and shaping the landscape of industries worldwide.

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) (Table 1). In the contemporary milieu, humanity's reliance on plastics is profound, with their pervasive application spanning virtually all sectors. The foremost sectors driving global demand include packaging (44%), building and construction (18%), automotive (8%), and electronics (7%)

(Plastics Europe, 2022) (Table 2).

Conversely, the menace of 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; S. Xu et al., 2020).

Table 1. Types and densities of polymers (Crawford & Quinn, 2016).

Plastic	Abbreviation	Chemical formula	Density (g/cm^3)
Polyethylene	PE	$(C_2H_4)_n$	0.92–0.97
Polyethylene - low density	LDPE	$(C_2H_4)_n$	0.91-0.93
Polyethylene - high density	HDPE	$(C_2H_4)_n$	0.94-0.97
Polypropylene	PP	$(C_3H_6)_n$	0.88–1.23
Polyvinyl chloride	PVC	$(C_2H_3Cl)_n$	1.15–1.70
Polystyrene	PS	$(C_8H_8)_n$	1.04–1.50
Polyethylene terephthalate	PET	$(C_{10}H_8O_4)_n$	1.30–1.50

Table 2. Distribution of plastic production by segments in Europe 2021 (PlasticsEurope, 2022)

Segment	Percentage (%)
Packaging	44
Building and construction	18
Automotive	8
Electrical and electronics	7
Household, leisure, and sports	7
Agriculture	4
Others	12

Plastics can be classified into two primary groups based on their thermal characteristics: thermoplastics and thermosets. Thermoplastics are materials that exhibit a tendency to soften upon heating and solidify upon cooling, maintaining their original structural integrity across a range of temperatures due to their linear and branched molecular composition. These materials comprise molecules that lack chemical interconnections, rendering themamenable to recycling. Among the commonly encountered thermoplastics are PE, PS, PVC, PP, polyamide (PA), expanded polystyrene (PS-E), thermoplastic elastomers (TPE), polyarylsufone (PSU), PET, and others (Ebewele,2000).

In contrast, thermosets are capable of transitioning from a liquid state, referred to as prepolymers, into a specific shape through the application of heat and pressure.

However, once they solidify, they become impervious to reshaping and reverting to their initial form. Characterized by their rigidity and durability, thermosets are crafted through crosslinking of polymers and, regrettably, are not amenable to recycling. Instances of thermosets include urea-formaldehyde (UF), phenol formaldehyde (PF), Polyurethane (PU), silicone, melamine, and epoxies (Ebewele, 2000).

Microplastics (MPs) defined as plastic particles with a size of less than 5 mm, have emerged as a significant environmental issue, mainly in aquatic ecosystems. These tiny particles can originate from the degradation of larger plastic debris, as well as from the direct release of MPs-containing products. Their ubiquitous presence in oceans, rivers, lakes, and other water bodies poses a considerable threat to aquatic life and ecosystems and leads to different impacts on aquatic life including physical harm, chemical contamination, and ecological consequences (described in more detail in Chapter 2) (Alfaro-Núñez et al., 2021; Barnes et al., 2009).

Freshwater mussels such as Unionidae family can serve as valuable bioindicators for assessing the presence and impact of MPs in aquatic ecosystems (Cera & Scalici, 2021; Staichak et al., 2021), they play a crucial role in maintaining the health and balance of their habitats, and they intricately connected to the nutrient cycles within their environments, particularly with the sediment they inhabit near bottom suspended sediments (NBS) and deposited sediments (Goldsmith et al., 2021).

Freshwater mussels, which are partially embedded in the bottom sediments ingest organic nutrients, minerals, and MP particles from both the continuously flowing NBS sediments and the deposited sediments that have been resuspended by their burrowing, and actively contribute to these nutrient cycles. One of their primary roles is in the recycling of organic matter. As filter feeders, mussels extract suspended particles, including organic detritus and bacteria, from the water column. This organic material becomes incorporated into the sediment, enriching it with essential nutrients such as carbon, nitrogen, and phosphorus (Vaughn, 2018). Moreover, freshwater mussels play a pivotal role in regulating the levels of nitrogen and phosphorus nutrients in their habitats. By filtering water and accumulating organic particles, mussels sequester nitrogen and phosphorus, preventing their excessive accumulation in the water column. This process helps maintain water quality and prevents eutrophication (Hoellein et al., 2017). Further, freshwater mussels contribute to sediment dynamics through bioturbation. The burrowing and movement of mussels within the sediment enhance sediment turnover. This bioturbation has cascading effects on nutrient availability. The

mixing of surface and subsurface sediment layers promotes oxygen penetration and microbial activity, leading to the breakdown of organic matter and the release of nutrients. In turn, these nutrients become available for uptake by plants and algae, creating a dynamic feedback loop within the ecosystem (Brim Box & Mossa, 1999).

At this point, the relationship between freshwater mussels and their habitat is integral to using them as effective bioindicators for MPs. Therefore, the second part of this work (chapter 4) investigated the situation of this habitat by studying the nutrients and heavy metals concentration in order to provide valuable insights into the state of aquatic ecosystems and the impact of anthropogenic activities on freshwater environments.

Aims of the research

Our study aims to evaluate two freshwater mussel species, Unio Crassus and Unio Tumidus, as biomonitors for microplastic (MPs) contamination and two sediments layers as habitats for the studied mussels in four locations along the Tisza River (Hungary). The overall objectives of this research were the following:

1- Investigate whether the two selected mussel species, coexisting in the same sampling sites and exposed to identical environmental conditions, yield consistent analytical information on MPs contamination or ifthe accumulation of MPs varies depending on the mussel species.
2- Determine the physical and chemical properties of near-bottom suspended sediments (NBS) and bottom sediments (BS).
3- Investigate the relationships between the grain size of sediments and concentrations of nutrient elementsand metal contaminants.
4- Identify the main source of nutrient elements on the basis of C/N ratios determined in sediments.
5- Evaluate the potential risk of metal contaminants for mussels as bottom-dwelling animals considering theConsensus-Based Sediment Quality Guidelines.

Structure of the dissertation

The first chapter comprehensively addresses the background of plastic pollutants, from their substantial contribution to marine litter and environmental concerns to their

diverse applications, production statistics, and the distinct thermal behaviors of thermoplastics and thermosets.

The second chapter will be a general introduction and literature review and will be divided into three main parts. The first part will be a broad background of plastic and MPs pollution, classification (primary, secondary), sources, the role of MPs as a transportation agent for different chemical pollution, and the concerns about the biological effects of MPs with giving more attention on the freshwater environment mainly rivers. The second part will describe MPs pollution in river sediments, starting with a general background. In addition, different sampling, preparation, and identification methods will be discussed. Finally, the characteristic results of MPs on river sediments will be provided. The last part of the introduction concerns the MPs pollution on river mussels, describing the current situation around the world with provides the available analytical method, and then the characteristic results of MPs on river mussels will be discussed.

The third chapter highlights the potential of Unio crassus and Unio tumidus mussels as sentinel species for monitoring MPs contamination in the Tisza River, with their selective ingestion of fibers and microfibers providing valuable insights into the presence and composition of these particles in the ecosystem.

The fourth chapter will seek to understand the environments of Unio crassus and Unio tumidus mussels, their nutrient availability and potential metal pollutants were assessed through the analysis of near-bottom suspended sediments (NBS) and bottom sediments (BS) at four sites along the Tisza River.

Finally, the fifth chapter will provide a general conclusion for the two studies.

Chapter 2. Literature Review

2.1 Microplastics

As per the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP), MPs 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. The determination of size thresholds remains an ongoing subject of scientific discourse, leading to various definitions in the literature. These encompass diameters below 5mm (GESAMP, 2015), a range spanning from 1 µm to 5 mm (Frias & Nash, 2019; Thompson et al., 2009), and between20–200 µm (Bermúdez & Swarzenski, 2021). Hence, the pressing imperative to achieve a harmonized consensus on the size definitions of MPs is undeniable.

While a standardized classification for plastic particles based on shape remains elusive, fragments, pellets, spheres, foam, and fibers emerge as prevalent shapes of MPs across global studies. Among these, fibers claim the spotlight as the dominant MP type in sediment and biota environments, as evidenced by a range of research endeavors (Harris, 2020; Hartmann et al., 2019).

MPs intricate chemical composition encompasses diverse polymer types, underscoring the importance of pinpointing pollutant sources and characterizing specific sample compositions. The determination of MPs' chemical makeup primarily relies on a spectrum of analytical methods, a comprehensive exploration of which will be expounded upon in the subsequent section (Hidalgo-Ruz et al., 2012).

Color serves as an additional attribute deemed valuable in describing MPs and discerning potential origins. Nonetheless, relying solely on color for the classification of MPs proves imprecise due to the potential for humanerror during visual assessment and the susceptibility of plastics to discoloration caused by aging and various weathering mechanisms (Lusher et al., 2017).

The origin of MPs constitutes the fundamental criterion for their categorization, irrespective of whether the particles were initially small or have undergone fragmentation. Consequently, MP classification is based on their original size scale, leading to the distinction between two distinct classes: primary and secondary MPs.

Primary MPs are intentionally manufactured at micrometer sizes, encompassing acrylic and polyester beads utilized for sandblasting, as well as microbeads integrated into cosmetic and personal care products (Eerkes- Medrano et al., 2015; Horton, Walton, et al., 2017). Given their diminutive size range, primary MPs can readily elude wastewater treatment systems, subsequently being released into aquatic environments (Murphy et al., 2016). In contrast, secondary MPs typically enter marine environments as larger particles, progressively breaking down over time into smaller particles and fragments. The degradation of plastics within the environment stems from diverse processes, including biodegradation, photodegradation, chemical alterations, thermal impacts, and mechanical forces. This degradation renders plastics vulnerable to transformations in their physical and chemical attributes, such as color, surface morphology, particle size, and density (Figure 1) (Andrady, 2015; Cole et al., 2011).

MP particles are subject to biological degradation by various organisms, including bacteria and fungi. For instance, fungi have shown promise as bioremediation tools due to their effective plastic waste degradation capabilities, as exemplified by the work of Zhang et al., 2014. Notably, Paço et al., 2017 highlighted the capacity of the marine fungus Zalerion maritimum for biodegrading polyethylene (PE) MPs. Additionally, research suggests that Aspergillus spp. possess the potential to biodegrade high-density polyethylene (HDPE) MPs by releasing extracellular enzymes that modify the plastic's surface, as demonstrated by Sangeetha Devi et al., 2015. Furthermore, Auta et al., 2018 conducted an assessment on two bacterial species, Bacillus sp. and Rhodococcus sp., and their efficacy in degrading PE MPs from mangrove sediment. Their findings underscored the efficient degradation of PE MPs by both bacterial strains.

Solar radiation, particularly under UV wavelengths, instigates photodegradation in MPs. Nevertheless, the propensity for photodegradation varies across diverse polymer types of MPs. This degradation process hinges upon the interplay of MPs' physicochemical attributes and the photon flux emitted by UV sources (Norma D. Searle, 2003). Paramount among the factors influencing MP degradation in water is pH and salinity levels; heightened acidity or alkalinity increases the likelihood of MPs undergoing hydrolytic degradation. Moreover, pH and salinity hold the potential to influence MPs' behavior within water and their interactions with environmental contaminants, as highlighted by Zhang et al. (2021).

The factors outlined above collectively contribute to the mechanical degradation of MPs resulting from interactions with sand or rock particles. Consequently, mechanical degradation precipitates a reduction in particle size, thereby amplifying the specific surface area of degraded polymers. This phenomenon augments MPs' capacity to adsorb an escalated amount of chemical pollutants onto their surfaces. However, a notable predicament arises from the limitless nature of plastic degradation, ultimately leading to the formation of MPs and even Nano plastics. This progression renders the origin of these particles untraceable and engenders formidable challenges in their removal from aquatic environments. The intricacy of this issue underscores the gravity of uncontrolled plastic fragmentation (Klein et al., 2018; K. Zhang et al., 2021).

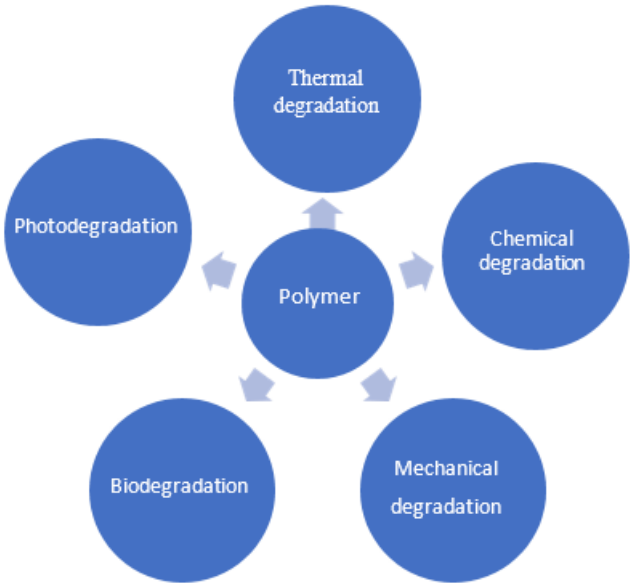

Figure 1: Degradation sources of polymers in the aquatic environment (Norma D. Searle, 2003)

2.2 Sources of Microplastics Pollution

Deficiencies in waste management, coupled with inadequate human activities and inadvertent contamination, collectively contribute to the relentless influx of plastics into the environment (Barnes et al., 2009). Anthropogenic pollution bears responsibility for the pervasive presence of marine debris, with plastics forming the predominant component, accounting for approximately 95% (Galgani et al., 2015)

Depending on their origins, MPs enter the environment in diverse shapes, sizes, and hues, leading to their global prevalence. Notable instances include their discovery in deep-sea habitats (Woodall et al., 2014), surface waters (S. Zhao et al., 2014), sediments (J. Wang et al., 2017), marine organisms (Gall S.C. & Thompson R.C., 2015), and even Antarctic snow (Aves et al., 2022). The influx and accumulation of MPs in aquatic systems stem from two primary sources: aquatic-based and land-based (Thushari & Senevirathna, 2020). The former encompasses oil and gas platforms, commercial shipping, aquaculture sites, and fishing operations (Hinojosa & Thiel, 2009; Vlachogianni et al., 2018), constituting approximately 20% of marine debris. In contrast, land-based sources contribute a substantial80% of plastic pollutants in marine environments, originating from various outlets such as stormwater runoff, tourism, industrial activities, and municipal sewage (Cole et al., 2011; GESAMP., 2010; Verla et al., 2019). Rivers emerge as significant conduits for transporting plastic debris from terrestrial to marine realms, responsible for an estimated 70-80% of the overall plastic loading, (Horton et al., 2017).

2.3 Microplastics as a transportation agent of chemical pollutants

The plastic manufacturing process often involves the incorporation of various chemical additives, including hexabromocyclododecane (HBCD), phthalates, polybrominated diphenyl ethers (PBDEs), and bisphenol A (BPA), aimed at enhancing properties such as color, thermal stability, humidity resistance, temperature resistance, and toughness (Fries et al., 2013; Hahladakis et al., 2018; K. Zhang et al., 2021). Regrettably, these additives are associated with hazards and can leach into the environment, posing risks to aquatic organisms (Setälä et al., 2014). Once released into the environment, MPs persist without significant degradation and interact distinctively with surrounding organic pollutants and heavy metals (M. Wagner et al., 2014). Capitalizing on their robust adsorption capacity, MPs serve as vectors, transporting chemical pollutants like persistent organic pollutants (POPs), heavy metals, and additives into aquatic organisms and ultimately bioaccumulating within the food chain (Y. Tang et al., 2021).

Research into the adsorption of pollutants onto MPs has witnessed substantial growth. However, the intricate nuances governing how the adsorption process on MPs ameliorates their impact remain enigmatic.

Typically, experimental endeavors for this purpose employ virgin plastic (primary). However, it is noteworthy that the majority of MPs existing in the environment are secondary MPs subjected to diverse degradation factors. Consequently, these secondary MPs possess distinct properties compared to the pristine plastics utilized in laboratory settings, notably incorporating oxygen functional groups and specific surface area variations (Yu et al., 2019). This discord underscores the need for a more nuanced understanding of the adsorption dynamics of pollutants onto secondary MPs, considering their altered characteristics stemming from real-world exposure.

It is crucial to highlight that the adsorption process is fundamentally shaped by biofilm formation on the plastic surface, as well as a host of other factors that will be expounded upon in the subsequent section. In the context ofheavy metals, a plethora of studies have underscored their presence on the surfaces of MPs. For instance, (Gao et al., 2019) identified that PVC, PP, PE, PA, and POM are adept at adsorbing heavy metals like Pb, Cu, and Cd.Notably, their research illuminated that the adsorption capacity is intricately linked to three pivotal factors: metalconcentrations, exposure duration, and particle size. This interplay results in distinct adsorption capacities for each polymer towards various heavy metals. Similarly, Ahechti et al., 2022 delved into the adsorption capacities offour heavy metals - Cu, Cd, Zn, and Pb - onto virgin plastics of PP and PE, exploring the influences of exposuretime, pH, and salinity. Their findings accentuated the paramount role of exposure time in dictating adsorption capacities, whereby longer exposure led to heightened adsorption levels. Moreover, the study by Godoy et al., 2019 embarked on assessing the potential of MPs to adsorb heavy metals in diverse aqueous environments, including irrigation water, wastewater, and seawater. Their investigation unveiled that PE, PP, PS, and PVC exhibit superior heavy metal adsorption compared to other polymers, particularly in irrigation water. The study attributed this phenomenon to specific factors such as surface area, porosity, and morphology inherent to these polymers. Furthermore, the introduction of organic matter has been demonstrated to induce the adsorption of heavy metals, observed both in irrigation water and wastewater scenarios. However, in seawater, the interplay of salinity and solution chemistry with the adsorption of heavy metals by polymers remains an ongoing area of investigation without conclusive evidence. Building on these findings, Brennecke et al., 2016 similarly affirmedthat MPs can indeed function as vectors, facilitating the transport of heavy metals within aquatic systems.

In respect of temperature influence, numerous researchers have underscored that heavy metal adsorption follows an endothermic reaction, a phenomenon where the adsorption capacity escalates with rising temperatures. For instance, Wang et al. (2020) examined the adsorption capacity of metal ions Cu and Zn on PET. Their findings unveiled that as the temperature increased, the adsorption capacity demonstrated a corresponding increase, a trend attributed to the endothermic nature of the process. Moreover, Oz et al., 2019 delved deeper into the adsorption capacity of Pb and Al onto three distinct types of MPs- PET, PA, and EVA - while subjecting them to varying temperatures (25, 35, 45, and 55°C). The outcomes underscored that the experiment conducted at 55°C yielded the highest adsorption capabilities, further corroborating the temperature-dependent nature of heavy metal adsorption.

With regards to pH, Holmes et al., 2014 investigated the impact of varying pH levels on the adsorption capacity of six heavy metals (Cd, Co, Cr, Cu, Ni, and Pb) onto both virgin and aged PE. The outcomes indicated that as the pH increased, the adsorption capacity exhibited an upsurge for Cd, Co, Ni, and Pb, while conversely, it decreased for Cu, with no discernible effect observed for Cr. Additionally, the study noted that the adsorption capacity was notably higher for aged pellets as compared to virgin ones.

The influence of salinity on the adsorption of heavy metals onto MPs has also been a subject of investigation. Holmes et al. (2014) explored the effects of salinity on the adsorption capacity of PE towards various heavy metals (Pb, Cd, Cr, Cu, Ni, and Co). Their findings highlighted distinct interactions between each type of PE and the individual metals in the presence of salinity. Notably, the adsorption of Cr exhibited an increase with salinity, while Cd, Co, and Ni adsorption demonstrated a decrease, with no significant effect observed for Cu and Pb. A similar observation was made concerning the adsorption capacity of Cd onto HDPE, where the introduction of NaCl led to a noteworthy reduction in Cd adsorption (F. Wang et al., 2019). Nevertheless, in spite of these outcomes, the ability of MPs to adsorb organic contaminants and heavy metals appears to remain unaffected by salinity (S. Tang et al., 2020). Additionally, a separate study indicated that the adsorption capacity of heavy metals was higher in aged MPs compared to virgin ones due to the augmented surface area and the presence of biofilm covering the MPs' surfaces (J. Wang et al., 2021; Q. Wang et al., 2020).

Numerous studies have documented the presence of diverse organic pollutants on the surfaces of MPs, encompassing compounds such as Polyaromatic hydrocarbons (PAHs) (Fisner et al., 2013), Polychlorinated biphenyls (PCBs) (Endo et al., 2005),

Dichlorodiphenyltrichloroethane (DDT) pesticide (Heskett et al., 2012), and Hexachlorocyclohexanes (HCH) isomers (H. Lee et al., 2014). Additionally, the International Pellet Watch has noted that certain persistent organic pollutants (POPs) can indeed be adsorbed onto the surfaces of MPs (Ogata et al., 2009). Similarly, the work of Mato et al., 2001 underscored the high levels of contaminants, including PCBs and DDE, present in PP resin pellets. This observation emphasizes that these plastic resins can indeed serve as vectors, transporting pollutants throughout aquatic environments. The ability of MPs to sorb various organic pollutants is contingent upon a range of factors. Most interactions between organic pollutants and MPs are underpinned by the process of adsorption (Y. Xia et al., 2023). These factors encompass characteristics of both the MPs and the pollutants, as well as prevailing environmental conditions such as temperature, pH, and salinity (Guo & Wang, 2019b; Luo et al., 2022).

In the realm of particle size, the interaction between various plastic polymers and pollutants is characterized by distinct affinities. A pertinent example is furnished by Munoz et al., 2021, whose findings underscore the pivotal role of MPs particle size in determining their adsorption capacity for organic pollutants. The study's outcomes reveal that as particle size decreases (20–1000 μm), the adsorption capacity proportionally increases due to the larger specific surface areas of the MP particles. Furthermore, aged MPs have been observed to exhibit heightened sorption affinities for pollutants in comparison to their virgin counterparts (Guo & Wang, 2019a). However, a study by L. Ding et al., 2020 revealed that aged PS may not effectively adsorb PAHs. This phenomenon is attributed to oxygen-containing functional groups introduced during the aging process, which readily form hydrogen bonds with surrounding water molecules, rendering it challenging for hydrophobic organic contaminants to displace the adsorbed water molecules.

