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Review

Review of mayflies (Insecta Ephemeroptera) as a bioindicator of heavy metals and microplastics in freshwater

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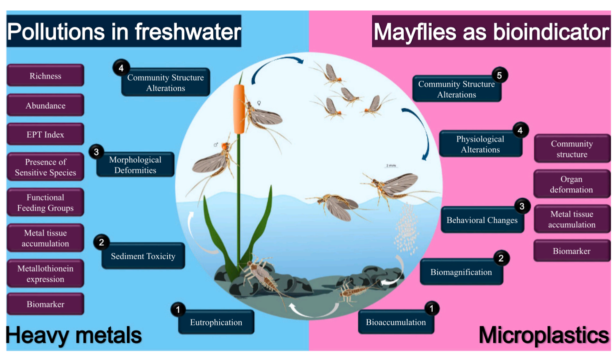
HIGHLIGHTS

- Microplastics are a vector of heavy metals in freshwater ecosystems.
- Mayflies are sensitive to environmental changes, making them a prominent bioindicator.
- The use of mayflies as a heavy metal and microplastic pollution indicator is limited.
- Community structure, behaviour, molecular-level effects and bio-accumulation can be used as matrices.
- Lack of bioindicators' sensitivity and standardised protocols are the current challenges.

GRAPHICAL ABSTRACT



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ABSTRACT

Heavy metal and microplastic pollutions are prevalent in freshwater ecosystems, with many freshwater bodies being contaminated by one or both of these pollutants. Recent studies reported extreme detections of Cd, Pb and Zn, high concentrations of Cr, Pb and Cu and microplastics acting as vectors of pollutants, including heavy metals. Mayflies can serve as bioindicators of heavy metal contamination in freshwater ecosystems because changes in their community structure, physiology, and behaviour can reflect and help predict the concentrations of metals in these environments. This review discusses the ecological alterations induced by tissue metal concentration in mayflies and other macroinvertebrates. As sensitive taxa to heavy metal contamination, mayflies can reflect the impacts of this pollution through their ethology and relationship to the substrate, highlighting issues such as eutrophication, alterations in community structure, inhibitory effects and sediment toxicity. Mayflies are also highly affected by microplastic exposure, which leads to ingestion, bioaccumulation, biomagnification, habitat and community alteration, behavioural changes, physiology alteration and toxicity. Mayflies bioindication metrics for assessing the impact of heavy metals and microplastics include the

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examination of community alteration, functional feeding behaviour, molecular structure, dietary and toxicity impacts, bioaccumulation and biomagnification and biomarkers. Current challenges for the utilization of mayflies as bioindicators include temporal variations in sensitivity, lack of universally recognised protocols and need for standardised protocols for microplastic analysis. Additionally, the applicability of mayflies as bioindicators may vary across different ecosystems, emphasising the need for selecting suitable indicators that align with the unique characteristics of the ecosystem.

1. Introduction

The extensive development of industrial activities improves the quality of human life but also causes major environmental problems (Abu Hasan et al., 2022). The heavy metal (HM) pollution of surface water associated with industrial wastewater discharges has been widely discussed (Ramli et al., 2023a, 2023b; Wibowo et al., 2023). HMs are dangerous to aquatic organisms because they may cause chronic and even acute effects (Jaishankar et al., 2014; Ramli et al., 2023b; Yunus et al., 2020). Jeong et al. (2023) summarised the chronic effects of the exposure of aquatic organisms to HMs and metalloid, including bioaccumulation, and various underlying physiological disruptions, such as reduced growth and development (e.g. decrease body length), diminution of reproductive rates and poor offspring viability. All these effects can lead to behavioural changes (Ghannem et al., 2023) and alterations in community structure and reduction in species diversity (Schmidt et al., 2011).

The two kinds of HMs that occur in aquatic ecosystems are: (1) HMs sensu stricto, which have high atomic mass, are non-essential for life and have high toxicity (e.g. Cd, Hg and Pb) and (2) metals with low atomic mass (trace elements) that are vital and can become quickly toxic when their concentration increases (e.g. Cu, Zn, Mo, Mn and Co) (Lévêque, 1996). The primary origins of these metals are human activities in terrestrial environments, including land spreading agricultural soils with trace elements (soil leaching source), discharges of residual sludge from wastewater treatment plants, discharges from factories processing non-ferrous metals (Cu, Zn, Pb, Cr and Cd) and effluents from tanneries (e.g. Cd and Cr) or paper mills (Hg). Another is the atmospheric fallout from pollution linked to human activities (particularly industrial activities) and rainwater runoff on roofs and roads (Zn, Cu and Pb) (Lévêque, 1996).