Concerning salinity, evidence suggests that high salinity concentrations can facilitate the sorption of specific pollutants onto PE and PS (F. Wang et al., 2015). J. Q. Hu et al., 2017 similarly illuminated that augmented salinity levels enhance the sorption capability of lubricating oil on the surfaces of nano-PE and micro-PS.

Given that many pollutants dissociate under specific pH conditions, variations in pH significantly modulate their fate and behavior within the environment. pH emerges as a pivotal factor influencing the sorption of organic pollutants onto MPs. Notably, higher pH levels impede the sorption of acidic pollutants featuring hydroxyl or carboxyl

groups, while neutral pH levels typically lead to peak sorption for aromatic contaminants possessing hydroxyl and amino groups (F. Wang et al., 2020). Additionally, the pH factor appears to have no discernible impact on Tetrabromodiphenyl ether (BDE-47) sorption onto PE, PP, PS, and PA (P. Xu et al., 2019).

2.4 Biological effects of microplastics

Currently, the biological impacts of MPs on aquatic organisms within both marine and freshwater ecosystems are being comprehensively investigated across various trophic levels on a global scale. Nevertheless, it's notable that research endeavors concerning MPs and their associated contaminants in organisms have predominantly concentrated on marine organisms, with comparatively less emphasis on their freshwater counterparts (Sarijan etal., 2021). Within this context, numerous studies have unveiled the biological effects of MPs on a diverse range of aquatic organisms, encompassing both marine and freshwater ecosystems. Among the studied organisms are

birds (Basto et al., 2019; Tanaka et al., 2020), fishes (Barboza et al., 2020; Güven et al., 2017), mollusks (Abidli et al., 2019; Magni et al., 2018), and crustaceans (Iannilli et al., 2019; Jemec et al., 2016).

The diverse array of characteristics exhibited by MPs, including factors like size, density, and shape, underpins their widespread occurrence throughout aquatic environments, spanning from surface waters to the depths of the ocean. This pervasive distribution enables MPs to come into contact with an extensive spectrum of aquatic organisms, facilitating their bioaccumulation across various trophic levels (Cole et al., 2011; de Sá et al., 2018; Thompson et al., 2009).

Research focusing on the biological effects of MPs has predominantly been conducted within controlled laboratory settings. These studies have centered on encapsulating a spectrum of aspects, including ecotoxicological impacts, uptake pathways, analytical methodologies, and the prevalence of MPs within distinct organs (Auta et al., 2017; Carbery et al., 2018; Cole et al., 2011; Crawford & Quinn, 2016; Wright et al., 2013). Upon entering aquatic environments, MPs can interact diversely with surrounding organisms due to their diminutive size, subsequently influencing their potential bioavailability. Predominantly, the primary route through which aquatic organisms acquire MPs is via ingestion. This can occur directly, driven by an inability to distinguish between MPs and their regular food sources, or indirectly, through trophic

transfer along the food chain (Auta et al., 2017; Galloway et al., 2017; Ribeiro et al., 2019). A concerning statistic suggests that over 250 marine species could be endangered by plastic ingestion (Laist, 1997). Documentation abounds on the ingestion of MPs by numerous aquatic organisms. Examples include cetaceans (Gambardella et al., 2017; Hossain et al., 2020), mollusks (Browne et al., 2008; Scott et al., 2019), echinoderms (Graham & Thompson, 2009), zooplankton (Cole et al., 2013; Desforges et al., 2015; Sun et al., 2017), and corals (Hall et al., 2015).

MPs exert detrimental effects on aquatic organisms, encompassing a spectrum of repercussions, including reduced feeding rates, impaired predatory performance, physical harm, induction of oxidative stress, reproductive disturbances, pathogenic developments, altered enzyme production, metabolic changes, and even mortality (Lei et al., 2018; Welden & Cowie, 2016). Several factors intricately influence the ingestion of MPs by aquatic organisms, encompassing aspects such as food concentration, taste preferences, species variations, and feeding strategies (e.g., filter and deposit feeders) (Wesch et al., 2016). Following ingestion, MPs tend to accumulate predominantly within the digestive system of organisms, with subsequent translocation to various tissues and organs, notably the stomach, intestine, and digestive tract (L. Hu et al., 2016).

MP toxicity to aquatic organisms is shaped by a nexus of factors, including particle size, polymer composition, MP concentration, exposure duration, and the species under scrutiny (Lagarde et al., 2016; Long et al., 2017; Y. Mao et al., 2018; C. Zhang et al., 2017). The attributes of MPs, such as color and shape, also play a role in influencing their uptake by aquatic organisms, with fibers being the most prevalent shape found within these organisms and potentially inflicting greater harm than other shapes (Crawford & Quinn, 2016; Gray & Weinstein, 2017; Herzke et al., 2021). The size-dependent toxicity of MPs has been observed, with smaller microbeads exhibiting greater toxicity than their larger counterparts (Jeong et al., 2016). The polymer densities of MPs contribute to their vertical distribution in aquatic environments, leading to variations in the types of plastics consumed by different organisms. Pelagic species such as zooplankton, for instance, are more prone to ingest low-density plastics like PE and PP, whereas benthic species such as mollusks tend to consume higher-density plastics like PVC and PET (Cole et al., 2011). It's noteworthy that the phenomenon of biofouling, driven by microbes, algae, and invertebrates, can impact plastic buoyancy by forming biofilms on the surface, elevating density and causing the plastics to sink

(Fazey & Ryan, 2016; Wright et al., 2013). Diverse factors dictate the rateof biofouling, including polymer surface energy, hardness, water conditions, and biological influences (Kaiser et al., 2017; H. Zhang, 2017).

2.5 Microplastics contamination in sediments

2.5.1 Background

The initial identification of MPs smaller than 5mm within sediments was identified by Thompson et al., 2004. Subsequent to this pivotal study, concerns surrounding the contamination and prevalence of MPs have garnered heightened attention. Presently, reports of MP presence span diverse aquatic systems, encompassing oceans as (Barrett et al., 2020; Chouchene et al., 2021), estuaries (Firdaus et al., 2020; Hope et al., 2021), rivers (Guerrantiet al., 2017; Kabir et al., 2022), as well as lakes (Clayer et al., 2021; R. Mao et al., 2021). Nonetheless, in comparison to the abundance of research concerning marine sediments, scant attention has been directed toward the investigation of MPs within freshwater sediments, with a particular dearth in studies focusing on river sediments (Blair et al., 2019; Blettler et al., 2018). Table 3 is a summary of relevant studies elucidating the abundance and characteristics of MPs within sediment across various global river systems.

Rivers serve as the vital interface connecting land and open oceans. A substantial proportion, approximately 80%,of plastic debris introduced into oceans originates from terrestrial origins, predominantly through riverine pathways. Consequently, rivers play a pivotal role as the primary conduit for plastic transportation to the marine environment (Andrady, 2017; Rochman, 2018; S. Wagner et al., 2019). Notably, the average concentration of MPs within rivers is notably higher, a staggering 50 times greater, than the maximum concentration observed in oceanic environments (Schmidt et al., 2017). The contribution of rivers to the influx of MPs into oceans is substantial. Estimations reveal that annually, ranges from 1.15 to 2.41 million tons of plastic debris are transported to the oceans from rivers worldwide, with a significant proportion originating from Asian nations (Lebreton et al., 2017). Correspondingly, another investigative endeavor determined that the global influx of plastic debris from rivers to oceans varies between 0.41 to 4 million tons annually (Schmidt et al., 2017). Predominant sources of plastic debris within freshwater systems encompass effluents from wastewater treatment plants (WWTPs), fisheries activities, atmospheric deposition, beach littering, and runoff originating fromagricultural, industrial, and urban areas (M. Wagner et al., 2014).

However, despite the integral role rivers play in transporting MP particles, comprehensive studies concerning the sources, distribution, and fate of MPs withinriver sediments remain limited. Simultaneously, the endeavors to comprehend rivers' dual role as both sources and sinks of MP pollution remain insufficient to bridge the existing knowledge gaps (Akdogan & Guven, 2019; Klein et al., 2015; Mani et al., 2015).

In the broader context, when plastics infiltrate aquatic environments, those with higher density gravitate and settlewithin the bottom sediments. On the other hand, plastics with lower density tend to remain buoyant, floating on the water's surface or within the water column. Notably, even plastics of lower density can find their way into sedimentary deposits due to the development of biofilm. Furthermore, as a consequence of gravitational settling, the accumulation of diverse pollutants on the surface of MPs contributes to an augmentation in their overall density. This dynamic process underscores the role of sediments as a primary sink and reservoir for MPs (Shruti & Kutralam-Muniasamy, 2019). The significance of sediment, however, extends beyond mere containment; it also encompasses the potential for resuspension and transportation of MPs. An illustrative example of this phenomenon is evident in instances where surface sediments containing MPs are disrupted, prompting their resuspension and subsequent vertical remobilization into deeper sediment layers or overlaying water bodies (F. Xia et al., 2021). As a result, the distribution patterns of MPs within river systems exhibit variability contingent upon the sources of MPs, prevailing environmental conditions, and unique characteristics of catchment areas. These attributes encompass geomorphological features, flow velocities (D. He et al., 2021), water depths, and occurrences of flood events (Dai et al., 2018; B. He et al., 2021; Kowalski et al., 2016; Rodrigues et al., 2018). For instance, an observation by Hurley et al., 2018 underscored the pivotal role of flood events in exporting approximately 70% of MPs from riverbeds.

Sediments assume a pivotal role within the aquatic ecosystem, facilitating the essential functions of nutrient and contaminant transfer and storage. Beyond this, they provide a habitat for a diverse array of organisms, encompassing mussels, larvae, and invertebrates, contributing to the intricate fabric of aquatic life (Buendia et al., 2013; Vidmar et al., 2017). These sedimentary deposits are broadly classified into two categories: suspended and deposited materials. Suspended sediments merit particular attention due to their role as significant conveyorsof nutrients and metals present within the water column. In contrast, deposited sediments settle on the riverbed, constituting an integral part of the aquatic landscape. The interplay between MPs, sediments, and organisms that ingest sediments assumes a position of paramount importance within

freshwater systems (Hauer et al., 2018; Palmer et al., 2000). The interaction is dynamic, with MPs potentially influencing sediment characteristics, including traits such as water-holding capacity and bulk densities. Furthermore, the chemical composition of sediment can undergo modification due to the weathered surface of adherent MPs, which accumulate a spectrum of organic pollutants, heavy metals, and original additives like plasticizers, retardants, antioxidants, and photo stabilizers. Consequently, the introduction of MPs into soil environments can yield the release of toxic additives, thus posing a threat to ecosystems and exerting lasting impacts on soil quality over the long term (B. He et al.,2021).

Table 3. Studies on MPs abundance and characteristics in river sediments

Study area	Abundance items/kg	Shape	Size mm	Color	Polymer	Reference
Six rivers in Tibetan Plateau, China	50-195	Fibers	< 1	White	PET	(Jiang et al., 2019)
Brisbane River, Australia	10-520	Films	< 3	White	PE	(B. He et al., 2020)
Ntuã River, Portugal	18-629	Fragment, fibers	N/A	Colored	PE, PP	(Rodrigues et al.,2018)
Ganga River, India	99.27-409.86	Fibers	N/A	N/A	PET, PE	(Sarkar et al., 2019)
Tisza River, Central Europe	2825 ± 1991	Fibers 94-98%	N/A	N/A	N/A	(Kiss et al., 2021)
Amazon rivers, Brazil	417 - 8178 0 – 5725	Fibers	0.063–5 0.063–1	White/ crystal	N/A	(Gerolin et al., 2020)
Kelvin River, UK	161–432	Fibers (88%)	< 0.09	Colored	N/A	(Blair et al., 2019)
Pearl River, China	685±342	Film	1.0-5.0	White/transparent	PP and PE	(Fan et al., 2019)
ai and Tebrau River, Malaysia	200 ± 80 And 680 ± 140	Film	1.0-5.0	Blue	N/A	(Sarijan et al., 2018)
Rhine-Main River, Germany	228-3763	Fragments, spheres	0.63-5	N/A	PP, PE, and PS	(Klein et al., 2015)
Japanese Rivers	8-1010	Fragments	1.0-5.0	N/A	PVC, PE, PP	(Kabir et al., 2022)
Yushan River, China	30-70	Films,fibers	N/A	Transparent	PE, PP, PET	(Niu et al., 2021)
Yongfeng River, China	5-72	Films	<1	Green	PE, PP	(Rao et al., 2020)

Haihe River, China	1346-11,917	Fibers	<1	Black	PE	(Liu et al., 2020)
Pearl River, China	80-9597	Fibers	0.02–1	Yellow, white	PE, PP	(Lin et al., 2018)
Wei River, China	360-1320	Fibers	< 0.5	N/A	PE, PVC, PS	(L. Ding et al., 2019)
Nakdong River, South Korea	1971 ± 62	Fragments	< 300	N/A	PP, PE	(Eo et al., 2019)
Thames River,Ontario, Canada	6-2444	Fragments,	0.063-2.38 0.18-8.70	Blue, white Black, blue	PE PET	(Corcoran et al., 2020)
Ciwaleng ke River, Indonesia	30.3±15.9	Fibers	0.05-0.1	N/A	Polymer mixure, PET, PA	(Alam et al., 2019)
Ottawa River, Canada	220	N/A	0.5-3	N/A	N/A	(Vermaire et al., 2017)
Rhine River, Germany	260-11,070	N/A	<0.075	N/A	APV	(Mani et al., 2015)
Bloukrans River,South Africa	160.1±139.5	N/A	N/A	N/A	N/A	(Nel et al., 2018)
Beijiang River, China	178 ± 69 544 ± 107	N/A	N/A	N/A	PE, PP	(J. Wang et al., 2017)
Wen-Rui Tang River, China	32,947±15,342	Fragments	<0.3 0.2-0.1	N/A	N/A	(Z. Wang et al., 2018)
Thames Rives, UK	660	Fragments, fibers	1-2	N/A	PET, PP, PE	(Horton et al., 2017)
Qin River, China	0-97	Sheets, fibers	1-5	White, blue	PP, PET	(L. Zhang et al., 2020)
Ebro River, Spain	2052±746	Fibers	<1mm	Colored, transparent	PA, PE	(Simon-Sánchez et al., 2019)
Ombrone River, Italy	57-1069	Filaments, fragments	0.5-10	Black	N/A	(Guerranti et al., 2017)
River Tame, UK	165	Fragments, fibers	0.25-1	N/A	PE	(Tibbetts et al., 2018)
Milwaukee River, USA	32.9 - 6229	Foams	0.125−0.3549	Black	PET	(Lenaker et al., 2019)

Po River, Italy	2.92 - 23.30	Fragments	1-5	N/A	PE, PS	(Piehl et al., 2019)
Elbe River, Czech Republic	3.35×10^6	Spheres, fragments	0.125-5	N/A	PE, PS	(Scherer et al., 2020)
Lawrence River, Canada	65-7562	Microbeads, fragments	0.25- 0.5	N/A	N/A	(Crew et al., 2020)
Fengshan River, Taiwan	508-3987	Fibers	0.05-0.297	N/A	ER, PET ,PR	(Tien et al., 2020)
Magdalena River, Colombia	0-105	Fibers	0.014-0.024	N/A	PET, PP, PE	Silva & Nanny., 2020)
Citarum River, Indonesia	16.666 ± 0.577	Fibers	1-5	Black	PE, PP	(Sembiring et al.,2020)
Ganga River, India	17-36	Films	2.5–5	White	PE	(Singh et al., 2021)
Vistula River, Poland	190-580	Fibers	0.05-5	Black	PS, PP	(Sekudewicz et al., 2021)

2.5.2 Sampling of sediments

Numerous methodologies for investigating MPs within sediment have been documented in the literature. However, the absence of standardized protocols remains a prevalent challenge. Notably, procedures for extracting MPs from sediments exhibit similarity across marine and freshwater environments (Stock et al., 2019). Consequently, the variation in sampling methods is conspicuous, spanning differences in sampling depth, location, scale, and sample volume (Hidalgo-Ruz et al., 2012). Hence, the selection of an appropriate sampling technique primarily hinges on the specific research objectives (Campanale et al., 2020).

In this context, it is prudent to emphasize the value of collecting an ample number of samples to gain comprehensive insights into the concentration and distribution of MPs within sediments (Stock et al., 2019). These sediment samples can be sourced from either beach environments or riverbeds. Nevertheless, the more frequent preference leans toward sandy beaches, attributed to their accessibility and the relative simplicity of thesampling process, as compared to riverbeds (Mai et al., 2018).

Diverse marine environments, encompassing the tideline, intertidal, and supralittoral zones, provide potential sources for collecting sediment samples (Mai et al., 2018). Direct sampling from beaches is a prevalent approach, employing stainless steel tools such as shovels, forceps, spatulas, and spoons. It is of paramount importance to don latex gloves and cotton clothing during collection to curtail sample contamination (A. B. Silva et al., 2018). To ensure precise estimation of MPs concentration within

sediment samples, certain key parameters warrant consideration: measurement of sampling depth, replication count, and sample weight. The weight of sedimentsamples varies across studies, ranging from 25g to 3000g, while the corresponding volume spans from 0.05 to 1.2L. A recommendation from NOAA stipulates a sampling weight of 400g per replicate, followed by subsequent drying and measurement. As for the replication count, the MSFD technical subgroup advocates for the use of fivereplicates for sediment sampling (Prata et al., 2019). Notably, the standard sampling depth frequently adopted is the uppermost 5cm of beach sediments, a practice supported by studies such as Hidalgo-Ruz et al., 2012 andBesley et al., 2017.

Emerging from the seabed, sediments are recognized as a significant sink for MPs (Woodall et al., 2014; Siegfried et al., 2017; Näkki et al., 2017). To capture these sedimentary insights, various sampling techniques are employed from vessels, often utilizing instruments like grabbers such as Ekman, VanVeen, and box corer (Maes et al., 2017; Pagter et al., 2018) (Figure2). Furthermore, corers have gained traction for generating depth profiles in both marine and freshwater settings (Willis et al., 2017; Zheng et al., 2020). The metric used to quantify MPs abundance hinges on the chosen sampling approach. The determination of MPs measurement units is inherently tied to the sampling method adopted. Consequently, the abundance of MPs is reported in terms of MP particles within specific units of surface area (m^2), sediment weight (kg), and sediment volume (L) (Hidalgo-Ruz et al., 2012). For preservation and subsequent analysis in the laboratory, sediment samples are commonly stored at a temperature of -20°C and shielded from light exposure (Lorenz et al., 2019).

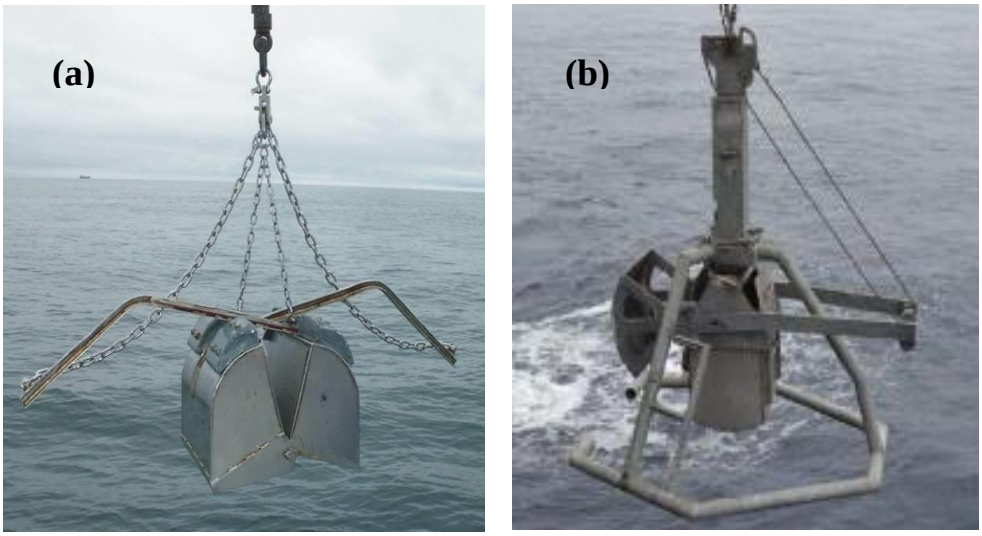

Figure 2: Sediment sampling instruments: (a) Van Veen grab; (b) box corer.

2.5.3 Sediment samples preparation

Following the sampling phase, a crucial step involves drying sediment samples in an oven set at 50°C before analysis, ensuring the precision of weight measurements during MP analysis (L. Yang et al., 2021). It's worth noting, however, that high temperatures can adversely impact the integrity of MPs (Zobkov & Esiukova, 2018). Importantly, extraneous substances present within sediment samples can hinder the quantification and identification of MPs (Hale et al., 2020; Lv et al., 2021). To address this, the extraction process involves separating MPs from their original matrix (sediment, water, biota). This process encompasses separation, size fractionation through filtration or sieving, and purification (A. B. Silva et al., 2018). For MPs larger than 5mm,

Visual methods are sometimes employed, using tweezers under the naked eye or a microscope (Maes et al., 2017). However, it's important to recognize that this technique, while swift and economical, may not yield precise results and could lead to overestimation or underestimation of MP quantities (Hidalgo-Ruz et al., 2012). In contrast, for MPs smaller than 5mm, other extraction techniques are recommended, such as density separation combined with filtration based on the protocol established by Thompson et al. (2004) (Hanvey et al., 2017). The efficient separation of MPs is significantly based on the choice of salt solution employed. Notably, the separation efficacy increases with the density of the chosen salt solution, enabling the separation of a greater number of plastic polymers. Saturated Sodium Chloride (NaCl) solution stands as the predominant choice for density separation due to its prevalence and advantages. It is particularly recommended by The Marine Strategy Framework Directive (MSFD) Technical Subgroup for its cost-effectiveness and environmental compatibility in comparison to alternative solutions (Ivleva et al., 2017). However, it's worth noting that NaCl's low density (1.2 g/cm^3) renders it inadequate for the separation of high-density polymers like PVC, POM, and PET. To address this limitation, alternative high-density salt solutions come into play. These solutions include:

- Zinc Chloride ($ZnCl_2$) emerges as a commonly employed solution for density separation due to its density of 1.6 g/cm^3 (Gerolin et al., 2020; Imhof et al., 2012; Syakti et al., 2018). However, there are significant challenges associated with the use of $ZnCl_2$, primarily its expense and toxicity (Imhof et al., 2012; Liebezeit & Dubaish, 2012). To address these concerns, (Rodrigues et al., 2020) put forth a strategy to mitigate the method's cost by proposing a reuse approach for $ZnCl_2$, enabling it to be utilized up to five times. This recycling method yielded an efficiency rate of over 95%. The reuse of $ZnCl_2$ not only contributes to cost

reduction but also plays a crucial role in minimizing the environmental hazards associated with its disposal(Prata et al., 2019).

- Sodium Iodide (NaI) stands out for its high separation efficiency due to its density of 1.95 g/cm^3, particularly when dealing with specific types of MPs. Notably, NaI can be recycled when cellulose filters are not utilized in the separation process. Nonetheless, it's important to mention that the use of NaI still entails challenges related to its cost and associated hazards (Lv et al., 2021; Nuelle et al., 2014).
- Sodium polytungstate solution (SPT), characterized by a density of 1.4 g/cm^3, finds application in densityseparation processes. However, it's important to note that SPT is not universally suitable for all polymer types, including polyvinyl chloride (PVC) and polyformaldehyde (POM). Additionally, SPT is considered expensive (Eo et al., 2019; Horton, Walton, et al., 2017).

- Calcium Chloride ($CaCl_2$), boasting a density of 1.4 g/cm^3, offers the advantages of being cost-effective and non-toxic. However, it's important to note that $CaCl_2$ might not exhibit high efficiency in separating MPs from samples rich in organic content (C. Li et al., 2020; Scheurer & Bigalke, 2018).

Indeed, the choice of salt solution holds pivotal significance, considering its implications on recovery rate, cost, and potential impacts on both the environment and human health. Therefore, a judicious selection process is essential. Furthermore, the size of MPs can exert an influence on the recovery rate (Quinn et al., 2017). Among the available options, the $ZnCl_2$ solution stands as a favored choice for density separation due to its remarkable efficiency, reasonable cost, and safety considerations.

Beyond density separation, alternative extraction methods have been explored in the realm of MP analysis. Elutriation followed by flotation (Claessens et al., 2013; Nuelle et al., 2014), utilization of novel instruments like the Microplastic Sediment Separator (MPSS) (Löder & Gerdts, 2015), oil extraction protocols (OPE), and improvedpressurized fluid extraction (PEE) have all been investigated (B. Zhang et al., 2020). The efficiency of these extraction methods varies, contingent on factors such as particle shape, size, and polymer type. The introductionof new extraction methods, as pioneered by (Imhof et al., 2016) (Nuelle et al., 2014), and (Claessens et al., 2013),has showcased recovery rates surpassing those of classical methods like the one conducted by Fries et al. (2013). The latter struggled to recover smaller MP particles as necessitated. To address this limitation, Nuelle et al. (2014) developed a two-step method. It involves initially extracting MPs from sediment samples using the air-induced overflow (AIO) method with NaCl solution and subsequently utilizing NaI solution with a density of 1.80 g/cm3 for floatation. This technique yielded impressive recovery rates ranging from 67% to 99%. These innovative approaches underscore the continuous evolution of extraction methodologies to accommodate the unique challenges posed by diverse MP characteristics.

In this context, it is advisable to consider the repetition of extraction steps to enhance the recovery rate for MP particles measuring less than 500 µm (Browne et al., 2011; Claessens et al., 2013; Nuelle et al., 2014). Notably, the MPSS method circumvents the need for repetition steps and showcases a high recovery rate of 96% (Imhof et al., 2016). Furthermore, X. Zhang et al., 2020 introduced a novel heating-assisted separation technique employing Monosodium phosphate (NaH_2PO_4) to extract MPs from sediment samples, yielding an impressive recovery rate. These nuanced methods underline the evolving landscape of MPs extraction, each offering distinctadvantages in terms of efficiency and practicality. Furthermore, an oil extraction process may be used to remove MPs from sediment samples. For instance, Crichton et al., 2017 used vegetable oil instead of salt solution, whichprovides a high recovery rate (>90%) for all

MPs types. In general, practical strategies to reduce MPs misidentification and underestimation caused by the separation methods are urgently needed. After the MPs extraction step, vacuum filtration is generally used to collect the floated plastic particles (Hanvey et al., 2017). In this regard, Zobkov & Esiukova, 2018 recommended using a filter with a diameter of 15 cm and a maximum pore size of 47 μm. Moreover, it is necessary to wash the vessel's inner wall with pure water to prevent sticking floated particles in it. After MPs extraction from samples, a size fractionation is recommended to compare the studies. Hence (MSFD Technical Subgroup on Marine Litter., 2013) suggested separating the MPs into fractions of 1-5 mm and 20 μm–1 mm.

Size fractionation, a technique applicable to water, sediment, and biota samples, can be conveniently achieved through sieving using stainless steel sieves or a series of mesh sizes in a cascading manner (McDermid & McMullen, 2004). When dealing with samples rich in biological material, a preliminary purification step is crucial to prevent potential clogging of sieves. This purification step aims to enhance the identification of MPs by eliminating organic matter that density separation might not effectively isolate (Herrera et al., 2018). A common solution utilized for this purpose is 30% H_2O_2 (Liebezeit & Dubaish, 2012; Mathalon & Hill, 2014; Stolte et al., 2015; S. Zhao et al., 2014). Notably, Nuelle et al., 2014 demonstrated that a 35% H_2O_2 solution is the most efficient in removing organic matter from the sample; however, it can alter the color of MPs. Additionally, Cole et al. (2014) recommended the use of enzymatic digestion as a means to dissolve organic matter adhering to MPs. These approaches highlight the strategies available to enhance the precision of MPs analysis by minimizing the impact of organic material.

2.5.4 Analysis (Identification)

The identification and quantification of MPs occur following the extraction process, involving the assessment of their size, color, shape, concentration, and chemical composition. Several methods are employed for the identification and quantification of MPs:

1. Visual Identification: Direct observation is a basic method for identifying and categorizing MPs.

2. Pyrolysis Gas Chromatography Coupled to Mass Spectrometry (Py-GC-MS): This thermoanalytical technique aids in mass-based identification of MPs' chemical composition (Nuelle et al., 2014; Pipkin et al., 2021).

3. Raman Spectroscopy; This technique enables particle-based identification through the analysis ofmolecular vibrations (Imhof et al., 2016; Tong et al., 2020).

4. Fourier-Transform Infrared Spectroscopy (FTIR): FTIR is commonly used for both the identificationand quantification of MPs from water and sediment samples (Chen et al., 2020; Qiu et al., 2016).

5. Thermal Extraction Desorption-Gas Chromatography-Mass Spectrometry (TED-GC-MS).

The choice of method depends on the research objectives. Mass-based techniques like Py-GC-MS and TED-GC-MS are suitable for determining the chemical composition of MPs. On the other hand, particle-based techniques like Raman and FTIR are more apt for analyzing the characteristics of individual particles (Campanale et al., 2020) (Table 4).

Table 4. Methodologies used for the characterization of MPs: advantages and disadvantages of each technique are described (Mariano et al.,2021; Ricciardi et al., 2021)

Methodology	Advantages	Disadvantages	Lower Size Limit
Fourier transform infraredmicro-spectroscopy (FTIR)	• Confirmation of the composition of the MPs • No false positive ornegative data • Detection of small plasticparticles (less than 20 μm) with with μ-FTIR • Non-destructive analysisof materials	• Expensive • Wavelength radiation canbe a limiting detection factor • Time consuming to analyseall particles on a filter	~10–20 μm
Raman micro-spectroscopy	• Detection of small MPs (1μm) • No false positive ornegative data • Non-destructive analysisof materials • Analysis of samples in solution, gas, film, surface, solids and singlecrystals is possible	• Expensive instrumentation • Time-consuming • Interference with pigments • Possible fragments releasedby adhesive polymers	~1 μm

Pyrolysis–Gas Chromatography-Mass spectrometry (Py–GC–MS)	• A more holistic approachto characterize, in a singleanalysis, additives, and plasticizer, in addition to polymer category • Powerful for mass determination • Suitable for biologicalmatrices	• No particle number information No evaluation of size andshape • The analysis requires expert personnel • Destructive • Time consuming	~50/100 µm
Thermal Extraction Desorption–Gas Chromatography-Mass Spectrometry (TED–GC–MS)	• Characterization of low-solubility MPs and additives • Powerful for mass determination	• No particle number information, no evaluation of size andshape • Complex data • Destructive • Expensive	~50/100 µm

2.5.4.1 Visual identification

The visual sorting method is conducted subsequent to the extraction and purification steps, often employed by researchers to identify suspected MPs for further analysis (Qiu et al., 2016). However, relying solely on naked- eye evaluation for assessing MP particles is not recommended. The use of a stereomicroscope becomes imperative in this context to ensure accuracy. It's important to note that visual identification is a time-consuming process and can yield imprecise outcomes, particularly when samples aren't thoroughly purified. Similarities between MP particles and organic or inorganic particles can lead to false positives and overestimations (Hidalgo- Ruz & Thiel, 2012; J. Li et al., 2018a; Shim et al., 2017; Song et al., 2015). In this context, quantification of MP particles using a stereomicroscope is a commonly adopted practice. Certain criteria need to be met to confirm the presence of MPs:

1. Shape and Color: MPs should exhibit characteristic shapes and colors distinct from natural particles.
2. Transparency: MPs should be transparent or translucent under the microscope, unlike most mineralparticles.
3. Irregularities: Look for irregularities, such as cracks or surface roughness, which are indicative ofsynthetic materials.
4. Flexibility: Flex or manipulate the particle using a fine probe; plastics will often exhibit more flexibilitycompared to minerals.
5. Burn Test: Applying a flame test can provide further insights; plastics will melt and burn with distinctcharacteristics.
6. Density Separation Confirmation: If density separation was performed, confirming that the particle wasextracted using density separation adds to the confidence of its identification.

MP particles that appear transparent or white warrant closer examination under higher magnification and fluorescence microscopy to rule out the possibility of an organic origin (KIMO Sweden, 2007; H. C. Lu et al., 2021). It's a crucial step to ensure accurate identification. Despite its utility, the identification of MPs under a stereomicroscope does come with drawbacks stemming from personal factors, the quality of microscopy equipment, and the nature of the sample matrix. Notably, as particle size decreases, the likelihood of errors increases (Hidalgo-Ruz et al., 2012). However, many studies continue to employ microscopy as a sorting and quantification method (H. C. Lu et al., 2021). It's important to note that while visual sorting is valuable, it does not yield

chemical information about the polymer composition of the MPs. Moreover, the risk of misinterpretations rises when the clarity and color of plastics are altered during the extraction or purification process (J. L. Xu et al., 2019). As such, while visual identification serves as a useful initial step, combining it with complementary analytical methods is essential to provide a more comprehensive understanding of the MPscontent and composition in samples (Table 5).

Table 5. Different types of microscopes used to study MPs: (Based on Qiongxuan Qiu et al., 2016)

Microscope	**Illumination source**	**Color**	**Resolution**	**Advantages/ Limitation**
Ordinary microscope	Light	Dark view	Poor	Low cost with the worst observation
Stereomicroscope/ Dissected microscope	Light	Clear view	Moderate	Stereoscopy with clear discrimination
Fluorescent microscope	Light	Bright colors	Good	Fluorescence with best observation and accurate counting
Scanning electron microscope	Electron beam	Only blackand white	Great	Great stereoscopy. High-resolution to gain the chemical and morphological characterization of MPs with the highest cost

In regards to MP detection, visual identification is often suitable for MP particles larger than 1 mm, while microscopy is more appropriate for MPs smaller than 1 mm. When dealing with MP particles smaller than 1 mm, the presence of inorganic and organic components can lead to interference, potentially resulting in a higher likelihood of missing small plastic particles during sorting (Shim et al., 2017). It's important to note that visual identification is not recommended for particles smaller than 500 μm due to a significant risk of misidentification(Renner et al., 2018). When dealing with small MP particles, chemical or spectroscopic methods are recommended for identification (Song et al., 2015). Notably, (Zobkov & Esiukova, 2018) indicated that MP particles larger than 1 mm could be detected using a microscope, particularly when fibers have a uniform color or particles exhibit clean and uniform coloration. However, in the case of white and transparent particles, they recommended using a fluorescence microscope at a higher magnification for examination. These nuances underline the importance of tailoring the detection method based on the size, color, and characteristics of the MP particles to ensure accurate and reliable results.

The initial step in MP identification often involves examining the surface texture and structural characteristics. This can be accomplished through visual identification via naked-eye observation or optical microscopy (Song et al., 2015). This approach enables the documentation of MPs' attributes like color, shape, surface texture, and other distinguishing features that aid in differentiation from non-MP particles within the sample. Furthermore, itoffers insight into their potential origin (A. B. Silva et al., 2018), making it a useful pre-selection step before proceeding to chemical composition identification (Prata et al., 2019; A. B. Silva et al., 2018). To enhance the efficiency of visual inspection, various techniques have been employed. These include using needles to prod particles (Shim et al., 2017), testing plastics with a heated needle tip (Campbell et al., 2017), and even gently melting plastics (at around 130°C for 3-5 seconds) within the sample to facilitate detection (S. Zhang et al., 2018).

These approaches highlight the innovative strategies researchers adopt to ensure accurate and efficient MPs identification.

Visual identification, while valuable, comes with certain limitations and potential pitfalls. The quality of data obtained through visual sorting is influenced by numerous factors, including the individual conducting the counting, the quality and magnification of the microscope used, and the characteristics of the sample matrix (water, sediment, biota). One significant drawback of visual identification is its size limitation. Given the small size of MP particles, those below a certain size threshold may be indistinguishable from other materials or impractical to sort manually. Additionally, the visual identification method is time-consuming, which can impact the efficiency of the analysis. Numerous studies have reported false identification rates for plastic-like particles observed through a microscope, which can be as high as 70%, especially as particle size decreases (Hidalgo-Ruz et al., 2012). Furthermore, differentiating between synthetic fibers (such as polyester) and natural fibers (like colored cotton) solely through microscopy can be challenging. Fibers, which are a dominant type of MPs in water, sediment, and biota, can present difficulties in accurate identification (Shim et al., 2017). There's also a debate regarding the classification of white, transparent, and black particles as plastics, as they can easily be mistaken for biological materials or other substances. This can potentially lead to an underestimation of the actual number of MPs present, highlighting the need for caution when interpreting results obtained through visual identification (Zobkov & Esiukova, 2018). In light of these limitations, researchers often combine visual identification with other analytical techniques to enhance accuracy and reliability in MP detection and characterization.

2.5.4.2 SEM-EDX

Scanning electron microscopy (SEM) is a powerful technique for imaging plastic particles, providing clear and high-magnification images of MP morphology. Additionally, it offers valuable insights into their elemental and chemical composition through the use of an energy-dispersive X-ray spectroscopy (EDS) detector. This compositional information is particularly useful for distinguishing carbon-dominant polymers from interfering inorganic materials (Girão et al., 2017; Gniadek & Dąbrowska, 2019). The SEM/EDS combination can reveal the chemical composition of the MPs being studied, including the presence of inorganic additives on their surfaces. However, it's important to note that SEM/EDS equipment can be expensive, and both sample preparation and evaluation can be time-consuming and labor-intensive, which could limit the number of samples that can be effectively analyzed (Gniadek & Dąbrowska, 2019). It's also worth mentioning that plastic colors cannot be relied upon as identifiers in SEM (Shim et al., 2017). While SEM and related techniques can characterize the surface of plastics, there's currently no established method to determine how long a particle has been in the marine environment. The classification of MP particles into different categories varies widely depending on the goals of the study. Morphological characteristics, such as origin, type, shape, color, and degree of degradation, often play a role in categorization (Zobkov & Esiukova, 2018). This underscores the multifaceted nature of MPs' characterization and the need for a comprehensive approach to understanding their presence and impact.

2.5.4.3 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy is a robust technique that provides valuable insights into the chemical composition of particles. It is especially effective for identifying carbon-based polymers, as it reveals specific chemical bonds within the particles. This method allows for distinguishing plastics from other organic and inorganic particles based on their distinct spectra (Shim et al., 2017). The FTIR spectrum library not only confirms the presence of plastics but also enables the identification of specific polymer types. When dealing with small MPs, micro-FTIR(μ-FTIR) is necessary for accurate analysis (Song et al., 2015). Employing IR spectra to "fingerprint" each plastic-like particle reduces the risk of false positives and aids in detecting plastic particles with no color. FTIR can identify the polymer composition, abundance of MPs, sample source, and functional groups present in MP polymers (Song et al., 2015).

FTIR spectroscopy is particularly effective for identifying MPs larger than 10 µm, as it allows for quick and direct identification of polymer types by comparing their spectra with known plastics (Qiu et al., 2016). During FTIR analysis, the sample is irradiated with infrared light in the 400-4000 cm^{-1} wavenumber range for Mid-IR. The molecular structure of the sample absorbs some of the IR light, generating a spectrum measured in transmission or reflection mode (Käppler et al., 2016). The resulting infrared spectrum provides information about molecular vibrations and dipole moments (Prata et al., 2019). FTIR spectroscopy offers three optimizing modes: Transmission mode, which requires infrared-transparent substrates for high-quality spectra; Reflectance mode, suitable for thicker samples; and Attenuated Total Reflectance (ATR-FTIR), enhancing information on irregular MP and applicable to thicker or opaque samples. Micro-FTIR produces high-resolution maps of samples, down to 20 µm, without requiring a pre-selection step. ATR-FTIR is suitable for identifying MPs larger than 500 µm, while Micro FT-IR is used for smaller MPs. However, micro-FTIR can apply pressure that may damage fragile MPs or dislodge them from the filter (J. Li et al., 2018b; Prata et al., 2019; Shim et al., 2017).

FTIR spectroscopy is widely utilized in studies related to MPs identification in biota and is recommended by the Marine Strategy Framework Directive (Hanke et al., 2011). Nevertheless, the main drawbacks of micro-FTIR include the time and effort it consumes, the potential risk of damaging expensive instruments if crystals come into contact with inorganic particles, and the need for data pre-treatment to eliminate interference from IR-activewater (J. Li et al., 2018b).

2.5.4.4 Raman Spectroscopy

Raman spectroscopy is a non-destructive technique that complements FTIR in the field of MPs analysis (Löder & Gerdts, 2015). This method relies on the interaction between a laser beam and the molecular structure of an object, producing back-scattered light with different frequencies that correspond to the molecular vibrations and atoms present in the sample. Each polymer generates a unique spectrum in Raman spectroscopy. The technique has been used to identify the polymeric composition of ingested MPs in marine species such as fish (Dantas et al., 2020; Nie et al., 2019).

Raman measurements are non-destructive and do not require extensive visual pre-sorting. Similar to FTIR, they can directly identify MPs on filters and provide polymer composition profiles for each sample. However, any remaining biological material must

be removed to prevent fluorescence during Raman measurements (Käppler et al., 2016). Techniques like confocal laser-scanning microscopy can also be combined with Raman data (Cole et al., 2013). Raman spectroscopy excels at identifying very small MPs till 1 μm with high spatial resolution and relatively low sensitivity to water interference, especially when coupled with micro-Raman microscopy (Imhof et al., 2016; J. Li et al., 2018b; A. B. Silva et al., 2018).

Despite its advantages, Raman spectroscopy has limitations. UV exposure during Raman measurements can potentially damage the sample. Additionally, the technique suffers from low signal quality due to fluorescence induced by biological, organic, and inorganic impurities. The presence of additives and pigment chemicals in MPs can interfere with the identification of polymer types using Raman spectroscopy (J. Li et al., 2018b; Shim et al., 2017). Raman spectroscopy is more responsive to non-polar, symmetric bonds compared to FTIR, which is better at identifying polar groups (Lenz et al., 2015). Moreover, Raman spectroscopy offers wider spectral coverage, better resolution, and lower water interference compared to FTIR (A. B. Silva et al., 2018).

2.5.4.5 Gas Chromatography Coupled with Mass Spectrometry Detector (GS/MS)

Pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) is a destructive method that has been utilized to identify the polymer types of MPs by analyzing their thermal degradation products (Qiu et al., 2016). Unlike FTIR and Raman methods, Py-GC-MS does not require pre-treatment of the sample; it directly examines the polymer composition of the sampled MPs. A small sample mass (5-200 μg) is typically analyzed in a single measurement. This technique can identify both the polymer type and organic plastic additives simultaneously (A.

B. Silva et al., 2018). However, Py-GC-MS has limitations. It cannot assess the number, type, or morphology of MPs since it provides only the mass of polymer present in the sample. Therefore, MPs must be pre-selected using optical techniques before applying Py-GC-MS. Currently, Py-GC-MS is mainly used as a confirmation method to verify the composition of suspected MPs (A. B. Silva et al., 2018).

Py-GC-MS works by thermally decomposing (pyrolyzing) polymers under inert conditions. The gases produced are cryogenically trapped and then separated on a chromatographic column. The resulting chromatograms from the samples are compared to reference chromatograms of known polymer samples to identify the polymer types. While Py-GC-MS can identify certain polymer types, it does not provide information about the number, size, or shape of MPs (Prata et al., 2019). It's important to note that

because Py-GC-MS is a destructive method, additionalanalyses of the same MP samples are not possible (Shim et al., 2017). Compared to FTIR and Raman spectroscopy, Py-GC-MS requires a well-trained and experienced operator and involves more time and effort forinstrument runs and data processing.

2.5.5 Characteristics results for river sediments (Features of microplastics inriver sediments)

The concentration of MPs reported in studies of river sediments worldwide can vary significantly due to various factors, including seasonal variations, environmental conditions, inputs from wastewater treatment plants (WWTPs), and the methods used in the experiments (Mani & Burkhardt-Holm, 2020; Pojar et al., 2021). Rivers play a critical role in transporting MPs from land to the ocean, and their contribution to marine plastic pollution has been well documented.