Microplastic (MP) pollution also occurs in surface water due to the extensive use of plastic in anthropogenic activities (Castro-Castellon et al., 2022; Ma et al., 2020). Some studies mentioned the effects of MP to freshwater organisms, including problems on the intestine, gills, and liver of *Pseudobagrus fulvidraco* (Lee and Kim, 2023), alteration of oxidative stress on *Ictalurus punctatus* (Zheng et al., 2023) and changes in antioxidant level on *Cyprinus carpio* (Yedier et al., 2023). Among aquatic invertebrates, chronic exposure to MP affected the growth, reproduction and mortality of *Daphnia magna* (Procházková et al., 2024). Reduced fertilization rate and high degree of larvae malfunction were observed on *Mytilus galloprovincialis* due to extensive exposure to MPs (Romdhani et al., 2024), and the interaction among temperature, CO_2 and MPs increased the mortality rate of *Phylloicus* (Firmino et al., 2023). The biomagnification of MP is also a hot topic to be discussed because humans might be indirectly affected through the consumption of aquatic organisms, such as fish (Qaiser et al., 2023). MP also acts as a vector of HMs due to its adsorption capacity (Brennecke et al., 2016), increasing its potential toxicity to the water ecosystem.

Regular monitoring is one of the efforts to tackle surface water pollution. Previous research reported the monitoring of freshwater lake polluted by Cu in China (Wang et al., 2022). The levels of As, Cd, Cr, Cu, Mn, Pb, Zn and Fe levels in the Yellow River, China (Zhao et al., 2021) and 12 HMs (Cd, Pb, Cr, Hg, Zn, Cu, Ni, Al, Fe, Mn, As, and Co) in the surface water in Bangladesh (Hossen and Mostafa, 2023) were regularly monitored. Several research also reported the monitoring of MP pollution in surface water such as in Karnataka, India where fibres were monitored in estuaries (Vaisakh et al., 2023), MP abundance in spring water in Batu, Indonesia (Yanuar et al., 2024) and polymer levels in drinking water source in southern China (Zhao et al., 2024). Most of the monitoring methods for HMs and MPs involved the utilization of chemicals, such as peroxide and acid extractions, and sophisticated technologies, such as atomic absorption spectrometry (AAS), inductively coupled plasma (ICP), Fourier-transform infrared spectroscopy (FT-IR) and Raman spectroscopy, which are more reliable than the other methods (Vaisakh et al., 2023; Wang et al., 2021; Zhou et al., 2020). Biological monitoring (using bioindicators or biomarkers) also shows good reliability in indicating HM contaminations [using *Leptodictyum riparium* (Maresca et al., 2018)] and MPs [analysing non-indigenous *Squalius vardarensis* (Koutsikos et al., 2023)].

Aquatic macroinvertebrates are well-established assessment tools for the extent of human-induced stressors across various biological levels, ranging from the molecular to the ecosystemic level (Carter et al., 2006; Resh and Rosenberg, 1984). Every living organism obeys the ecological principle of Shelford's Law of Tolerance, which describes the relationship between organisms and environmental factors and states that the success of an organism is determined by its ability to tolerate and function within a range of environmental conditions; every organism has its tolerance range and its maximum and minimum threshold, and toxicity and death are expected if these tolerance limits are crossed (Shelford, 1911; Spence and Tingley, 2020; Yuan, 2004). A bioindicator is a living organism that provides information about the quality and ecological state of the environment in compliance with its tolerance range and the stressor (factor) intensity (Chowdhury et al., 2023; Parmar et al., 2016; Sagova-Mareckova et al., 2021). Bioindicators play a crucial role in environmental risk assessments and the development of conservation and management strategies (Bartell, 2006; Parmar et al., 2016; Pinilla-Cortés and Moreno-Gutiérrez, 2019).

To the best of our knowledge, research and reviews on the use of mayflies as a bioindicator of MP and HM pollutions in freshwater ecosystem are scarce. Thus, the present work aimed to highlight the use of mayflies as a bioindicator of HM and MP pollutions in surface water (freshwater), focusing on the used taxa and its sensitivity. This review also described the current limitations and future approaches that may enrich future discussions and research. The findings would enrich the knowledge of the monitoring of HM and MP pollutions, focusing on surface freshwater bodies and the use of bioindicator as monitoring techniques.