In the context of European rivers, different studies have estimated the amounts of MPs entering the oceans. For example, Simon-Sánchez et al. (2019) estimated that The Ebro River contributes 2.14×10^9 MPs/year to the Mediterranean Sea. The Danube River was found to contribute around 1533 tonnes of MPs per year to the Black Sea (Lechner et al., 2014). The Rhone River was reported to introduce about 0.7 tonnes of floating plastic into the Mediterranean Sea annually (Castro-Jiménez et al., 2019), and the Rhine River released between 20 and 31 tonnes of plastic particles to the North Sea (Van Der Wal et al., 2015). Regarding the characteristics of MPs in European river sediments, the reported concentrations have shown a wide range, from 2.9 items/kg (Piehl et al., 2019) to 11070 items/kg (Mani et al., 2019). The most prevalent shapes of MPs reported in European rivers are fibers and fragments. Commonly reported polymer types in sediment samples from European rivers include (PE), (PP), and (Ps). MP colors that are often observed include colored and black particles (Table 3).

Various sources of MP pollution in the sediments of European rivers have been identified in published studies. These sources include effluents from WWTPs (Blair et al., 2019; Kiss et al., 2021; Rodrigues et al., 2018), industrial activities (Scherer et al., 2020), and waste management systems (Simon-Sánchez et al., 2019). However, it's worth noting that there can be inconsistencies in findings between studies. For instance, Klein et al. (2015) did not find a significant relationship between MPs concentration and the effluents of WWTPs and industrial areas. Hence, the variability in reported concentrations and sources of MPs in European river sedimentsunderscores the complex and multifaceted nature of MPs pollution, which can be influenced by a range of factors specific to each river and its surrounding environment.

2.6 Microplastics contamination in mussels

2.6.1 Background

Freshwater mussels belong to the bivalve species and are known for their filter-feeding or suspension-feeding behavior. They have the ability to filter water, which enables them to extract food particles suspended in the watercolumn. This filtration capacity can vary within the range of 0.1 to 3 liters per hour (Zieritz et al., 2019). Freshwater mussels rely on a diverse range of particles present in their surrounding water as a source of food, including both organic components like phytoplankton and detritus, as well as inorganic particles like silt (Strayer, 2014). These mussels are widespread and can be found in various river ecosystems, with an approximate density of about 100 mussels per square meter (Atkinson & Vaughn, 2015). Despite their seemingly high abundance, freshwater mussels are susceptible to the risk of extinction due to various anthropogenic activities that can negatively impact their growth and overall population health. One of the significant threats to freshwater mussels is the increased sediment load in water bodies resulting from human activities. High sediment levels can affect water quality, decrease the availability of suitable habitats, and directly impact mussel growth and reproductive success (Goldsmith et al., 2021). The vulnerability of freshwater mussels highlights the delicate balance of aquatic ecosystems and the need for conservation efforts to protect these important organisms and the ecosystems they inhabit.

Freshwater mussels play a crucial and multifaceted role in the dynamics of freshwater ecosystems, exerting profound effects on habitat provision and nutrient cycling (Pan & Wang, 2004; Zieritz et al., 2019). With their ability to interact with both organic and inorganic particles, mussels contribute to habitat formation and nutrient redistribution, ultimately shaping the intricate trophic interactions within aquatic environments. This is underscored by their capacity to remove, generate, and influence the movement of particles and dissolved materials, thereby impacting benthic and pelagic habitats, as well as the broader stream trophic chain (Pan & Wang, 2004; Zieritz et al., 2019). A notable mechanism through which mussels influence freshwater ecosystems is by modulating nutrient dynamics. By engaging in various ecological functions, including filter-feeding on particulate matter, nutrient excretion, biodeposition, and the decomposition of pseudofeces, mussels play a critical role in nutrient cycling (Atkinson et al., 2013; Atkinson & Vaughn, 2015; James et al., 2021). These activities result in the release of both limiting and non-limiting nutrients, which can have far-reaching effects on

nutrient availability and distribution within stream ecosystems. The extent of mussels' influence on nutrient cycles, particularly nitrogen (N) and phosphorus (P), varies depending on the ecosystem's characteristics and the dominant species present (Zieritz et al., 2019). Consequently, freshwater mussels contribute to the delicate balance of N and P stoichiometry, a critical factor in maintaining the health and functionality of aquatic ecosystems. Moreover, mussels serve as conduits for the transfer of nutrients and energy from the water column to benthic compartments, thereby influencing the dynamics of the trophic pyramid (Atkinson & Vaughn, 2015; T. J. Hoellein et al., 2017; James et al., 2021; J. Li et al., 2021; Ozersky et al., 2015). As our understanding of these intricate interactions continues to evolve, it is clear that freshwater mussels are vital drivers of ecosystem structure and function. By modifying habitat characteristics and regulating nutrient availability, they contribute to the overall health and resilience of freshwater systems. Recognizing the importance of conserving these organisms becomes imperative in safeguarding the delicate balance of nutrient cycles and trophic interactions within our aquatic environments.

Freshwater mussels possess a set of remarkable attributes that make them attractive candidates for bioindication purposes. Their widespread distribution, accessibility, extended lifespan, and remarkable resilience to a broad spectrum of environmental conditions, including salinity, temperature, oxygen levels, and food availability, position them as promising tools for monitoring aquatic pollution(J. Li et al., 2019; Pastorino et al., 2021). As such, the scientific community has increasingly turned to mussels as bioindicators for assessing MPs contamination in both marine and freshwater environments (Bonanno & Orlando-Bonaca, 2018; Bråte, Blázquez, et al., 2018; Cera & Scalici, 2021; Staichak et al., 2021; Su et al., 2018). Despite the promising potential of mussels as biomonitoring tools for MP contamination, several challenges and limitations must be acknowledged. Several critical factors influence their applicability and the accuracy of the results obtained, including:

1. Particle Characteristics: The efficiency of particle capture by mussels is intricately linked to particle size, shape, and surface properties (Jørgensen et al., 1984; Rosa et al., 2013, 2017, 2018). The diverse attributes of MPs in terms of these characteristics may impact how effectively mussels accumulate them.
2. Selective Particle Sorting: Mussels possess the ability to sort particles based on both physical and chemical factors (Rosa et al., 2018; Ward, 1996; Ward & Shumway, 2004). This sorting behavior could introduce bias in the collected data,

potentially leading to underestimations or overestimations of MP concentrations.

3. Differential Retention: Different sizes of MPs can exhibit varying retention patterns within the digestive tracts of mussels (Kinjo et al., 2019). This variability in retention can affect the accuracy of quantifying MP concentrations in mussel tissues.

Navigating these complexities is essential to ensure the reliable and accurate use of mussels as bioindicators for MPs contamination. Recognizing the potential limitations and developing strategies to address them is crucial for advancing the efficacy and credibility of using mussels in biomonitoring efforts. By addressing these challenges, researchers can harness the unique attributes of mussels to provide valuable insights into the extent and impact of MPs pollution in aquatic ecosystems. Similar to sediment-based studies, research focused on MPs contamination in freshwater mussels remains relatively limited when compared to the extensive body of work available for marine ecosystems. Table 6 presents a concise summary of selected studies that have reported on MPs found in various species of river mussels across the globe.

Table 6: A summary of the abundance and characteristics of microplastics in bivalves from a riverine environment

Studyarea	**Bivalve Type**	**Abundance**	**Dominant Shape**	**Size**	**Dominant Color**	**Chemical composition**	**Reference**
Grand River, Canada	Mussel *Lasmigona costata*	0-0.16 items/g 0-7 items/individual	Fragments	21-298 µm	White	co-EPPP	(Wardlaw& Prosser,2020)
öje River, Sweden	Mussel *nodonta anatina*	4-142 plastic fibers/individual	Fibers	N/A	Black	N/A	(Berglund et al., 2019)
Saint John River, Canada	Mussel *M. margaritifera* L	0-0.6 microfibers/g 0-10.9 microfibers/g	Microfibers	> 100 µm	Blue	N/A	(Doucet etal., 2021)
St. Lawrence River, USA	*Dreissena polymorpha* and *D. bugensis*	N/A	Not found	N/A	N/A	N/A	(Schessl etal., 2019)
Milwaukee River, USA	Mussel *Dreissena sp*	ems/g0.6 items/individual	Fibers	N/A	White	cotton natural cellulose-basednatural PET	(T. Hoellein etal., 2021)

Yangtze River, China	Asian clam *Corbicula fluminea*	0.3-4.9 items/g 4-5.0 items/ individual	Fibers	250-1000 µm	Blue	PET 33% PP 19% PE 9%	(Su et al., 2018)
Thames River, UK	Asian clam *Corbicula fluminea*	0-24 items/ individual	Fibers	N/A	N/A	PP 57% PE 9% Nylon 8% Polyallomer 8% PVP 6% Others 12%	(McCoy etal., 2020)
Pearl River, China	Oyster Saccostrea cucullata	1.5-7.2 items/g 1.4-7 items/ individual	Fibers	< 100 µm	Transparent	PET 34% PP 19% Pe 14% PS 8% Cellophane 8% PVC 6% Polyamide 4% Expanded polystyrene 3%	(H. X. Li et al., 2018)

It is evident that the studies listed in Table 6 highlight the growing interest in exploring MPs contamination in freshwater mussel species across different geographical locations. Despite the relatively limited number of studies compared to marine counterparts, these investigations provide valuable insights into the prevalence, distribution, and characteristics of MPs in river mussels. However, due to the diverse nature of freshwater environments and mussel species, further research is necessary to establish a comprehensive understanding of MPs pollution in these ecosystems and its potential impacts on mussels and the broader aquatic environment.

2.6.2 Sampling of Mussels

Despite the substantial amount of research conducted on MP pollution over the past two decades, the lack of standardized protocols for sample collection, preparation, and identification remains a significant challenge. This absence of standardization hinders effective comparison between studies and a comprehensive understanding of the current state of MP pollution (Stock et al., 2019). Technical difficulties in the extraction and characterization of MPs from original samples further compound these challenges (Stock et al., 2019). Another complicating factor is the inherent heterogeneity of MPs, which come in a variety of sizes, shapes, colors, densities, and chemical compositions (Hidalgo-Ruz et al., 2012).

It's notable that a substantial portion of studies examining MPs in mussels is conducted

under controlled laboratory conditions rather than in natural field environments (Lusher et al., 2017). This difference is crucial to acknowledge, as laboratory conditions often yield higher MPs concentrations compared to real-world settings, making direct comparisons challenging (Stock et al., 2019). Choosing an appropriate sampling method depends on factors such as mussel size, research objectives, and available resources. In river studies, manual collection of mussels is a common approach (Berglund et al., 2019), followed by careful placement in clean PP bags (Schessl et al., 2019), sterile Whirl-Pak bags (Doucet et al., 2021), or glass beakers (Pastorino et al., 2021) to prevent contamination. Once collected, mussel samples are typically stored at -20°C until they undergo MP analysis (Suet al., 2018). Recording mussel characteristics like shell length, shell weight, growth stage, and soft tissue weight is recommended after sampling to provide valuable context (Stock et al., 2019). Moreover, maintaining the integrity of laboratory procedures is crucial; all instruments and equipment used in pre-treatment should be thoroughly rinsed with ultrapure water to prevent any potential sources of contamination during subsequent laboratory work (Hermsen et al., 2018). Researchers should also don cotton lab coats to minimize the risk of cross-contamination during the handling and analysis of samples. In essence, the challenges associated with standardized protocols, variations in laboratory and field conditions, and potential sources of contamination necessitate a meticulous and cautious approach when conducting research on MP in mussels. Addressing these challenges will help ensure the reliability and comparability of results across different studies.

2.6.3 Mussels samples preparation

The extraction of MPs from biota samples differs from the procedures used for water and sediment samples. Extracting MPs from biota involves a series of steps, including digestion to remove organic matter, density separation, and filtration (Fu et al., 2020). The initial step in extracting MPs from biota samples is digestion, which is crucial for breaking down organic components and isolating the plastic particles. When it comes to mussel samples, several methods have been employed to extract MPs, including dissection, depuration, homogenization, and tissue digestion using chemicals or enzymes (Lusher et al., 2017). Visual sorting is then employed to distinguish plastics from other materials present in the sample, such as organic debris (like shell fragments, animal parts, dried algae, seagrasses, etc.) or other items (metal paint coatings, tar, glass, etc.). Visual sorting can be performed either with the naked eye or under a microscope. Quantifying MPs in mussel samples is particularly challenging due to the high presence of biological materials, microbial biofilms, and debris. To accurately quantify MPs, it is

crucial to separate them from other materials in the sample. Environmental samples, including biota samples, often contain a significant amount of organic matter (Prata et al., 2019). Therefore, a purification step is necessary after the initial sample extraction to prevent the overestimation of MPs during analysis. This purification step removes impurities like biofilms and other organic and inorganic residues attached to the surface of MPs (L. Yang et al., 2021). Chemical or enzymatic degradation methods can be employed for this purification process. Among the commonly used substances, 30% H_2O_2 at controlled temperatures stands out as the most frequent choice for purifying samples (C. Li et al., 2020; Y. Zhang et al., 2020). Various substances are used for the digestion of biota samples to extract MPs, each with its advantages and limitations. Some commonly used substances include acids like nitric acid (HNO_3) and hydrochloric acid (HCl), as well as alkaline substances like potassium hydroxide (KOH) and sodium hydroxide (NaOH). Acids like HNO_3 and HCl have been employed for digestion (Berglund et al., 2019). However, these acids have certain drawbacks. HCl, for instance, is less efficient in removing organic matter, and both sulfuric acid (H_2SO_4) and (HNO_3) can potentially damage the morphology of the MPs (Andrady, 2017; Besley et al., 2017; J. Li et al., 2018b). On the other hand, digestion with alkaline substances has proven effective. Potassium hydroxide (KOH) at 10% concentration, as well as sodium hydroxide (NaOH), are commonly used for digestion
(Doucet et al., 2021; Foekema et al., 2013; H. X. Li et al., 2018; McCoy et al., 2020) . NaOH has been noted for its high digestion efficiency on biota samples (Cole et al., 2014). However, it can also cause damage to certain polymers like nylon fibers, polyethylene, and PVC. In a comparative study by Thiele et al. (2019), various methods of recovering MPs from bivalves were assessed, including trypsin, proteinase-k, KOH, and H_2O_2. The study found that using KOH was the most efficient extraction method. Similarly, Dehaut et al. (2016) compared different protocols for MPs extraction from biological organisms and concluded that using a KOH solution is the most effective method. In summary, the choice of digestion method for biota samples depends on factors such as the type of samples, target polymers, and potential damage to the MPs' morphology. Both non-oxidizing acids and alkaline substances have been used, with alkaline solutions like KOH often showing higher efficiency in extracting MPs while minimizing damage to the samples.

For the extraction of MPs from biota samples, digestion methods often involve the use of oxidizing substances such as 30% hydrogen peroxide (H_2O_2), potassium persulfate

($K_2S_2O_8$), and sodium hypochlorite (NaClO) (Beer et al., 2018; T. Hoellein et al., 2021; Su et al., 2018). Enzymatic methods have also been employed for digestion, offering an alternative to acid or base-based approaches that may damage certain polymers (J. Li et al., 2018b; Miller et al., 2017). Enzymatic purification is recommended due to its effectiveness and polymer-friendly nature. Cole et al., 2014 utilized a single enzymatic step involving proteinase-K for purification. Another study by Löder & Gerdts, 2015 employed enzymes such as lipase, amylase, proteinase, chitinase, and cellulase to purify biota and sediment samples, effectively removing the biological matrix.

Enzymatic digestion has emerged as the most efficient method for digesting mussels while preserving the integrity of MP polymers (Cole et al., 2014; Courtene-Jones et al., 2017; Löder et al., 2017; Wardlaw & Prosser, 2020). This method offers distinct advantages, although there is a need for standardization and improvement due to challenges related to enzyme cost and maintaining optimal reaction conditions. In the pursuit of effective enzymatic purification, researchers have explored different enzymes and their digestion efficiency. Courtene- Jones et al., 2017 compared the performance of three enzymes (trypsin, papain, and collagenase) on biological samples and found trypsin to be the most efficient enzyme, offering a good balance between digestion effectiveness, preservation of polymer integrity, and reasonable time and cost considerations. Similarly, Cole et al., 2014 optimized an enzymatic digestion protocol using Proteinase K, which demonstrated digestion efficiency exceeding 97% for biota samples without compromising MP particles' structural integrity. Another approach proposed by Loder et al. (2017) involved employing a series of grade enzymes, including protease, cellulose, and chitinase, for the purification of MPs. The basic enzyme purification protocol (BEPP) achieved a digestion efficiency of 89.3% for surface water samples, effectively removing organic impurities present in the sample matrix.

Catarino et al., 2017 conducted a study comparing the effectiveness of different digestion methods for recovering MPs from mussel soft tissues. Their investigation evaluated three digestion methods: 1M NaOH, 35% HNO_3, and the use of a protease enzyme. The findings indicated that the protease enzyme exhibited the highest digestion efficiency and MPs recovery rate, preserving the integrity of MPs without causing destruction. In a similar vein, Cole et al., 2014 undertook a comprehensive assessment of various digestion techniques for separating MPs from plankton-rich water samples and marine zooplankton under controlled laboratory conditions. Their study encompassed acid, alkaline, and enzymatic digestion methods. The results demonstrated that utilizing a proteolytic enzyme, specifically proteinase-K, proved to be the most efficient approach,

achieving over 97% digestion for both water and biota samples without causing harm to the MPs present. However, it's worth noting that according to von Friesen et al., 2019, the chemical digestion methods mentioned earlier can potentially damage the polymer structure of MPs. Meanwhile, enzymatic methods entail high costs and time investments. To address these challenges, they proposed an alternative approach involving the use of pancreatic enzymes and a pH buffer (Tris) for efficient digestion. This method stands out for its simplicity, cost- effectiveness, minimal environmental impact, and time efficiency. During the digestion process, heating and shaking are crucial to enhance purification efficiency. Heating can be accomplished using tools like a water bath, oscillation incubator, or oven at temperatures ranging from 40-80°C. Shaking velocity typically falls within the range of 80-100 rpm. As for the digestion time, there is variability across studies, spanning from 40 minutes to 7 days. Following digestion, certain studies incorporate a flotation step, while others opt for density separation using salt solutions like NaCl solution (1.2 g/cm^3) or a $ZnCl_2$ solution (1.5 g/cm^3) to isolate the MPs further.

In conclusion, the extraction and purification of MPs from biota samples, particularly mussels, require a tailored approach that considers the unique challenges posed by the presence of biological materials and organic debris. By carefully applying methods such as digestion, visual sorting, and purification, researchers can achieve accurate and reliable quantification of MPs in biota samples. The choice of digestion and purification methods depends on factors such as the target polymers, the potential for polymer damage, and the desired level of extraction efficiency. Enzymatic methods provide an attractive solution for purification due to their ability to effectively remove impurities while being gentler on the MPs structural integrity.

2.6.4 Analysis (Identification)

Indeed, conducting chemical characterization is paramount in distinguishing plastics from other particles in mussel samples. Just as seen in sediment samples (as discussed earlier), various identification techniques are at hand for identifying MPs in mussel samples (as summarized in Table 4). However, among these methods, FT-IR and Raman spectroscopy emerge as the most commonly employed approaches. FT-IR spectroscopy has been frequently utilized for the chemical identification of MPs in mussel samples. Studies by Su et al. 2018 and McCoy et al., 2020 have harnessed FT-IR to effectively discern plastic particles in mussel samples. This spectroscopic method involves

exposing the samples to infrared light, which interacts with the molecular structure of the materials, generating a distinctive spectral pattern that aids in identifying the polymer composition of the MPs. Similarly, Raman spectroscopy has gained prominence as a valuable technique for identifying MPs in mussel samples. Wardlaw & Prosser, 2020 as well as Mercogliano et al., 2021 have employed Raman spectroscopy to successfully differentiate plastic particles in their mussel samples. Raman spectroscopy works by subjecting the samples to laser light, leading to the scattering of light at different frequencies depending on the molecular structure of the particles. This scattering pattern offers insights into the composition and identity of the MPs present. Both FT-IR and Raman spectroscopy provide robust methods for determining the chemical nature of MPs within mussel samples. These techniques enable researchers to effectively discriminate between plastic particles and other materials present in the samples, thus contributing to a more comprehensive understanding of MPs contamination in aquatic ecosystems.

2.6.5 Characteristic results for river mussels

Despite the growing body of research on MPs in mussels, there remains a notable dearth of studies focusing on MPs in river-dwelling mussels, with the majority of investigations being conducted within laboratory settings. Concerning the specific characteristics gleaned from studies of MPs in river mussels, the abundance of MPs exhibited substantial variability across different investigations. This variation spans from instances of no observed plastic items per individual of Lasmigona costata mussel collected from Grand River, Canada (Wardlaw & Prosser, 2020), to instances where as many as 142 plastic items were found per individual of Anodonta anatina mussels collected from Höje River, Sweden (Berglund et al., 2019). Among the findings from various studies, a consistent pattern emerges regarding the shapes of MPs found within mussel samples. Fibers were the predominant shape encountered, followed by fragments. Polymer types were also consistently reported, with PET, PP, and PE emerging as the most prevalent polymers within mussels collected from river environments. In terms of color, the most frequently reported hues were black, blue, and white. Furthermore, the reported size distribution of identified MPs typically fell within the range of particles smaller than 100 µm, as documented in Table 6. It is evident that while strides have been made in understanding MPs in mussels, there remains much variability in terms of MP abundance, characteristics, and polymer types across different river systems and mussel species. The scarcity of studies focused on river mussels underscores the need for more comprehensive investigations in these environments to

gain a more holistic view of MPs contamination in aquatic ecosystems((Berglund et al., 2019).

Chapter 3. Comparison of Freshwater Mussels Unio tumidus and Unio crassus as Biomonitors of Microplastic Contamination of Tisza River (Hungary).

3.1 Background

Among the many adverse effects of anthropogenic activities on the aquatic environment, plastic pollution is a prominent one. While plastics are an inexpensive material with innumerable applications and provide many social benefits, they have also emerged as a persistent pollutant in the 'plastic age' due to the mismanagement of discarded plastics (Kinjo et al., 2019; Wardlaw & Prosser, 2020). In recent years, the focus has been on MPs (plastic particles with a size < 5 mm), which are prevalent in many different environments such as seawater, riverine, sediments, soil, polar ice, and land (Alfaro-Núñez et al., 2021; Gomiero et al., 2019; H. X. Li et al., 2018; Su et al., 2018). MPs have a variety of shapes (spherical, angular, fragment, pellet, sheet), but a large proportion of MPs in the aquatic environment are in the form of fibers arising from clothing material (Ward, Zhao, et al., 2019; Wardlaw & Prosser, 2020). Due to their small size, a large fraction of MPs remains suspended in the water column and are easily ingested by a variety of organisms such as plankton, zooplankton, invertebrates, fishes, and mammals (Costa et al., 2020; Dris et al., 2016; H. X. Li et al., 2018; Meaza et al., 2021; Zantis et al., 2021). A number of these organisms could serve as bioindicators providing information about MP pollution in their environment; however, large-sized organisms can be opportunistically sampled only in small numbers, unlike the smaller organisms, where the sampling of a large number of individuals is possible. Among invertebrates, musselsare important organisms that have been used to indicate pollution levels because they are sensitive to physical and chemical alterations in the aquatic environment (J. Ding et al., 2021; Kühn et al., 2017; Su et al., 2018; Vandermeersch et al., 2015; Wardlaw & Prosser, 2020).

Several papers in the last decade have reported the biomonitoring of MP pollution by mussels both in the marine (Bonanno & Orlando-Bonaca, 2018; Bråte, Hurley, et al., 2018; J. Li et al., 2021) and freshwater (Cera & Scalici,2021; Staichak et al., 2021; Su et al., 2018; Wong et al., 2020) environments. Due to their broad geographical distribution, sessile lifestyle, easy accessibility and sampling method, tolerance to a considerable salinity range, high-stress resistance, and excessive accumulation of a wide range of pollutants, mussels are the ideal test organisms for environmental biomonitoring in the aquatic environment (J. Ding et al., 2021; Vandermeersch et al.,

2015). Mussels are suspension feeders (filter feeders) that are able to process large volumes (~24 L) of suspension originating from the suspended sediment daily through their filter system (Naidu, 2019; Riisgård et al., 2011), but 40 L/day rates have also been reported (Tankersley~ & Dimock, 1993). During this filtration process, not only are phytoplankton, bacteria, and particulate organic matter ingested, but also non-digestible MPs (fragments, films, and fibers), sand, and silt particles are taken up. On the basis of the literature data, the retention time of ingested MPs in mussels varies from a few hours to 40 days, depending on their particle size and the mussel species (Kinjo et al., 2019). However, the larger particles covered with mucus as pseudofeces are removed from the mantel cavity within a few hours (Ward, Zhao, et al., 2019). In spite of the fact that freshwater organisms are directly affected by terrestrial run-off, wastewater, and other industrial discharges potentially containing a high level of MPs and other contaminants, ecological studies have mostly focused on marine organisms (Fu et al., 2020). Although the use of mussels as sentinel species for large-scale monitoring programs in the marine environment has recently been recommended (J. Li et al., 2019), there are more limiting factors that hamper the reliable applicability of mussels for the biomonitoring of MP contamination (Ward, Rosa, et al., 2019). The most critical points are the following: (1) The capture efficiency is influenced by the size, shape, and surface properties of particles (Jørgensen et al., 1984; Rosa et al., 2013, 2017, 2018); (2) mussels are able to sort particles based on their physical and chemical factors (Rosa et al., 2018; Ward, 1996; Ward & Shumway, 2004); (3) differentially sized MPs are retained differently in the digestive tract of mussels. The ingestion of suspended particles in the mussel–particle relationship has been widely studied during the last 40 years; however, the characterization of the mussel's habitats, namely that these animals serve as 'living sampling devices', is less investigated. 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.

According to the published data of eight research groups, the bivalves (mussels, clams, and oysters) collected from different rivers primarily contained fibers (Berglund et al., 2019; Doucet et al., 2021; T. Hoellein et al., 2021;

H. X. Li et al., 2018; McCoy et al., 2020; Schessl et al., 2019; Su et al., 2018; Wardlaw & Prosser, 2020). Their length and number /individual values changed in the range of 20–1000µm and 0–142 (Table 6). This means that the ingestion of natural or synthetic

fibers is preferred to particles with other shapes, and these fibers have a longer residence time in the organisms.

Considering the literature data mentioned above, the high amount of plastic litter transported by the Tisza River and its tributaries from the neighboring countries, the high concentration of MP particles (3177 ± 1970 items/kg) in the bottom sediment of this river, and the dominant role of fiber contamination (Kiss et al., 2021), we decided to investigate the applicability of freshwater mussels as the characteristic invertebrates of the Tisza River for the biomonitoring of fiber. For this purpose, Unio crassus and Unio tumidus belonging to the family Unionidae were selected. They are present in the entire European mainland inhabiting running waters of different sizes and depths, particularly channels with low shear stress and fine mineral substrate (Lopes-Lima et al., 2017; Zając et al., 2018). They have a relatively high occurrence in the studied aquatic environment. The main objective of our study was to determine whether these two mussel species grown simultaneously at the same sampling sites under the same environmental conditions provide the same analytical information on MP contamination or whether the number of MPs accumulated in these mussels is actually species-dependent.

3.2 Materials and Methods

3.2.1 Sampling

Along the Tisza River, four sampling sites at the settlements of Tímár (1), Tokaj (2), Csongrád (3), and Szeged

(4) were selected, which are located at river km of 552, 544, 244, and 160, respectively (Figure 3). The choice of these sampling sites provided an opportunity to study the potential effect of the tributaries (Bodrog, Sajó, Zagyva, Körös, Maros) on the MP load. These rivers flow through industrial and agricultural areas, and more small WWTPs as potential contamination sources are located at their banks. The mussels were collected during a 3-day campaign in August 2021, within a 5–10 m coastal strip and at a depth of 0.8–1.2 m, by applying a stainless steel trowel and a long-handled deep net with a brass mesh. From about 60–80 mussels at all the sampling sites, 10 Unio crassus and 10 Unio tumidus mussel species of nearly similar sizes were selected, rinsed with distilled water, placed in an aluminum foil, transferred to the laboratory on ice, and then stored at -20 ◦C until analysis. The other mussels were placed back into the river.

Figure 3: Hydrographic map of Tisza River and its tributaries arriving from Slovakia (Bodrog, Sajó) and Romania (Körös, Maros). The four samplingsites located at settlement Tímár (1), Tokaj (2), Csongrád (3), and Szeged (4) are marked with red circles.

3.2.2 Chemicals and Reagents

A Wasserlab Automatic unit (Labsystem Ltd. Budapest, Hungary) was used for the production of ultrapure water(resistivity 18.2 MΩ cm−1). For the production of a 10% KOH solution, appropriate amounts of solid KOH granulates (VWR International, LCC) were dissolved in ultrapure water. A particle-free $ZnCl_2$ solution with a density of 1.5 g cm^{-3} was prepared by dissolving solid $ZnCl_2$ (VWR International, LCC, Radnor, PA, USA) 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).

3.2.3 Sample Preparation

To avoid any contamination of the samples from the air, all the steps of sample preparation were carried out in a laminar box (AC2-4G8 Airstream® Class II, Thermo Fischer Scientific, Waltham, MA, USA). The shell lengthsof the defrosted mussels were measured, and then all of the soft tissues were removed from the shells using a steel spoon and placed separately into 750 mL glass beakers, covered with a watch glass. After measuring their weights, the wet mass of the soft tissues was calculated. Based on

the reported literature data for the digestion of soft tissues, 10% KOH was deemed the most suitable among the other commonly used chemicals (H_2O_2, HNO_3, KOH, proteinase K, and trypsin) (Kühn et al., 2017; Thiele et al., 2019). The tissues (6–12 g) were separately digested in 150 mL 10% KOH at 40 ˚C for 24 h and incubated at room temperature for another 24 h. In order to increase the efficiency of this digestion procedure, as a first step, an ultrasonic treatment at a frequency of 37 kHz was applied for 30 min. To separate the MPs from inorganic or organic residues, a 400 mL particle-free $ZnCl_2$ solution with a density of 1.5 g cm^{-3} was added to the samples, and the mixture was covered with an aluminum foil. After the 48 h long density separation procedure, a 200 mL supernatant was filtered by applying pressure filtration with high-purity synthetic air (4 bar) in order to reduce the risk of contamination from laboratory air. The particles and fibers were concentrated on a Whatman GF/C glass fiber filter with a diameter of 21 mm and pore size of 1.2 µm. Following filtration, the wet-loaded filters were placed on Petri dishes lined with crumpled aluminum foil and covered with glass lids. The filters were then dried at 60 ˚C in a laboratory oven to obtain a constant weight and stored in airtight Petri dishes at room temperature until the analysis. The procedure of blank samples was carried out using the same experimental conditions as mentioned above.

3.2.4 Analysis of Residues via Optical Microscopy and Raman Spectrometry

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 microspectrometer 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, was selected. The spectral resolution of measurements varies 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).

3.2.5 Statistical Analysis

Figures were drawn with Excel, while statistical analyses were performed with R

statistical software (Team, 2020). Exact Poisson tests (C-test with the poisson.test function of the 'stat' package) and Poisson regression model (with the glm function of the 'stat' package) were used to compare the mean number of fibers found in the mussel individuals of different species and habitats. The Bonferroni–Holm correction was used to avoid the problems of multiple testing (Holm, 1979) (with the p.adjust function of the 'stat' package).

3.3 Results

The length of the shells and the wet mass of the soft tissues of Unio crassus and Unio tumidus mussels collected at the four different sampling sites are listed in Table 7.

Table 7. Mean values and standard deviations of shell lengths, wet weight of soft tissues of *Unio crassus* and *Unio tumidus* mussels (n = 10), and the numbers of fibers found in these bivalves related to individuals or 1 g soft tissue. Letters (i.e., ^{a}, ^{b}, ^{c}, ^{d}) indicate the results of the statistical analysis. Values sharing the same letters are not different significantly.

Mussel	Site	Shell Length (mm)	Soft Tissue WetWeight (g)	Number of Fibers/ind	Number of Fibers/g
Unio crassus	Tímár	62 ± 5	10.94 ± 1.75	2.8 ± 0.5 ^{a}	0.25
	Tokaj	68 ± 3	9.85 ± 2.34	2.7 ± 0.5 ^{a}	0.27
	Csongrád	64 ± 7	9.53 ± 2.72	4.9 ± 1.2 ^{bc}	0.51
	Szeged	71 ± 4	12.82 ± 2.12	3.8 ± 0.8 ^{ab}	0.29
Unio umidus	Tímár	67 ± 5	7.15 ± 1.74	5.2 ± 1.4 ^{bcd}	0.72
	Tokaj	63 ± 6	6.84 ± 1.33	6.0 ± 1.3 ^{cd}	0.87
	Csongrád	70 ± 8	6.94 ± 2.18	7.2 ± 1.9 ^{d}	1.03
	Szeged	77 ± 8	7.95 ± 2.33	7.1 ± 2.4 ^{cd}	0.89

Procedure blank 0.45 fibers/individual.

The average length of the mussel shells (mm) was higher for Unio tumidus than for Unio crassus, but an opposite trend was observed for the soft tissue wet weight (g). The numbers of particles per individual and per gram of the soft tissues found in these mussels are demonstrated in Figures 4 and 5. Despite the higher soft tissue mass in Unio crassus compared with that in Unio tumidus, the number of fibers and fragments in individuals collected from the same sampling sites showed a different picture. The particles including the synthetic and natural fibers had a length and diameter of 20–1000 µm and 10–75 µm, respectively, and the fragments had about two times higher concentration in Unio tumidus than in the other mussel species ($p < 0.001$, exact Poisson test). During the investigations of loaded filters using a stereomicroscope, nine fibers of different colors were found in the following proportion: blue (54.3%), black (22.2%), gray (10.6%), brown (3.8%), red (3.4%), white (3.4%), turquoise (1.3%), green (0.6%),

and pink (0.4%). Based on the Raman spectra of thereference samples, the indigo dye (Figure 6) and polyethylene terephthalate were identified as the basic materials of blue fibers in most cases (Figure 7). Among the cellulose-based fibers, two groups could be distinguished with diameters of 10–25 and 30–75 µm. The finer microfibers are characteristic of yarns made with a blend of polyester/cellulose. The thicker fibers with lengths of >200 µm are presumably the basic material of sacks used for packaging agricultural products (Figure 8). Figure 9 demonstrates a relatively large (length ~800 µm and diameter 100–120 µm) polyamide particle. It is likely that this large fragment, as pseudofeces, was even present in the mantle cavity during the sampling.This type of MP contaminant was detected in only 2 of the 80 mussels investigated.

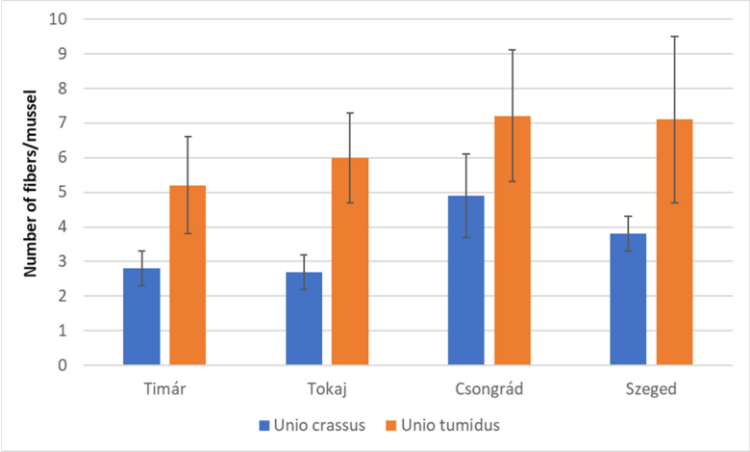

Figure 4: Number of fibers in mussels (n = 10) of Unio tumidus and Unio crassus collected at settlements ofTímár, Tokaj, Csongrád, and Szeged. The standard deviations are marked on the bars.

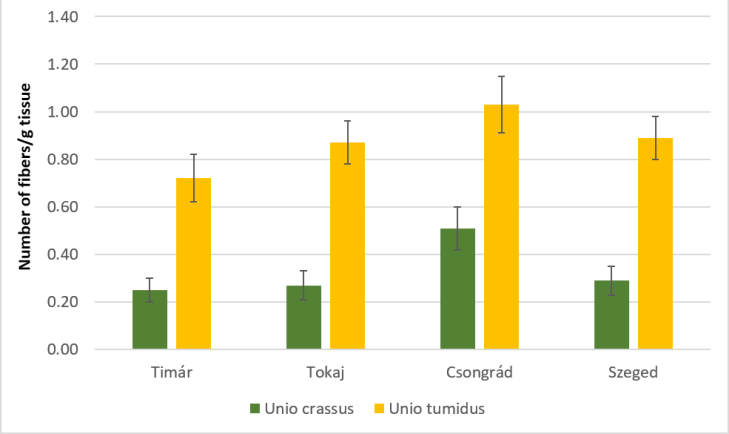

Figure 5: Number of fibers/g soft tissue (n = 10) of Unio tumidus and Unio crassus collected at settlements ofTímár, Tokaj, Csongrád, and Szeged. The standard deviations are marked on the bars.

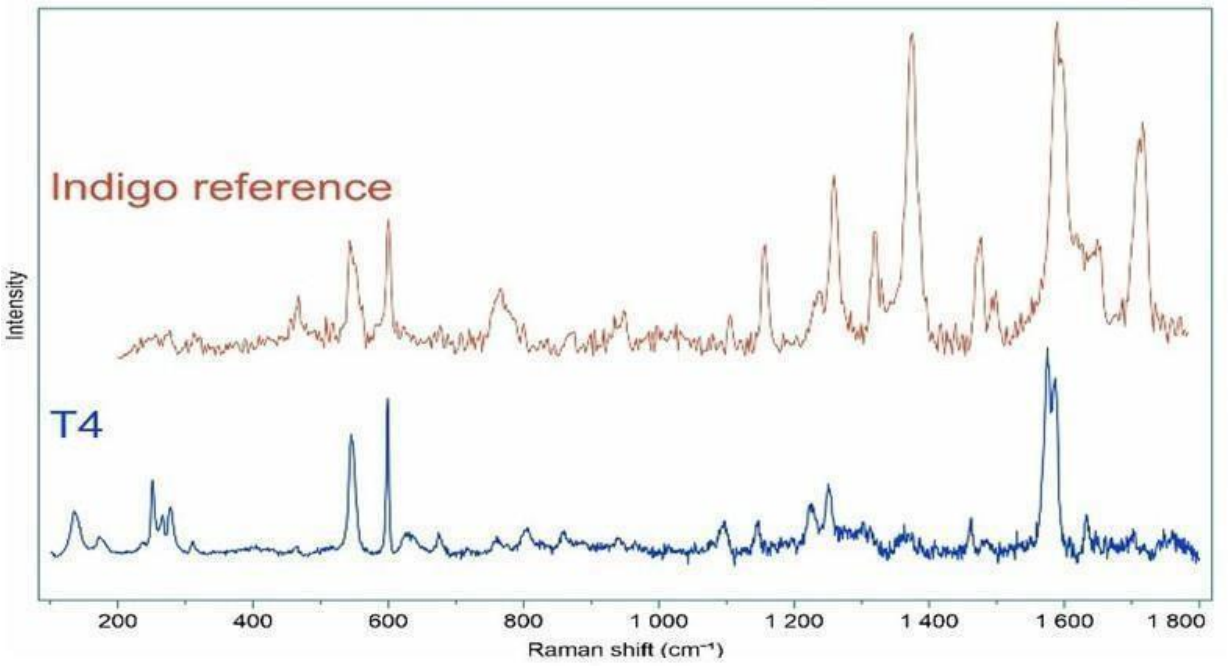

Figure 6: Stereomicroscopic picture and Raman spectrum of blue fiber found in Unio tumidus mussel collected at samplingsite Szeged and Raman spectrum of indigo dye reference material.

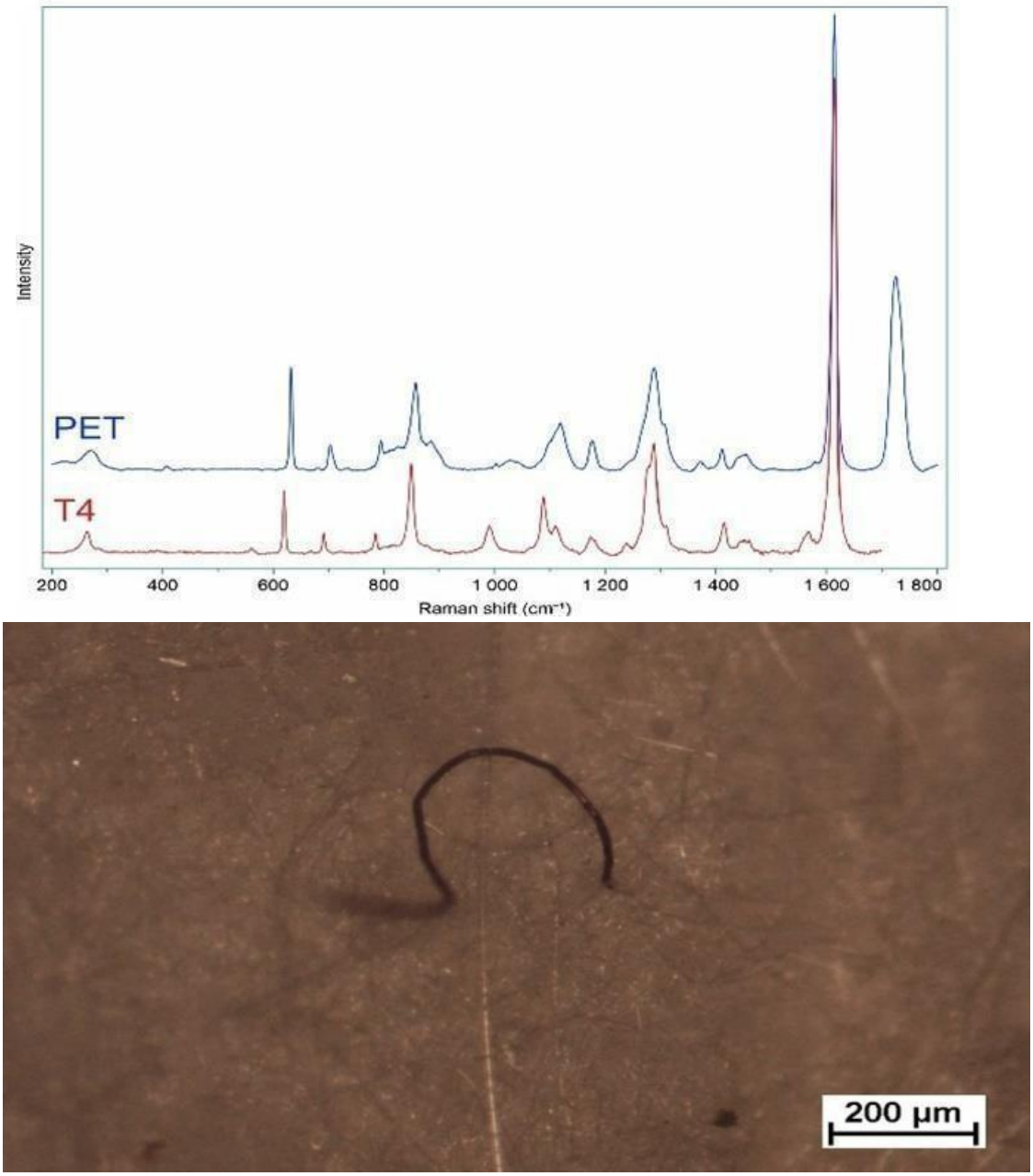

Figure 7: Stereomicroscopic picture and Raman spectrum of a fiber found in Unio tumidus mussel collected at sampling siteSzeged and Raman spectrum of PET reference material.