2. Mayflies as a bioindicator of environmental pollution

A close look on the biology, ethology and ecology of mayflies is essential to understand how and when these organisms can help indicate pollutant occurrence or impact. The father of modern ecology Eugene Odum said: 'A species' habitat is its address; its niche is its profession'. In freshwater ecosystems, macroinvertebrates' habitat depends on their development stage and on the seasons; thus, a given species can occupy different habitats and niches (function in the ecosystem) (Lévêque, 1996). Therefore, pollutants can biologically and spatially influence a given species during each development stage and season.

According to the River Continuum Concept (Dodds and Maasri, 2022; Vannote et al., 1980), macroinvertebrates, particularly those in watercourses, are organised into four functional feeding groups or trophic guilds and depending on their feeding habits: shredders, scrapers (or grazers), collectors and predators. The distribution of mayflies in running waters is also predominantly influenced by the physical characteristics of the substrate (Gallitelli et al., 2021). Mayflies represent a diverse group that can occupy different functional feeding groups depending on the taxon, exhibiting ecological versatility in freshwater habitats. They can act as scrapers or grazers, consuming algae and associated material found on or within substrates of varying sizes (such as rocks, pebbles, cobbles, sand and silt), as collectors–gatherers, collecting fine particulate organic matter (FPOM) from the streambed, or as collectors–filterers, using specialised body parts to gather FPOM from the water column, a few mayfly species are even predators, feeding on other organisms (Baptista et al., 2006; Bauernfeind and Soldán, 2012; Vilenica et al., 2018). Mayflies as caddisflies are gill breathers which enable them to directly absorb pollutants from the water. For instance, these functional guilds can experience different HM bioaccumulation patterns according to size and diet (Rodriguez et al., 2018) that is linked to their age, the type of their mouthparts, their locomotive behaviour and their relationship with the substrate throughout their life cycle.

Mayflies undergo various life stages in aquatic environments; thus, they may be differently affected by the contaminants in water and sediments at each stage of their life. They undergo incomplete metamorphosis, consisting of egg, nymph and two adult stages. Mayflies are benthos elements that are also part of the occasional hyporheos (i.e. subsurface zone beneath and adjacent to a watercourse); they dwell in this area during the early stages (first instars of larvae and embryonic stages) of their development cycle or even in the final stages to protect themselves from predators or harsh heterologous conditions (spates and low flow), while the later stages predominate in the benthos (Pugsley and Hynes, 1986, 1985; Williams, 1984). They can reach $\geq$10 cm deep in the sediments (Dudley Williams and Hynes, 1974). A considerable number of mayflies are positively thigmotactic (seeking contact with substrate) and spend a great part of their life cycle (above all the embryonic stage and the early larval stages) in a hypogeal environment (Gonser, 2001). The hyporheic zone is mandatory to ecological processes, nutrient cycling and water filtration. In standing waters as lakes and ponds, the hyporheic processes terminology can vary and is often referred to (in lakes) as the hypolimnion or hypolimnetic zone, which is the deeper layer of water below the thermocline. Combined with their close association with the substrate and sensitivity to changes in water and sediment quality due to pollutants, this vertical distribution of mayflies throughout the streambed and water column makes them excellent bioindicators, serving as sensitive early sensors of habitat alteration.

3. HM and MP pollutions in freshwater ecosystems

Surface water pollution caused by HMs and MPs can be found in many places. Polluted freshwater may cause additional problems because the amount of available freshwater in some areas is already limited. Table 1 summarizes some recent studies on HM and MP pollutions in freshwater ecosystems.

Table 1 shows that the freshwater bodies in some particular regions were contaminated by HMs, MPs or both. Bawa-Allah (2023) reported the extreme detection of Cd, Pb and Zn in some freshwater sources, in which the concentrations should be <0.003, 0.003 and 3 mg/L, respectively, according to the local standard. Hossen and Mostafa (2023) also reported the high concentrations of Cr, Pb and Cu exceeding the maximum limits of 0.066, 0.03 and 0.5 mg/L, respectively according to Bangladesh standard for freshwater bodies. In freshwater river ecosystem in Asia, MP pollution reached 11.128 items/m^3 (Cera et al., 2020). Previous research mostly mentioned the abundance and compositions of MPs in some particular areas; to date, no hazard category of MP pollution based on abundance has been established. Yuan et al. (2022) highlighted the MP hazard category based on previous published data and concluded that the smaller the particle size and the more complex polymer in MPs, the higher the potential hazard. Kabir et al. (2021) reported the abundance of MPs in rivers up to 1061 particles/L, and Wang et al. (2017) reported the abundance of 8925 particles/m^3 in a river in Japan.

MPs are also vectors of pollutants, including HMs. The surface of MPs facilitates the attachment of some other pollutants, including HMs. Patidar et al. (2023) reported the abundance of MPs in freshwater source in India with a value of 22.3 particles/L, that is, the concentration of HMs per gram of MPs was high. Zuo et al. (2024) reports that various HMs can be found in MPs. The role of MPs as vector of other pollutants, especially HMs, was explained by Selvam et al. (2021), who concluded that polypropylene had the highest adsorption capacity for HMs (Cd > Mn > Pb > As>Zn > Co). The strength of the interactions between MPs and heavy metals depends on environmental factors and HM concentration, implying a high potential hazard to aquatic organisms (Patidar et al., 2023).

4. Mayflies as a bioindicator of HMs

Numerous studies stressed the multiple impacts of HM on macroinvertebrates; HM concentration can even predict the benthic community structure (Clements et al., 2000). HM can alter these smalls organisms' physiology and ethology and their community structure and population dynamics (e.g. reduced taxa richness) due their habitat alteration (Costas et al., 2018; Rodríguez-Olague et al., 2021; Rodriguez et al., 2018). These metal-induced ecological alterations are provoked by the tissue metal concentration as proven for some macroinvertebrates (Bervoets et al., 2016; De Jonge et al., 2013; Rainbow et al., 2012; Rodriguez et al., 2018; Schmidt et al., 2011). Hence, identifying bioindicator taxa and their reactions to HM is highly recommended for appropriate aquatic ecosystem management (Costas et al., 2018).

4.1. General effects of HMs on macroinvertebrates

The effects of HMs on macroinvertebrate populations and the aquatic environment (e.g. exacerbation of eutrophication) are undeniable and can be utilised as monitoring tools to assess HM contaminations. Some HMs effects on macroinvertebrates are summarised in Fig. 1.

Eutrophication involves the excessive fertilization of a water body with nutrients, leading to the generation of more organic matter than the natural self-purification mechanisms can manage (Chambers et al., 2001). Released phosphorus due to reducing environment, seasons changes and daylight is one of the main causes of eutrophication. Ammonia and nitrates are also factors of nutrient enrichment. Phosphorus can even form complexes with metals in the water column, creating suspended solids that will sediment in the bottom. Some macroinvertebrates contribute to the balance of nutrient cycles, and exposure to HMs may disrupt this balance by posing toxicity to the species (Tabassum et al., 2024). The relationships among HMs, macroinvertebrates and eutrophication are complex, and eutrophication degree is considered a key factor influencing HM levels in invertebrates, such as zooplankton (Gagneten, 2010). Through lacustrine core sediment analysis, Muneer et al. (2023) revealed a significant increase in lacustrine productivity in the Ahansar lake basin (Kashmir Himalaya region, India) from 1980 until 2020. This productivity rise coincided with an enrichment of HMs that is linked to regional urbanization history and changes in agricultural practices, particularly the use of fertilizers—major accelerators of eutrophication—which often contain HMs (e.g. manganese, zinc and copper). Driven by elevated nitrogen (N) and phosphorus (P) concentrations, amplified eutrophication significantly influenced the accumulation of HMs in periphyton within the highly polluted rivers of the Chaobai River Watershed, Northern China (Tang et al., 2014). Wang et al. (2020) showed that redox potential (Eh) and HMs may have had greater impacts on the microorganisms in the water and sediment than N and P. The combination of metals as Zn and Cu and eutrophic conditions was even more detrimental to these organisms, with an increase in N and P concentrations provoking the

Table 1
Heavy metal and microplastic pollutions in freshwater ecosystems.