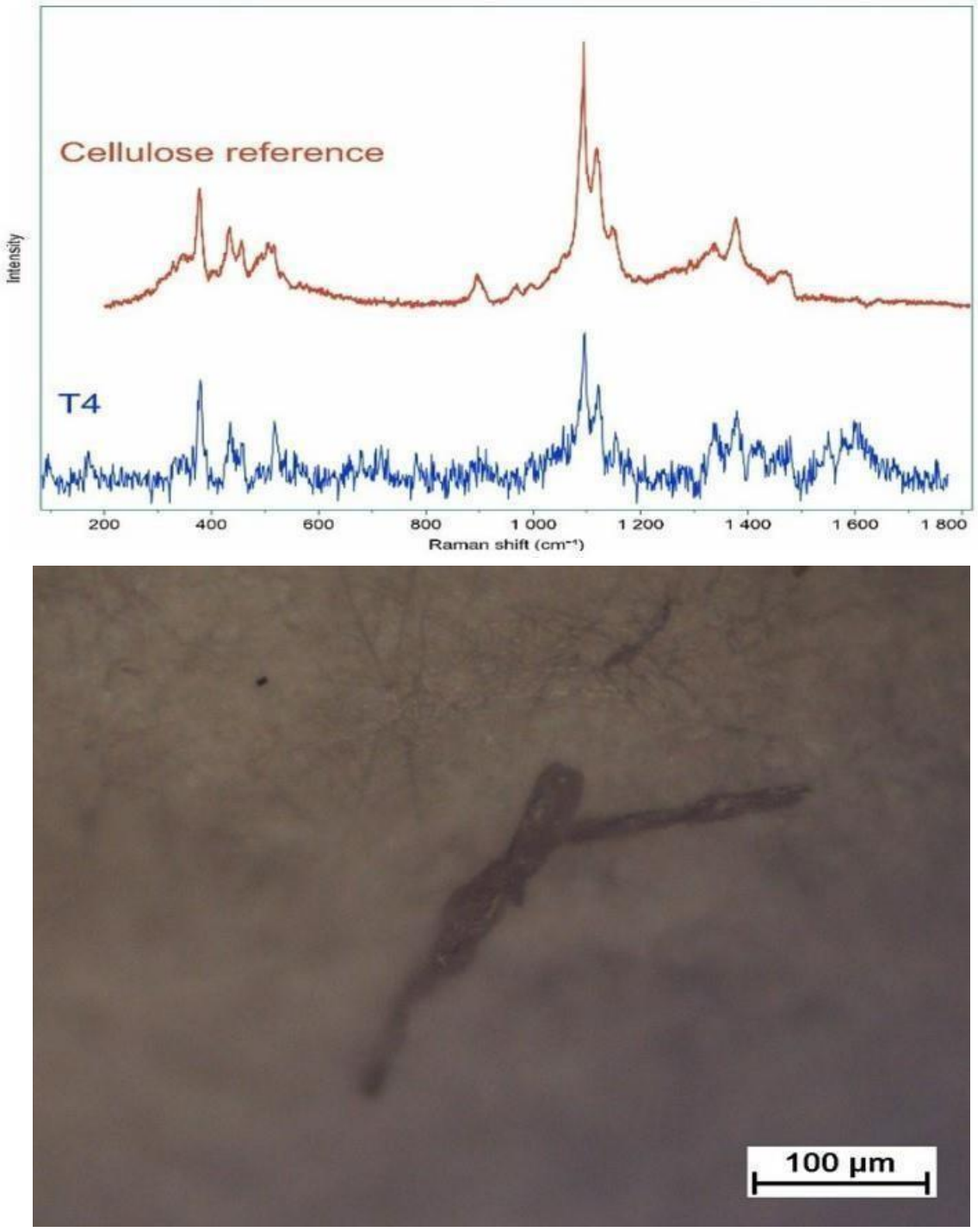

Figure 8: Stereomicroscopic picture and Raman spectrum of a thick fiber found in Unio tumidus mussel collected at sampling site Szeged and Raman spectrum of cellulose reference material.

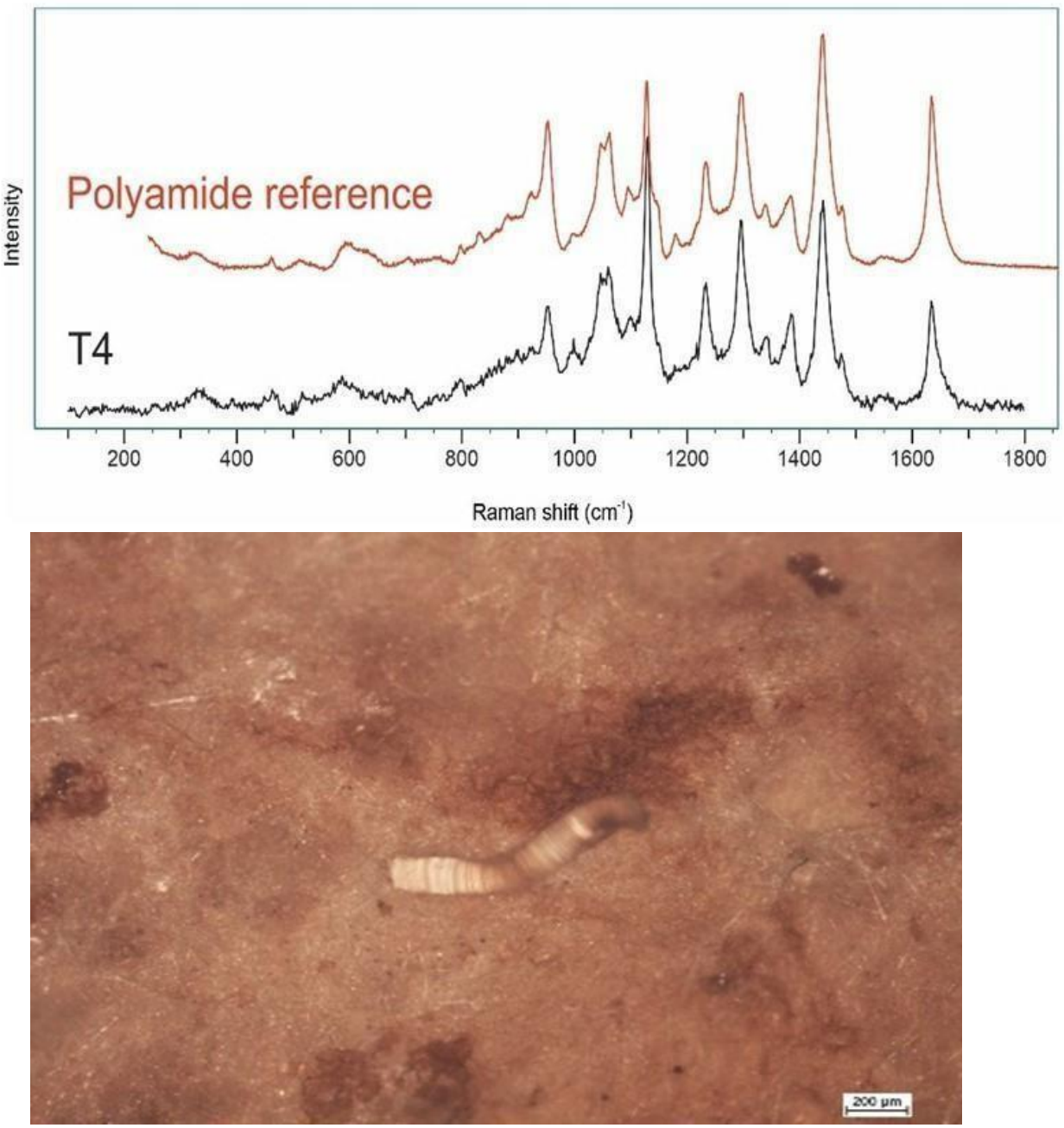

Figure 9: Stereomicroscopic picture and Raman spectrum of a fragment found in Unio tumidus mussel collected at sampling site Szeged and Raman spectrum of polyamide reference material.

3.4Discussion

The length of the shell depends on the age of the mussel species. In the majority of Unionoideans, the greatest shell growth occurs in immature individuals during the first 4–6 years of life. For example, if the shell length in the case of Unio tumidus amounts to 60, 70, or80 mm, the age of this mussel is about 4, 5, or 10 years (McMahon et al., 2001). This implies that the Unio tumidus mussels in our study with a shell length of 63–77 mm were about 4.5–5.5 years old. Comparing the wet mass of the soft tissues of these mussel species simultaneously collected at the same sampling sites, it can be established that in all the cases, the Unio crassus had 1.4–1.6 times higher soft tissue

mass than Unio tumidus. This can be explained by having a higher filtration rate (3.3–4.1 L/h) than Unio tumidus (2.1–2.4 L/h), which results in higher nutrient transport and the faster growth of organisms (Kryger & Riisgård,1988).

The anomaly in the soft tissue mass and particle accumulation might be explained by other varying factors, such as seasonality in growth, reproduction, and feeding behavior (filtration, rejection, and egestion) (T. Hoellein et al., 2021). Higher MP concentration in smaller-sized mussels was also reported in Dreissena polymorpha (Weber et al., 2021). The authors attributed this higher MP concentration to the fact that the higher relative feeding activity, higher relative pumping rates, and larger relative gill area of smaller mussels enable them to take up more MPs per body mass. The mussel condition index (mussel dry mass/shell length) provides a gross assessment of mussel health. The mussels near potential MP sources (urban rivers and treated wastewater) are expected to have lower condition indices than those found in other sites. It was reported that the condition index could be related to food availability or habitat conditions but was not related to the MP concentration in the mussels (T. Hoellein et al., 2021). Similarly, Wardlaw and Prosser (2020) also did not find a statistically significant relationship between the shell length and the MP abundance ($p = 0.09$) or between the soft tissue wet mass and MPs ($p > 0.5$) in the freshwater mussel Lasmigona costata (Wardlaw & Prosser, 2020). They concluded that there was not a discernible relationship between the size and length of mussels and the number of MPs in their study, which could be due to the low number of MPs. In contrast, Berglund et al. (2019) reported a positive correlation between the size of the mussel and the number of MP fibers accumulated by them. They explained that larger mussels filter larger volumes of water and, thus, accumulate more fibers (Berglund et al., 2019).

The measurement of MPs in mussels represents the internal exposure level of MPs to these organisms and can help in evaluating the ecological risks due to MP uptake (Su et al., 2018).

Comparing the literature data related to the riverine environment (Table 6) with our results, it can be established that the abundance of fibers and fragments in the mussels, clams, and oysters collected from different rivers changed in the range of 0–142 items/individual, while in our case, the calculated mean values using 10 samples from each sampling site amounted to 2.7–

4.9 and 5.2–8.3 items/individual for Unio crassus and Unio tumidus, respectively. These values were obtained after subtracting the procedure of blank samples (0.45 items/individual). A few other studies have reported the MP abundance in mussels in

relation to the mass of the mussels: 0–0.16 particles/g wet weight in Lasmigona costata, 0.3–4.9 MP/g wet weight in Asian clams (Corbicula fluminea) (Su et al., 2018), 1.5–7.2 items/g wet weight in the oyster Saccostrea cucullate (H. X. Li et al., 2018), and 0.35–0.38 fibers/g wet weight in Mytilus sp. (Vandermeersch et al., 2015). Our data changed in the range of 0.25–0.51 and 0.72–1.03 items/g soft tissue for Unio crassus and Unio tumidus, respectively. However, in the literature, there are no experimental data on the comparison of the stored amount of MPs in the different mussel species grown under the same environmental conditions. Schessl et al. (2019) conducted an experiment to study the microbead content in Dreissena polymorpha and Dreissena bugensis grown in the littoral zone of the Upper St. Lawrence River in the USA; however, they did not detect any MP particles in the mussels. Their results were explained by the relatively small size of the collected mussels (an average length of 17.33 mm) and the limited ingestion capability of these organisms (Schessl et al., 2019).

When evaluating the fiber content of the mussels in the direction of flow from Tímár to Szeged, only a slow increment in fiber concentration could be observed, except for the sampling site at Csongrád (Table 7), where the stream velocity was extremely low (less than 0.1 m/s) in a small bay, and the bottom of the bed was muddy. If we compare the data obtained from the sampling sites Tímár and Szeged, where the hydrological conditions were similar, it can be established that, due to the transport of contaminants between the tributaries (Bodrog, Sajó, Zagyva, Körös, Maros), along the 392 km section of the Tisza River, the number of fibers/g soft tissue values increased by 35% in both mussels species. Our observations are in agreement with the experimental data of Kiss et al. (2021), who observed about 20% higher MP amount in the sediments of these tributaries compared with the amount found in the main river (Kiss et al., 2021).

The frequent presence and predominance of fibers in mussels (Berglund et al., 2019; H. X. Li et al., 2018; Su et al., 2018) are not surprising if we consider that fibers make up to 64–100% of MP contaminants in the water phase of several rivers, e.g., Ciwalengke (Alam et al., 2019), Yellow (Han et al., 2020), and Antuã (Rodrigues et al., 2018). The high occurrence of fibers in mussels could be due to mussel ecology and the high amount of fibers discharged into the rivers from wastewater treatment plants. Microfibers primarily arise from washing clothes. De Falco et al. (2019) demonstrated that the microfibers released during washing range from 124 to 308 mg kg^{-1} of the washed fabric, and depending on the type of washed garment, it would correspond to a number of microfibers ranging between 640,000 and 1,500,000. The most abundant fraction of the microfibers shed was retained by filters with a pore size of 60 µm and

presented an average length of 360–660 µm and an average diameter of 12–16 µm (De Falco et al., 2019). The fibers, particularly the smaller ones, are not removed by the currently available wastewater treatment technologies and, thus, accumulate in the aquatic environment(Berglund et al., 2019; H. X. Li et al., 2018; Su et al., 2018).

Similar to our observations, the blue-colored fibers were predominant in the bivalves collected from St. John and Yangtze rivers (Doucet et al., 2021; Su et al., 2018). However, in other studies, the white- colored, light-colored, and transparent fibers were also dominant (T. Hoellein et al., 2021; H. X. Li et al., 2018; J. Li et al., 2018b). Since mussels are not able to selectively uptake fibers based on color, the observed proportion of colors likely represents a real picture of the proportion of dyed fibers in the ingestible size range. However, it is worth noting that the dyes affect the surface properties of the fibers and, thus, the biofilm formation on their surface. This can increase the specific gravity, thus causing a change in the depth distribution of the fibers in the water body of rivers. A debatable idea was put forward by Berglund et al. (2019) that mussels could have a color preference for food, and the color distribution of MPs in mussels could be a result of both the color preference of the mussels andthe dominant color in the water (Berglund et al., 2019). The color of the MPs ingested by mussels was also reported to be dependent on the season. In autumn and summer, the bivalvesingested more transparent MPs, while in winter, they ingested more blue MPs, and in spring, they contained an equal amount of both MPs (J. Ding et al., 2021).

The rejection of larger particles was demonstrated by Ward et al. (2019) in the mussel Mytilus edulis, where the mussel rejected a lower number of 19–113 µm sized MP spheres and a significantly higher number of 1000 µm sized MP spheres (Ward, Zhao, et al., 2019). The proportion of the MP spheres rejected in pseudofeces increased with an increase in the sphere size, while for the fibers, the rejection was variable and displayed no trend with regard to size.The ability of mussels to size-select is due to the presence of two digestive paths (intestinal andglandular path) and the microstructures in their digestive tract. The immediate bulk egestion oflarge MPs could occur through the intestinal path, while the longer retention of smaller MPs (1–10 µm) could occur through the glandular path (Kinjo et al., 2019).

Based on our observations, it can be established that, under the environmental conditions of theTisza River, Unio tumidus is a more efficient sample than Unio crassus to characterize the fibercontamination of rivers and follow their concentration changes during long-term monitoring.

Chapter 4. Particle-based nutrients and metal contaminants in the

habitat of Unionidae mussels in the Tisza River (Hungary)

4.1 Background

The freshwater mussels of the family Unionidae play a dominant role in freshwater ecosystems due to their suspension-feeding, waste excretion, and burrowing activities, as outlined by (Goldsmith et al., 2021). These mussels, which are partially embedded in the bottom sediments ingest organic nutrients, minerals, and MP particles from both the continuously flowing near bottom suspended (NBS) sediments and the bottom sediments that have been resuspended by their burrowing. It implies a continuous interaction between the mussels and sediments in the benthic habitat. For instance, sediment impacts on mussels cause smothering, a drop in fish abundance, and decline in feeding and respiration. The increased concentration of suspended sediments could have a deleterious effect on mussel growth, survival, and reproduction, which could eventually result in changes in diversity (Brim Box & Mossa, 1999). On the other hand, mussels-mediated nutrient dynamics, biodeposition, and bioturbation can alter the chemical composition and properties of sediments (Haag, 2012; T. J. 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).

In order to characterize the habitats of mussels in rivers, it is necessary to investigate the physical and chemical properties of the water phase, the NBS sediment, and deposited (bottom) sediments. However, it should be noted that there is a dynamic feedback interaction between the river bed, flow, and mobile sediments (Shu et al., 2020). Despite the fact that there is limited possibility to uptake dissolved organic carbon and trace elements from the water phase, as was demonstrated by (Roditi et al., 2000) in Zebra mussels, the primary process of nutrition is based on the ingestion of suspended organic particles (Pan & Wang, 2004).

To evaluate the nutrient supply of bottom-dwelling species, mostly the total organic carbon (TOC), total nitrogen (TN), total phosphorous (TP), as well as the TOC/TN concentration or molar ratios were measured in the bottom and suspended sediments. The majority of published data are on bottom sediments since sampling surface sediments using grab samplers or augers is a relatively straightforward process

compared to sampling suspended sediments (Table 8).

Table 8. Published TOC, TN and TP concentrations and C/N ratios determined in surface sediments of river

River	TOC (%)	TN (%)	TP (%)	C/N ratio	Reference
Orinoco, Venezuela	0.84-9.06	0.08-1.45	-	9.1	(Paolini, 1995)
Cedar-Ortega, USA	2.3-22.6	-	-	-	(Ouyang et al., 2006)
Odra, Poland	0.14-17.6	-	-	-	(Niemirycz et al., 2006)
Beijing, China	0.8-1.29	-	-	-	Chen et al., 2009
Danube, Serbia	0.2-2.82	-	-	-	(Relić et al., 2011)
Tigirs, Turkey	-	0.08	0.12		(Varol & Şen, 2012)
Pinang, Malaysia	2.16-5.33	-	-	-	(Ong Meng Chuan et al.,2016)
10 river basins, China	-	0.11	0.07	-	(Y. Yang et al., 2017)
Lower Lancang, China	0.9	0.09	0.05	10.0	(H. Lu et al., 2018)
Shenzen, China	1.7	0.19	0.16	8.9	(Wijesiri et al., 2019)
Betwa, India	-	-	-	0.9-77	(Venkatesh & Anshumali, 2020)
Jegricka, Serbia	-	0.46	0.09	-	(Savic et al., 2021)
Xiashan, China	-	0.5	0.04	-	(W. Li et al., 2020)
Serinhaem, Brasil	0.4-7.9	0.1-0.34	-	7.7-37	(Carneiro et al., 2021)
Warta, Poland	0.2-21.5	0.07-16.2	0.05-0.88	4.5-111	(Fiedler, 2021)
Vistula, Poland	1.44	0.13	-	11.5	(Kobierski & Banach-Szott, 2022)
Yangtze, China	3.12-6.43	-	-	-	(S. Zhang et al., 2020)
Red River, Vietnam	0.43 ± 0.31	0.09 ± 0.04	0.06 ± 0.02	4.77	(Le et al., 2022)
min-max range	0.14-22.6	0.07-16.2	0.04-0.88	0.9-111	

On the basis of these selected literature data, it can be established that in the surface layer of bottom sediments in riverine environment, the concentrations of TOC, TN, and TP, as well as the TOC/TN ratio, changed in a relatively wide range of 0.14%–22%, 0.07%–1.45%, 0.04%– 0.88%, and 0.9–111, respectively. At this point, it should be stressed that these nutrient concentration measurements only describe the surface sediments at the time of sampling, and we must estimate any short- or long-term changes brought on by physical and chemical (degradation) processes (Dalu et al., 2019; Islam et al., 2019).

In the case of suspended sediment (suspended particulate matter, SPM), which include both living (algae, bacteria) and non-living (e.g., inorganic minerals and organic detritus) compounds, we must take into account that their depth profiles in the rivers change continuously based on the hydrologic characteristics, rainfall or storm events, watershed pattern, vegetation, hydraulic conditions, physical characteristics (size, shape, and density) and hydrodynamic behaviour of particles(Kumar et al., 2021). Since the sampling procedure for suspended sediments is more sophisticated than for bottom sediments, it is challenging to receive reliable concentration data on depth profiles of nutrient elements and contaminants in rivers using different sampling devices and sampling strategies. Nevertheless, despite the

measurement uncertainty of element concentrations, the C/N concentration ratios provide reliable information on the origin of organic compounds. The potential sources of organic materials include (1) living or dead algae and bacteria, (2) degraded soil organic matter, and (3) plant materials.

In addition to the transport of nutrients, it is also important to study the sediment-associated transport of essential and toxic metals across the mussel habitat. Since all particles are blanketed with biofilms (Mages et al., 2004; Prieto et al., 2016), the metal ions can be immobilized in the extracellular polymer matrix by a variety of mechanisms, including biosorption, precipitation as sulfides or phosphates, and reductive microbial precipitation (van Hullebusch et al., 2003). The biofortification factor of biofilms formed on artificial substrata inthe Tisza River varied in the range of 103 and 105 for different heavy metals (Kröpfl et al., 2006). Because of these aforementioned processes in the biofilm layer, it is anticipated that theconcentration of metals and metalloids will be higher in the finer suspended sediments due to decreasing grain size and increasing specific surface area of particles, as compared to the deposited bottom sediments. The relationship between the physico-chemical properties of sediments and their metal content was studied by (Dendievel et al., 2022) in seven Western European Rivers. On the basis of a dataset combining long-time monitoring and scientific data (>12,000 samples), they quantified the influence of key factors (sediment matrix type, TOC content, grain size distribution, fractionation, location, and time) on the metal content of sediments.