Heavy metals	Microplastics	Analytical method	Location	Reference
Cd 0.73 mg/L Co 0.81 mg/L Cr 4.02 mg/L Cu 10.5 mg/L Fe 20 mg/L Mn 1.22 mg/L Ni 0.8 mg/L Pb 8.33 mg/L Zn 20.46 mg/L	–	AAS and ICP	Some freshwater bodies, Nigeria	(Bawa-Allah, 2023)
As 40.75 mg/L Cd 0.96 mg/L Cu 31.94 mg/L Pb 28.99 mg/L Zn 91.33 mg/L	–	ICP/AES	Yellow River, China	(Bai et al., 2012)
Cr 0.63 mg/L Cu 0.89 mg/L Fe 2.31 mg/L Mn 0.89 mg/L Pb 2.09 mg/L	–	ICP and AAS	Several rivers, Bangladesh	(Hossen and Mostafa, 2023)
Al 5010 μg/L As 2.4 μg/L Cr 1.3 μg/L Cu 2723 μg/L Fe 8420 μg/L Mn 0.83 μg/L Ni 0.7 μg/L Pb 17,620 μg/L Zn 0.7 μg/L	–	Graphite Furnace Atomic Absorption Spectrophotometer	Beyşehir Lake, Turkiye	(Şener et al., 2023)
Co 1.64 ppm Fe 0.14 ppm Pb 0.33 ppm Si 27 mg/L	–	AAS	Thuckalay, India	(Arumugham et al., 2023)
Cu 2.88 mg/L Zn 11.47 mg/L	–	AAS	Kongsfjorden Fjord, Norwegian	(Rajaram et al., 2023)
Cd 0–0.59 μg/L Cu 2.6–16.18 μg/L Fe 128–298 μg/L Mn 8.6–28.6 μg/L Zn 28.4–61.9 μg/L	–	AAS	Nile River, Egypt	(Goher et al., 2019)
Cu 0.14 mg/L Fe 0.29 mg/L Zn 0.09 mg/L	–	ICP-OES	South Florida, USA	(Yang et al., 2013)
As 1.7–61,000 μg/L Co 0.2–3400 μg/L Fe 6–500,000 μg/L Mn 1000–1,531,453 μg/L Ni 0.05–255,000 μg/L Pb 0.07–635,496 μg/L Sb 0.2–173,000 μg/L Ti 23.5–3065.7 μg/L U 91.6–36,300 μg/L V 0.2–63,200 μg/L Zn 0.75–278,388 μg/L	–	ICP-MS	Atoyac River Basin, Mexico	(Dueñas-Moreno et al., 2024)
–	0.17 particles/L	FTIR	Pantelleria, Italy	(Pierdomenico et al., 2024)
–	Max 180 particles/m^3	FTIR	Alkaline Lake, Argentina	(Alfonso et al., 2020)
–	0.78 particles/L	Microscope and FTIR	Northeastern Poland	(Pol et al., 2023)
–	Max 9 particles/L	Microscope and FTIR	Al-Hubail Lake, Saudi Arabia	(Picó et al., 2020)
–	Maximum 21.3 particles/L	Microscope	Sierra Nevada, Spain	(Godoy et al., 2022)
–	8.77 particles/L	ATR-FTIR	Kumaraswamy Lake, India	(Ephsy and Raja, 2023)
–	Max 11.9 particles/L	Raman spectroscopy	Adelaide, Australia	(Leterme et al., 2023)
–	99–1061 particles/L	Microscope and ATR-FTIR	Majime River, Japan	(Kabir et al., 2021)
–	1660–8925 particles/m^3	Microscope and FTIR	Hanjiang River and Yangtze River, Wuhan, China	(Wang et al., 2017)
–	1145 particles/L	FTIR and GC–MS	Flanders, Belgium	(Vercauteren et al., 2023)
–	34–64 particles/L	FTIR and SEM-EDS	Dawanshan Island, China	(Gong et al., 2023)
–	600–2500 particles/m^3	Microscope and FTIR	Himalayas, India	(Farooq et al., 2023)
–	5.57/m^3	ATR-FTIR	Elbe River, Czech Republic	(Scherer et al., 2020a)
–	5.5/m^3	μFTIR	Lake Balaton, Hungary	(Svigruha et al., 2023)
Fe 18.2–88.5 μg/g Mn 0.19–8.25 μg/g Cr 0.03–0.14 μg/g	Max 36 particles/L	Microscope and AAS	Vis, Croatia	(Maršić-Lučić et al., 2018)

(continued on next page)

Table 1 (*continued*)