Some characteristic pseudo-total concentrations of the most widely measured metal contaminants (Zn, Cd, Pb, Ni, Cu, and Cr) in river surface sediments are listed in Table 9. Their concentrations vary by many orders of magnitude in the range of 0.1–1,000 mg/kg, depending on lithogenic and anthropogenic sources, textural traits, organic matter contents, mineralogicalcomposition, and depositional environment of sediment.

Table 9. Pseudo total and total concentrations (mg/kg) in river surface sediments determined after acidic extraction with aqua regia and total digestion with mixture of HNO_3+ HCl +HF, respectively, using flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectroscopy (GF-AAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES) or inductively coupled plasma mass spectroscopy (ICP-MS) techniques

Study area	Thicknss of sampled layer	Sampling tool	Sample preparation	Analytical method	Zn	Cd	Pb	Ni	Cu	Cr	Reference
Pearl River, China	0-5 cm	Plastic spoo n	H_2O_2, HCl	ICP-MS and XRF	136	0.84	44.6	-	43.0	55.2	(G. Zhao et al., 2018)
Danube River, Central and Western Europe	-	Grab sampler or manual dredging	HNO_3	ICP-AES	83-622	1.10-25.9	14.7-108	24.6-143	31.3-663	35.3-139	(Woitke et al., 2003)
Tinto River, Spain	Core samples	Auger	HNO_3 +$HClO_4$	AAS	901	2.7	2330	17	805	56	(Morillo et al., 2002)
Yangtze River, China	0-5 cm	-	HNO_3+HF	ICP-MS	82.9	0.2	23.8	31.9	24.7	79.1	(H. Wang et al., 2015)
Danube River, Romania	0-10 cm	Stainless steel scoop	Aqua regia	AAS	32.1-207	0.1-1.3	0.42-77.7	11.9-93.5	7.24-86.5	4.09-68.2	(Ilie et al., 2016)
Danube River, Hungary	0-10 cm	Hand augerand plastic scoops	Aqua regia	AAS	186-388	< 0.02-1	12.9-32.2	23.4-43.0	22.1-39.4	20.4-63.4	(Nagy A. Szabó et al., 2018)
Tigris River, Turkey	0-5 cm	Sediment core sampler	Aqua regia	AAS	149-1061	0.77-7.90	146-660	122-534	98.7-2860	72.1-158	(Varol & Şen, 2012)
Odra River, Poland	Surface layer	-	Aquaregia	ICP-MS and AAS	1055	8.47	113.3	51	99.3	64.7	(Gielar et al., 2012)
Someșu l Mic River, Romania	0-20 cm	Stainless steel shovel	Aqua regia	AAS	97.7	0.16	35.5	26.6	-	-	(Barhoumi et al., 2019)
St. Lawrence RiverHarbor, Canada	0-10 cm	Birge-Ekman grab sampler	Aqua regia	ICP-MS	306	0.8	58.2	42.9	108	68.5	(Pourabadeh ei & Mulligan, 2016)
naíba River, Brazil	Surface layer and 50-95 cm	Van Veen grab sampler, manual piston corer and acrylic tubes	Aqua regia	AAS	13.4	-	5.9	-	6.8	18.0	(de Paul Filho et al 2015

Siete River, Ecuador	5-10 cm	Peterson grab sampler	Aquaregia	ICP-MS	133	0.73	20.3	596 1	484	-	(Pesantes et al., 2019)
Szamos and TiszaRiver, Hungary	5-10 cm	Gravity corer	HNO_3 +$HClO_4$ +HF	ICP-OES and ICP-MS	320-3095	1.9-23	63-374	55-85	54-664	98-159	(Kraft et al., 2006)
Indus River, Pakistan	0-10 cm	Hand auge r	HNO_3 +$HClO_4$ +HF	AAS	-	1.41	47.3	128	71.7	90.6	(Usman et al., 2021)
15 Rivers, Serbia	Bottom sediment	Van Veen grab sampler	HNO_3+HCl + HF	ICP-OES	66.7-1095	1.28 - 10.5	57.8 - 318	33.2-274	11.5 - 870	59.8-230	(Sakan et al., 2015)
Sava River, Centraland Southeast Europe	0-15 cm	Piston corer	HNO_3+HCl + HF	ICP-MS	50-300	0.2-1.0	10-120	20-200	10-50	20-250	(Vidmar et al., 2017)
3 Rivers, Central and Southeast Europe	0-10 cm	-	HNO_3+HCl + HF	ICP-MS	50-200	-	20-30	20-30	20-40	20-40	(Rügner et al., 2019)

In this work, our objectives were (1) to determine the physical and chemical properties of NBS and bottom sediments collected at four locations along the Tisza River within the habitats of Unionidae mussels (Unio tumidus, Unio crassus, Unio pictorum), (2) to investigate therelationships between the grain size of sediments and concentrations of nutrient elements and metal contaminants (3) to identify the main sources of nutrient elements on basis of C/N ratios determined in sediments (4) to evaluate the potential risk of metal contaminants for mussels as bottom-dwelling animals considering the Concensus-Based Sediment Quality Guidelines (MacDonald et al., 2000).

4.2 Materials and methods

4.2.1 Sampling sites and techniques

The Tisza River, which originates in the Bukovina segment of the Carpathian Mountains, is themain tributary of the middle Danube River. Its length and drainage area are 966 km and 157,186 sq. km, respectively. Along the Hungarian section of the Tisza River, four sampling sites at the settlements of Tímár, Tokaj, Csongrád, and Szeged, which are located at flow km of 552, 544,244, 160, respectively, were selected (Figure 3). 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).

Although this sampling method does not produce an isokinetic sample, it is more accurate when sediments are fine (<63 µm) and flows are turbulent. Van Veen grab sampler was used for sampling bottom sediments, allowing samples to be collected from a nearly 10 cm thick surface layer of bottom sediments. About 1 kg of sample was collected at each sampling point and transferred into plastic bags with zip-fastener. The suspension and sediment samples were stored on ice during transport to the laboratory.

For the characterization of the water phase, the water was measured on-site using a portable Ponsel-ODEON water quality meter (Fondriest Environmental, Ohio, United States). During the campaign, the flow velocity was measured by a rotating current meter (Global Water Flow Probe FP 211, Xylem, United States). The concentrations of selected anions, cations, as well as total organic C and N of water samples obtained after filtration of NBS sediments were determined by applying DIONEX 5000 ICS + dual channel ionchromatograph (Thermo Scientific, Massachusetts, United States) and a MULTI N/C 3100 TC/TN analyzer (Analytik Jena, Jena, Germany), respectively. The elemental concentrations were quantified using an inductively coupled plasma mass spectrometer (Analytik Jena, Jena, Germany). These parameters are listed in (Tables 10, 11).

Table 10. Physico-chemical properties of Tisza River at the selected four sampling sites

	Date	Sampling point	Temperature (°C)	pH	Redox potential (mV)	Dissolved oxygen (mg/L)	Oxygen saturation (%)	Electric conductivity (µS/cm)	Turbidity (NTU)
1	04/08/2021	Tímár	25.70	8.36	79.8	10.60	131	435	45.8
2	04/08/2021	Tokaj	25.95	8.48	81.0	11.34	160	428	55.3
3	05/08/2021	Csongrád	25.40	8.20	91.8	7.89	97.9	415	210
4	05/08/2021	Szeged	26.37	8.00	142	7.63	96.1	442	69.2

Table 11. Concentration of major cations and anions infiltrated water of Tisza River at the selected four samplingsites

	Tímár	Tokaj	Csongrád	Szeged
Na^+ (mg/L)	31	30	26	28
NH_4^+(mg/L)	<0.1	<0.1	<0.1	<0.1
K^+ (mg/L)	<1	<1	<1	<1
Mg^{2+} (mg/L)	<1	<1	<1	<1
Ca^{2+}(mg/L)	46	56	54	53
F^- (mg/L)	0.5	0.5	0.5	0.5
Cl^- (mg/L)	40	39	32	34
SO_4^{2-} (mg/L)	28	31	35	37
NO_3^- (mg/L)	1.0	2.7	3.5	4.0
NO_2^- (mg/L)	0.02	0.01	0.02	0.01

4.2.2 Chemicals and reagents

A WasserLab Automatic unit (Labsystem Ltd., Budapest, Hungary) was used for generating ultrapure water (resistivity of 18.2 MΩ cm−1). Nitric, hydrochloric, and sulphuric acids, hydrogen peroxide, and solid natrium hydroxide of analytical grade were purchased from VWR International Ltd. (Debrecen, Hungary). The internal and multi-elemental standard solutions were produced by Sigma-Aldrich Ltd. (Missouri, United States).

4.2.3 Determination of total suspended solids

The suspension 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 total suspended solids (TSS).

4.2.4 Determination of grain size distribution

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 cm^3 ultrapure water and subjected to 5 min of ultrasonic treatment at a frequency of 32 kHz. The cumulative and differential distribution functions were determined with the use of a Shimadzu SALD-2300 laser diffraction particle size analyzer (Shimadzu, Kyoto, Japan).

4.2.5 Chemical characterization of sediment samples

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 cm^3 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). The operating conditions are listed in Table 12. To characterize the reliability of this analytical procedure a recovery test was carried out for8 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 rangeof 82% and 114%.

Table 12. Operating conditions of ICP-MS system for elemental analysis of extracts obtained from sediment

Plasma power	1290 W
Outer gas (Ar)	7.5 L/min
Intermediate gas (Ar)	1.5 L/min
Aerosol carrier gas (Ar)	1.0 L/min
Reaction gas (He)	90 mL/min
Reaction gas (H_2)	110 mL/min
Sample uptake	0.30 mL/min
Nebulizer	Meinhard
Spray chamber	Double pass
Sampler cone	Ni. 1.1 mm orifice
Skimmer cone	Ni. 0.5 mm orifice
Analytical isotopes	^{7}Li; ^{27}Al; ^{52}Cr; ^{55}Mn; ^{56}Fe; ^{59}Co; ^{60}Ni; ^{65}Cu; ^{66}Zn; ^{75}As; ^{114}Cd; ^{118}Sn; ^{202}Hg; ^{208}Pb
Internal standards	^{45}Sc; ^{89}Y; ^{115}In

Data acquisition	Peak jumping
Dwell time	30 ms
Replicates	5×20

4.2.6 Statistical methods

Data were visualized using R statistical software (R Core Team, 2019). The relationship between TOC and metal/metalloid concentrations was examined by Person's correlation after checking the assumption of the normal distribution. Figures 10 A–D were drawn with the tkplotfunction of the "igraph" package (Csardi & Nepusz, 2006) using the Pearson's correlation coefficients and p-values as input data.

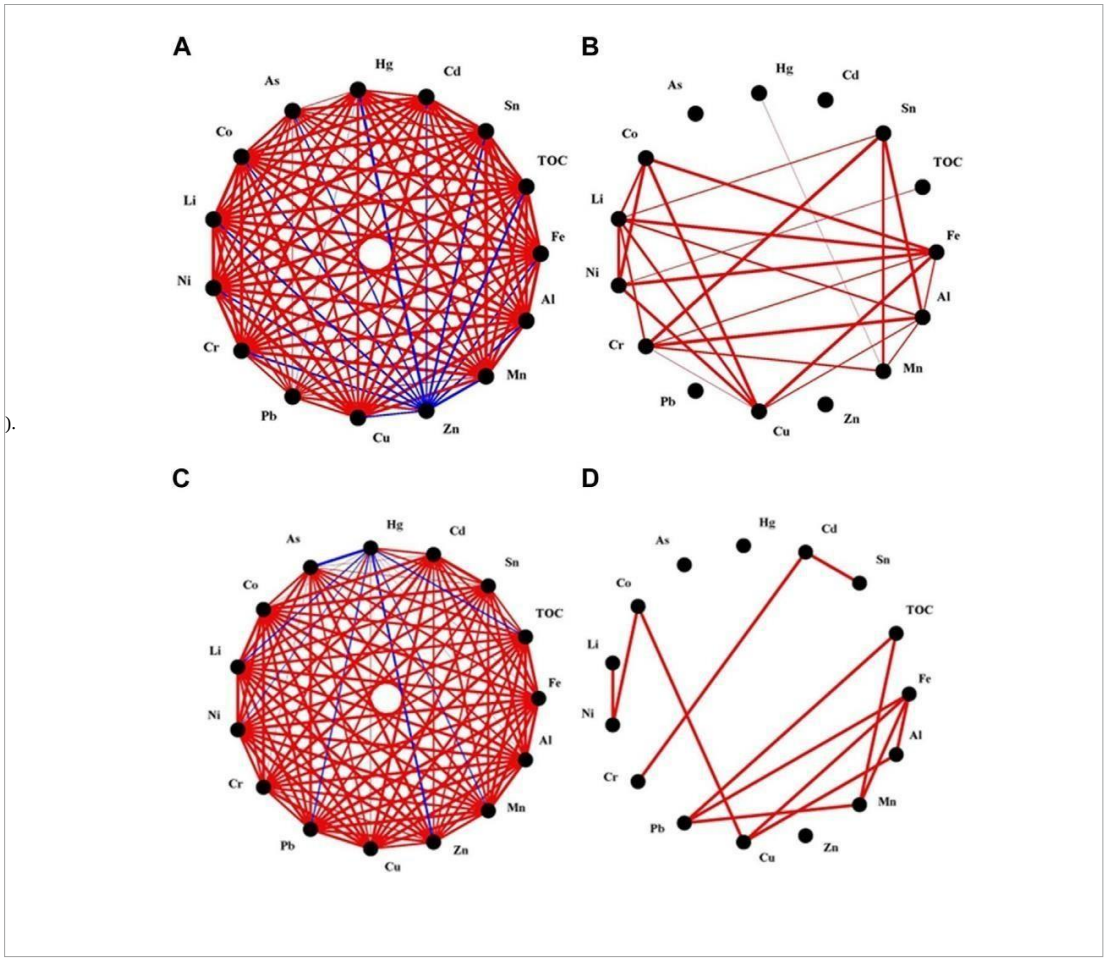

Figure10: Network representation of the relationships between the concentrations of elements investigated andTOC in the NBS (A,B) and bottom (C,D) sediments. Elements appear as nodes, while the connectinglines between them represent the correlations. In subfigures (A,C), all correlations are shown, whilein subfigures (B,D), only the significant correlations ($p < 0.05$) are depicted. The colours of the linksindicate the direction of the relationship: red linksdenote positive correlations while blue links signifynegative correlations. The width of the links is correlated with the strength of the correlation (i.e., the absolute values of the Pearson's correlation coefficients).

4.3 Results and discussion

4.3.1 Physical characterization of sediments

The mass concentration of NBS sediment particles fluctuated between 209 and 274 mg/L. These values are similar to TSS concentrations of 70–247 mg/L measured at middle depth in the Yellow River, however, considerably lower than the TSS concentrations in the 2:1:1 ratio mixed subsurface, middle and near-bed water samples of Yangtze River (X. Wang et al., 2012). The grain size distribution functions of suspended and bottom sediments originating from the same sampling site are depicted in Figures 11 A–D. The bottom sediments can be characterized by a high maximum value within the typical sand size range of 60–500 µm, while the NBS- sediments primarily consist of silt (size range 2–60 µm). In its grain size distribution functions, three different grain size groups can be observed, signifying the existence of particles originating from various sources. In all NBS sediments the maximum values are observable approximately at particle sizes of 6, 20, and 60 µm. Considering the particle capture efficiencyof mussels, even this size range is important for the nutrient supply of Unionidae mussels (Kryger & Riisgård, 1988). However, (Kryger & Riisgård, 1988), concluded in their paper that particle quality rather than size is what modulates suspension feeding in turbid waters. In the typical clay grain size range (<2 µm), particles were not detectable in the bottom sediments collected at Tokaj and Szeged. This phenomenon can be explained by the hydrodynamic sorting of the rivers (higher stream velocities at these sampling points (0.25 vs. 0.1 m/s). However, in all NBS sediment samples, around 5%–10% of the particles were found to be in this size range. In conclusion, silt predominates among the NBS sediments investigated, which is consistent with the finding made by (Bouchez et al., 2011) in the instance of the Amazon River.

It should be noted that our data on grain size measurements do not accurately reflect the grain size distributions that originally existed in the mussel habitats. This is because the cohesive suspended sediments are transported primarily as flocculated material (Deng et al., 2019; Droppo, 2001), but the flocs were damaged during the sample preparation by drying and resuspension of solid particles applying ultrasonic treatment before the grain size measurements. It implies that the effect of flocculation processes governed by extracellular polymeric substances (B. J. Lee et al., 2017) is partly eliminated. In order to avoid these undesirable disturbances and to obtain a true picture of the particle sizes of suspended

sediments, it would be necessary to apply in-situ methods (e.g., submersible particle size analyzer and submersible digital holographic camera), as was demonstrated by

(Safar et al., 2022). In the absence of such devices during our investigations, the suspended particles were collected and analysed in the conventional manner described in subchapters 4.2.1 and 4.2.4.

4.3.2 Chemical characterization of sediments

4.3.2.1 Study of nutrient elements

The suspension-feeder mussels can take the main nutrients (C, N, P) from three different sources: (1) particulate organic matter, e.g., algae, plant residues, bacteria, (2) biofilms formedon the surface of all inorganic and organic particles existing in the water, (3) dissolved organiccompounds from the water phase. This third source plays only a negligible role in the supply of nutrients, as it was established by Roditi et al. (2000) in case of Zebra mussels. However, the biofilms containing microbial cells and extracellular polymeric substances (polysaccharides, lipids, nucleic acids, etc.) serve as a well utilized source of nutrients. The chemical investigations were focused on the determination of nutrients (TOC, TN, and TP) andthe C:N:P molar ratio. The measured concentrations of these three nutrient elements in the NBS and the bottom sediments are listed in Table 13. It can be established that the TOC concentrations in the NBS sediments were higher by a factor of 1.7–3.4, compared to the bottom ones. An exception was the sampling site Csongrád, where the river's velocity was extremely low (≤0.1 m/s) in a small muddy bay. It should be noted that the TOC values of bottom sediments were consistent across all sampling sites and only varied in a narrow concentration range of 9.1–10.6 mg/g.

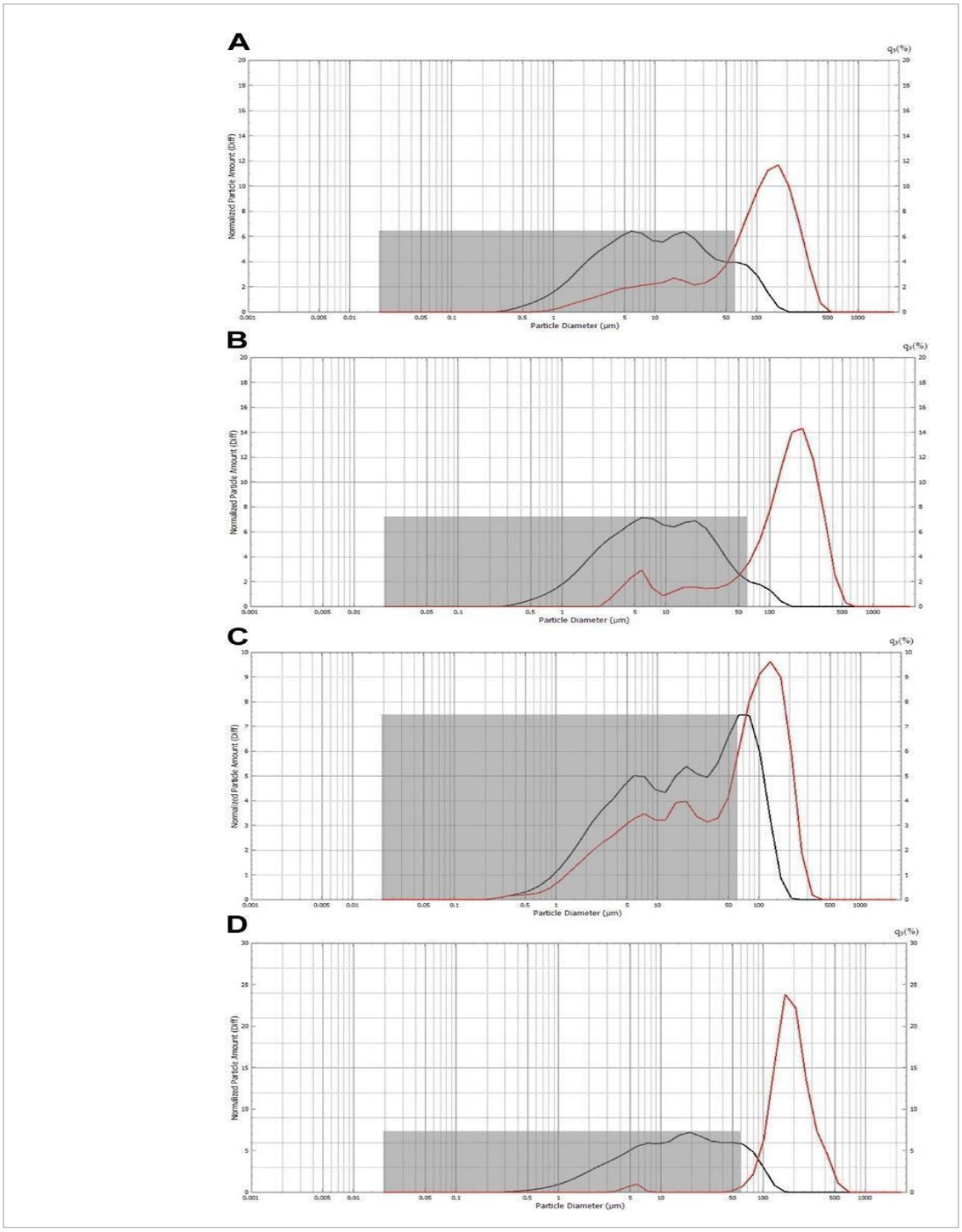

Figure 11: Grain size distribution of near-bottom suspended sediments (black) and bottom sediments(red) collected at sampling sites Tímár (A) Tokaj (B), Csongrád (C), and Szeged (D).

In the case of TN and TP content, the tendency of concentration fluctuations between the NBS and bottom sediments is similar to the TOC. The highest TOC and TN values were measured at the sampling site Tokaj, below the mouth of the tributary Bodrog, whereas the highest TP values were recorded at Szeged, below the mouth of the tributary Maros. Summarizing, it can be stated that the nutrient concentrations in the Tisza River's NBS sediments at sampling siteswith flow rates of 0.1–0.25 m/s are higher than those in the bottom sediments.

The C/N molar ratios were calculated (Table 14) to provide information on the bacterial-algal, soil-derived, or vascular land- plant origins of sedimentary organic matter. In reality, organic molecules from all three sources can participate in the formation of the current C/N values, albeit in varying proportions. Bacteria and algae typically have C/N molar ratios between 4 and 7, soil-derived organic compounds have between 8 and 20, and vascular land plants have 20 or greater (Meyers, 1994; Tyson, 1995). This distinction arises from the absence of cellulose in bacteria and algae and its abundance in vascular plants and the consequent relative richness of proteinaceous material in the aforementioned microorganisms. Since the C/N molar ratios in the NBS sediments and bottom sediments varied in the range of 13.7–18 and 13.1–16.3, respectively, it can be stated that the soil- derived organics are the dominant sources of organic matter in these samples. Similar C/N values (10–12) were published for Amazon (John I. Hedges et al., 1986), St. Lawrence (Pocklington & Tan, 1987), Yellow and Yangtze Rivers (X. Wang et al., 2012) as well as for rivers in Central United States (Onstad et al., 2000). Our higherratios can be attributed to the near-bottom sampling, while these literature data are related to surface or middle depth water samples.

The C/P and N/P molar ratios were also higher in the NBS sediments, with the exception of themuddy bay at sampling site Csongrád, and their highest values were also detected at sampling site Tokaj. This indicates that the highest nutrient supply for the suspension-feeder mussels is available at this settlement below the inflow of the tributary Bodrog.

Table 13. Total organic carbon (TOC), total nitrogen (TN), and total phosphorous (TP) concentrations of NBS and bottom sediments (BS) in the Tisza River.

Sampling site	Sediment type	TOC (mg/g)	TN (mg/g)	TP (mg/g)
Tímár	NBS	17.3 + 1.4	1.11 + 0.22	0.70 + 0.05
	BS	10.2 + 0.9	0.76 + 0.12	0.49 + 0.04
Tokaj	NBS	26.3 + 2.2	2.08 + 0.31	0.59 + 0.02
	BS	9.1 + 0.8	0.65 + 0.13	0.39 + 0.02
Csongrád	NBS	8.8 + 0.7	0.66 + 0.08	0.51 + 0.03
	BS	9.4 + 0.8	0.83 + 0.05	0.47 + 0.03
Szeged	NBS	16.2 + 1.7	1.37 + 0.18	0.98 + 0.07
	BS	10.6 + 1.1	0.75 + 0.04	0.68 + 0.05

Table 14. Molar ratios of nutrient elements in the near-bottom suspended sediment (NBS) and bottom sediments (BS) of the Tisza River.

Sampling site	Sediment type	C/N	C/P	N/P
Tímár	NBS	18.0	63.0	3.63
	BS	15.6	53.1	3.12
Tokaj	NBS	14.7	113	7.36
	BS	16.3	59.4	3.63
Csongrád	NBS	15.0	40.8	2.54
	BS	13.1	51.0	4.02
Szeged	NBS	13.7	42.2	3.03
	BS	15.7	39.5	2.42

4.3.2.2 Study of metal contaminants

The various metal and (As) contaminants (Table 15) were typically present in higher concentrations in the NBS sediments at Timár, Tokaj, and Csongrád than in the bottom sediments, with exception of Zn in samples collected at Tokaj. This metal is likely stored in the bottom sediments as a result of a former Zn contamination delivered by the tributary Bodrog. In the case of sediments collected at Szeged, a tributary effect of the Maros River was evident, resulting in higher concentration of Al, Mn, Cr, and Sn in the bottom sediments. The differences between the measured metal concentrations in the NBS sediments and the bottom sediments can be characterized by factor 1.1–2.2. The highest deviations for most metals in the suspendedand deposited sediments were measured at the sampling site Tokaj, where the TOC and TN concentrations also had maximum values (Table 13). To evaluate these measurement data it should also be considered, that the biofilms due to their free–COOH and–OH groups are able to bind dissolved metal ions as chelates or complexes. Therefore, decreasing grain size of sediment particles and increasing specific surface area of biofilms grown on sediment particles leads to increasing metal concentrations of the finer NBS sediments. The network representation of relationships between the concentrations of the investigated components in NBS and bottom sediments are demonstrated in Figure 3, while the calculated coefficients are listed in Table 16.On the basis of these statistical data, it can be stated that the majority of the metals investigated, with exception of Cd, Cr, Hg, and Sn in NBS and Pb and Zn in bottom sediments, displayed a strong positive correlation with TOC (correlation coefficient "r" ranged from 0.68 to 0.98). However, significant positive correlations ($r = 0.98$, $p = 0.04$) with TOC were found solely in the case of Mn and Pb in the NBS and Ni in the bottom sediments. Only Hg in NBS and Zn in bottom

sediments showed negative correlation with TOC. Our findings concur with those of (Dendievel et al., 2022) and (Miranda et al., 2021), who found that the key factors influencingmetal concentrations of sediments are their grain size and TOC content.

Table 15 Mean pseudo-total concentrations of major and trace elements in the near-bottom suspended sediments and bottom sediments of Tisza River at four different sampling sites.

Elements	Concentration(mg/kg)							
	Tímár		Tokaj		Csongrád		Szeged	
	NBS	BS	NBS	BS	NBS	BS	NBS	BS
Fe	36,902	27,674	40,188	21,117	25,626	21,135	36,130	36,879
Al	30,702	22,602	34,106	18, 08	20,877	19,803	32,064	38,444
Mn	1,117	754	1,300	599	807	771	1,077	1,126
Zn	273	269	240	524	148	134	166	162
Cu	39.8	27.6	42.0	20.8	22.9	18.6	41.8	39.9
Pb	33.0	24.8	39.2	29.6	22.2	18.2	30.0	29.2
Cr	32.3	24.7	32.9	20.0	22.9	22.3	37.8	40.6
Ni	33.2	23.9	31.9	19.3	22.3	19.4	29.4	28.9
Li	27.2	15.2	24.7	11.4	14.4	12.2	20.9	20.6
Co	12.2	9.09	11.6	7.54	8.47	7.20	11.6	10.9
As	11.5	10.0	11.6	8.44	9.80	8.49	9.50	9.27
Hg	2.71	1.75	2.52	1.25	2.94	2.66	4.16	3.93
Cd	1.47	1.45	1.35	1.00	0.987	0.900	1.63	1.53
Sn	0.706	0.274	0.615	0.116	0.460	0.235	0.747	0.87

Table 16. Correlation between TOC and metal/metalloid concentrations in bottom sediments (BS) and near-bottom suspended sediments (NBS) (dark red=strong positive correlation, r>0.5; light red: weak positive correlation, r=0.15–0.5; white: r=0.15– -0.15; light blue: weak negative correlation r=-0.15–0.5; dark blue: strong negative correlation r<-0.5)

	Sediment: BS														
	Fe	Al	Mn	Zn	Cu	Pb	Cr	Ni	Li	Co	As	Hg	Cd	Sn	TOC
Fe		0.97	0.91	-0.44	1.00	0.46	0.97	1.00	1.00	0.99	0.59	0.76	0.90	0.95	0.94
Al	0.97		0.96	-0.48	0.96	0.43	1.00	0.95	0.98	0.94	0.37	0.87	0.77	0.99	0.85
Mn	0.91	0.96		-0.69	0.89	0.18	0.98	0.90	0.94	0.86	0.34	0.96	0.68	0.98	0.85
Zn	-0.44	-0.48	-0.69		-0.36	0.58	-0.52	-0.45	-0.51	-0.36	-0.26	-0.78	-0.27	-0.56	-0.57
Cu	1.00	0.96	0.89	-0.36		0.55	0.96	0.99	0.99	0.99	0.56	0.72	0.90	0.93	0.92
Pb	0.46	0.43	0.18	0.58	0.55		0.39	0.45	0.40	0.52	0.15	-0.03	0.48	0.35	0.25
Cr	0.97	1.00	0.98	-0.52	0.96	0.39		0.95	0.98	0.93	0.37	0.88	0.76	1.00	0.86
Ni	1.00	0.95	0.90	-0.45	0.99	0.45	0.95		0.99	1.00	0.64	0.73	0.93	0.93	0.96
Li	1.00	0.98	0.94	-0.51	0.99	0.40	0.98	0.99		0.98	0.56	0.81	0.88	0.96	0.95
Co	0.99	0.94	0.86	-0.36	0.99	0.52	0.93	1.00	0.98		0.64	0.68	0.94	0.91	0.94
As	0.59	0.37	0.34	-0.26	0.56	0.15	0.37	0.64	0.56	0.64		0.11	0.86	0.32	0.78
Hg	0.76	0.87	0.96	-0.78	0.72	-0.03	0.88	0.73	0.81	0.68	0.11		0.44	0.91	0.68
Cd	0.90	0.77	0.68	-0.27	0.90	0.48	0.76	0.93	0.88	0.94	0.86	0.44		0.72	0.94
Sn	0.95	0.99	0.98	-0.56	0.93	0.35	1.00	0.93	0.96	0.91	0.32	0.91	0.72		0.84
TOC	0.94	0.85	0.85	-0.57	0.92	0.25	0.86	0.96	0.95	0.94	0.78	0.68	0.94	0.84	

	p-values and significance for BS														
	Fe	Al	Mn	Zn	Cu	Pb	Cr	Ni	Li	Co	As	Hg	Cd	Sn	TOC
Fe		0.03	0.09	0.56	0.00	0.54	0.03	0.00	0.00	0.01	0.41	0.24	0.10	0.05	0.06
Al	0.03		0.04	0.52	0.04	0.57	0.00	0.05	0.02	0.06	0.63	0.13	0.23	0.01	0.15
Mn	0.09	0.04		0.31	0.11	0.82	0.02	0.10	0.06	0.14	0.66	0.04	0.32	0.02	0.15
Zn	0.56	0.52	0.31		0.64	0.42	0.48	0.55	0.49	0.64	0.74	0.22	0.73	0.44	0.43
Cu	0.00	0.04	0.11	0.64		0.45	0.04	0.01	0.01	0.01	0.44	0.28	0.10	0.07	0.08
Pb	0.54	0.57	0.82	0.42	0.45		0.61	0.55	0.60	0.48	0.85	0.97	0.52	0.66	0.75
Cr	0.03	0.00	0.02	0.48	0.04	0.61		0.05	0.02	0.07	0.63	0.12	0.24	0.00	0.14
Ni	0.00	0.05	0.10	0.55	0.01	0.55	0.05		0.01	0.00	0.36	0.27	0.07	0.07	0.04
Li	0.00	0.02	0.06	0.49	0.01	0.60	0.02	0.01		0.02	0.44	0.19	0.12	0.04	0.05
Co	0.01	0.06	0.14	0.64	0.01	0.48	0.07	0.00	0.02		0.36	0.32	0.06	0.09	0.06
As	0.41	0.63	0.66	0.74	0.44	0.85	0.63	0.36	0.44	0.36		0.89	0.14	0.68	0.22
Hg	0.24	0.13	0.04	0.22	0.28	0.97	0.12	0.27	0.19	0.32	0.89		0.56	0.09	0.32
Cd	0.10	0.23	0.32	0.73	0.10	0.52	0.24	0.07	0.12	0.06	0.14	0.56		0.28	0.06
Sn	0.05	0.01	0.02	0.44	0.07	0.66	0.00	0.07	0.04	0.09	0.68	0.09	0.28		0.16
TOC	0.06	0.15	0.15	0.43	0.08	0.75	0.14	0.04	0.05	0.06	0.22	0.32	0.06	0.16	

	Sediment: NBS														
	Fe	Al	Mn	Zn	Cu	Pb	Cr	Ni	Li	Co	As	Hg	Cd	Sn	TOC
Fe		0.99	0.98	0.71	0.97	0.96	0.82	0.94	0.89	0.93	0.63	-0.08	0.76	0.75	0.92
Al	0.99		0.95	0.61	0.99	0.91	0.89	0.90	0.83	0.93	0.50	0.07	0.83	0.80	0.88
Mn	0.98	0.95		0.71	0.90	0.99	0.70	0.88	0.84	0.84	0.70	-0.23	0.61	0.60	0.98
Zn	0.71	0.61	0.71		0.60	0.76	0.31	0.87	0.93	0.74	0.94	-0.60	0.36	0.44	0.66
Cu	0.97	0.99	0.90	0.60		0.85	0.94	0.92	0.84	0.96	0.44	0.16	0.90	0.89	0.80
Pb	0.96	0.91	0.99	0.76	0.85		0.63	0.88	0.85	0.81	0.78	-0.33	0.54	0.53	0.99
Cr	0.82	0.89	0.70	0.31	0.94	0.63		0.74	0.64	0.87	0.10	0.50	0.98	0.94	0.59
Ni	0.94	0.90	0.88	0.87	0.92	0.88	0.74		0.99	0.97	0.72	-0.17	0.76	0.79	0.79
Li	0.89	0.83	0.84	0.93	0.84	0.85	0.64	0.99		0.94	0.79	-0.29	0.67	0.72	0.75
Co	0.93	0.93	0.84	0.74	0.96	0.81	0.87	0.97	0.94		0.54	0.06	0.89	0.91	0.72
As	0.63	0.50	0.70	0.94	0.44	0.78	0.10	0.72	0.79	0.54		-0.80	0.10	0.15	0.72
Hg	-0.08	0.07	-0.23	-0.60	0.16	-0.33	0.50	-0.17	-0.29	0.06	-0.80		0.51	0.45	-0.32
Cd	0.76	0.83	0.61	0.36	0.90	0.54	0.98	0.76	0.67	0.89	0.10	0.51		0.99	0.47
Sn	0.75	0.80	0.60	0.44	0.89	0.53	0.94	0.79	0.72	0.91	0.15	0.45	0.99		0.44
TOC	0.92	0.88	0.98	0.66	0.80	0.99	0.59	0.79	0.75	0.72	0.72	-0.32	0.47	0.44	

	p-values and significance for NBS														
	Fe	Al	Mn	Zn	Cu	Pb	Cr	Ni	Li	Co	As	Hg	Cd	Sn	TOC
Fe		0.01	0.02	0.29	0.03	0.04	0.18	0.06	0.11	0.07	0.37	0.92	0.24	0.25	0.08
Al	0.01		0.05	0.39	0.01	0.09	0.11	0.10	0.17	0.07	0.50	0.93	0.17	0.20	0.12
Mn	0.02	0.05		0.29	0.10	0.01	0.30	0.12	0.16	0.16	0.30	0.77	0.39	0.40	0.02
Zn	0.29	0.39	0.29		0.40	0.24	0.69	0.13	0.07	0.26	0.06	0.40	0.64	0.56	0.34
Cu	0.03	0.01	0.10	0.40		0.15	0.07	0.08	0.16	0.04	0.56	0.84	0.10	0.11	0.20
Pb	0.04	0.09	0.01	0.24	0.15		0.37	0.12	0.15	0.19	0.22	0.67	0.46	0.47	0.01
Cr	0.18	0.11	0.30	0.69	0.07	0.37		0.26	0.36	0.13	0.90	0.50	0.02	0.06	0.41
Ni	0.06	0.10	0.12	0.13	0.08	0.12	0.26		0.01	0.03	0.28	0.83	0.24	0.21	0.21
Li	0.11	0.17	0.16	0.07	0.16	0.15	0.36	0.01		0.06	0.21	0.71	0.33	0.28	0.25
Co	0.07	0.07	0.16	0.26	0.04	0.19	0.13	0.03	0.06		0.46	0.94	0.11	0.09	0.28
As	0.37	0.50	0.30	0.06	0.56	0.22	0.90	0.28	0.21	0.46		0.20	0.90	0.85	0.28
Hg	0.92	0.93	0.77	0.40	0.84	0.67	0.50	0.83	0.71	0.94	0.20		0.49	0.55	0.68
Cd	0.24	0.17	0.39	0.64	0.10	0.46	0.02	0.24	0.33	0.11	0.90	0.49		0.01	0.53
Sn	0.25	0.20	0.40	0.56	0.11	0.47	0.06	0.21	0.28	0.09	0.85	0.55	0.01		0.56
TOC	0.08	0.12	0.02	0.34	0.20	0.01	0.41	0.21	0.25	0.28	0.28	0.68	0.53	0.56	

Table 17 Average and concentration range of metals determined in the bottom sediments (BS) and near-bottom sediments (NBS) of Tisza River and the recommended sediment quality guideline values for metals and associated levels of concern to be used while conducting sediment quality assessment.

Metal/ metalloid	Concentration rangein bottom sediment (mg/kg)	Average concentration in bottom sediment (mg/kg)	Concentration rangein near-bottom sediment (mg/kg)	Average concentration innear-bottom sediment (mg/kg)	TEC (mg/kg)	PEC (mg/kg)
As	8.44–10.0	9.05	9.50–11.5	10.6	9.79	33.0
Cd	0.900–1.53	1.22	0.980–1.63	1.36	0.990	5.00
Cr	20.0–40.6	26.9	22.9–37.8	31.5	43.4	111
Cu	18.6–39.9	26.8	22.9–42.0	36.6	31.6	149
Fe	21,117–36,879	26,701	25,626–40,100	34,690	20,000	40,000
Pb	18.2–29.6	25.5	22.2–39.2	31.0	35.8	128
Mn	599–1,126	812	807–1,300	1,075	460	1,100
Hg	1.25–3.93	2.40	2.52–4.16	3.08	0.180	1.06
Ni	19.3–28.9	22.9	22.3–33.2	29.2	22.7	48.6
Zn	134–524	272	148–273	2067	121	459

Correlations between different elements revealed distinct pictures, depending on the sediment type. For instance, in the NBS and bottom sediments, Fe had significant correlations with Al, Co, Cu, Li, Ni, and Al, Cu, Mn, Pb, respectively. In the NBS sediments, Zn demonstrated negative correlations with all other elements (except Pb), while in the bottom sediments, Hg displayed negative correlations with As, Li, Mn, Ni, Pb, and Zn. However, As and Zn concentrations did not display any significant correlations with any other elements in both sediment types.

The Consensus-Based Sediment Quality Guidelines (CBSQGs) developed by MacDonald et al., 2000, might be usefully viewed in the context of evaluating the potential risk of metal contaminants. The CBSQGs contain threshold effect concentrations (TEC) and probable effectconcentrations (PEC) for 10 elements, which are the concentrations at which toxicity to benthic-dwelling organisms is predicted to be unlikely and probable, respectively. The concentration range of metal contaminants determined in the NBS and bottom sediment samples, as well as the TEC and PEC values are listed in Table 17. It can be established that the measured concentrations of As, Cd, Cr, Cu, Pb, and Ni in both type of sediments was lowerthan their PEC values. The Fe, Mn, and Zn concentrations were mostly between their respective TEC and PEC limits. However, the risk of Hg contamination can be predicted since its concentration in all sediment samples exceeded the PEC value.

Chapter 5. Conclusion

In the Tisza River, both species of mussels, Unio tumidus and Unio crassus, exhibited a prevalence of fiber particles in their collected samples. This trend of fiber dominance is consistent with findings from bivalve specimens sourced from various rivers worldwide, including the Thames in the UK, Yangtze and Pearl in China, St. John in Canada, Milwaukee in the USA, and Höje in Sweden. Notably, Unio tumidus, as a living sampling device, demonstrated a greater capacity for accumulating MPs compared to Unio crassus. As a result, Unio tumidus holds promise as a potential biomonitoring species within this specific catchment area, facilitating the study of variations in fiber contamination. Considering future projections for global fiber production until 2030, it is anticipated that polyester production will experience a substantial increase. In contrast, natural fibers like cotton, wool, and cellulosic materials are expected to exhibit minimal growth or even stagnation. Given that wastewater treatment plants situated along riverbanks are the primary sources of fiber contaminants in riverine environments, it becomes imperative to calculate the anticipated rise in emissions of polyester fibers into rivers. Concurrently, the higher concentration of these contaminants within suspension-feeding organisms needs to be assessed. In terms of biomonitoring different types of MPs contaminants, the utilization of living organisms for sampling (biomonitoring) presents several inherent limitations, as outlined by studies conducted by Ward and Hollein's research groups. To obtain accurate quantitative data regarding the total load of MPs in rivers, an alternative approach is recommended. Employing an automated sampling system capable of capturing all streaming-suspended particles across a wide size range and at various depths would provide a more comprehensive and reliable depiction of the MPs burden within rivers. This data could then be subject to appropriate analytical investigations to achieve a comprehensive understanding of the MPs load present in rivers.

It is important to highlight that the chemical composition recorded in bottom sediments reflects a longer integration period of nutrient and contaminant accumulation, spanning from several months to years, contingent upon the thickness of the analyzed sediment layer. The examination of sediments collected from NBS provides insights into both the momentary (temporal) concentration of contaminants and the concurrent concentration of seasonally fluctuating nutrients. Our observations based on the examination of NBS sediments from extensive shellfish fields within the Tisza River basin elucidate various facets. These include the geochemical context of the

catchment area, the interplay between bottom-dwelling organisms and their environmental surroundings, and the presence of anthropogenic pollutants stemming from diverse industrial and agricultural sources. Furthermore, the constituents sourced from soil and organic materials significantly contribute to the nutrient makeup within the habitat of Unionidae mussels. Notably, the concentrations of these constituents are notably influenced by the inputs from tributaries within the river system. In evaluating the contamination levels against the concentration thresholds outlined in the CBSQGs for heavy metals within sediment matrices, it is evident that only (Hg) contamination potentially poses a toxic risk to Unionidae mussels, given their benthic lifestyle and ecological niche.

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I. The data of the doctoral dissertation:

Name of the author: Wael Almeshal
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Title and subtitle of the doctoral dissertation: 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)
DOI-identifier[72]: 10.15476/ELTE.2024.028
Name of the doctoral school: Doctoral School of Environmental Science
Name of the doctoral programme: Environmental Chemistry Doctoral Program
Name and scientific degree of the supervisor: Dr. Gyula Záray Professor Emeritus
Workplace of the supervisor: Research Centre of Environmental Science

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