Heavy metals	Microplastics	Analytical method	Location	Reference
Ni 0.04–0.27 μg/g Pb 0.04–0.85 Cu 0.08–0.61 μg/g				
Ag 152.23 mg/g As 15.1 mg/g Cr 50.09 mg/g Cu 128.8 mg/g Fe 262.03 mg/g Ni 56.89 mg/g Pb 1.87 mg/g Zn 41.83 mg/g	22.3 particles/L	FTIR and XRF	Jharkhand, India	(Patidar et al., 2023)
Ba 97 mg/kg Cr 5 mg/kg Cu 3 mg/kg Mn 2 mg/kg Ni 0.4 mg/kg Pb 1 mg/kg Sb 32 mg/kg Zn 42 mg/kg	Unspecified release	SEM and ICP-OES	Shanghai, China	(Zuo et al., 2024)
Al 182.20 μg/kg Fe 597.26 μg/kg Ti 4208.87 μg/kg Zn 283.41 μg/kg	Unspecified release	FTIR and ICP-MS	Bari, Italy	(Campanale et al., 2022)

proliferation of opportunistic plant species. The authors later added that because eutrophic environments are endogenous, whether the intensification of eutrophication and salt nutrients would influence the release of HMs in the aquatic environment remains unclear. Carbonates, which help mitigate HM toxicity, are heavily consumed due to eutrophication that drives the exponential growth of opportunistic plants (Völker et al., 2013; Wang et al., 2020). Zhang et al. (2018) found significant correlations between HMs and nutrient levels [total organic carbon (TOC), δ13C, and total phosphorus TP] in lacustrine sediments from China, suggesting geochemical associations during transport and shared anthropogenic sources such as fertilizers. In Lake Mälaren, Central Sweden, historical eutrophication (high nutrient levels) and HM pollution were also linked to each other, with early nutrient enrichment and lead contamination largely attributed to 19th-century industrial metal production (Renberg et al., 2001). According to Marqués et al. (2003), the benthic macroinvertebrates with sensitivity or tolerance to mining disturbances react the same to organic pollution. Sensitive taxa, such as those from Ephemeroptera, Plecoptera, and Trichoptera (EPT) orders, are vulnerable to both types of disturbances, and pollutant-resistant taxa are generally the same, i.e. chironomids and Oligochaeta displaying high tolerance levels.

The response of benthic macroinvertebrates varies according to the extent of eutrophication; if this latter is mild to moderate, the usual response is an increase in abundance and taxa richness relative to reference conditions (Dodds, 2006; Lyche-Solheim et al., 2013; Ntitslidou et al., 2021; Yang et al., 2024). However, an exacerbated eutrophication reduces taxa richness and increases density, favouring pollution-tolerant macroinvertebrates such as oligochaetes, chironomids and nematodes. Meanwhile, the abundance and diversity of sensitive taxa, such as mayflies, stoneflies and caddisflies, decline. In the most severe stage of eutrophication, benthic organism abundance and taxon richness diminish due to smothering. During this stage, oxygen depletion from microbial decomposition of organic matter adversely impacts the fish and plant populations (Lowell et al., 2000).

Trace metals can be harmful to organisms because of their inhibitory effects (toxicity or smothering), leading to a decrease in taxa richness and abundance (Lowell et al., 2000) and a shift in the benthic community structure that results in a trivialised and predictable species composition, i.e. rarity or lethality of sensitive species and dominance of metal-tolerant ones (www.ec.gc.ca) (Canfield et al., 1994; Clements, 2004; Courtney and Clements, 2002; Pollard and Yuan, 2006).

In ecotoxicology, bioassays help determine the ecological impairment and the impact of contaminated sediments on benthic invertebrate communities' structure (e.g. sediment quality triad analysis, mesocosms or cage bioassays or a single species bioassay). Sediment toxicity bioassays comprise laboratory tests on living organisms (mostly benthic macroinvertebrates) to measure the sediment toxicity on these organisms and the community structure to analyse the state of living biota and the changes that can happen in this structure (Bartlett and Norwood, 2013; Chapman, 1992; Heß et al., 2024; Jean and Fruget, 1994; Jergentz et al., 2004; Sarkis et al., 2023; Van Den Berg et al., 2019).

In field, '*in situ* cage bioassays' refer to caged specimens in a their natural aquatic environment. This technique allows to distinguish between sediment and water effects and to measure the number of survivors, mean individual growth over the exposure period, estimation of reproduction and whole-body concentrations of relevant elements (www.ec.gc.ca). Several aquatic invertebrates have been used in field cages (e.g. amphipods, midges, leeches, snails and water fleas), but mayflies are not included. In a single taxa assay, the burrowing mayflies' *Hexagenia limbata* (Ephemeridae) survival and growth in the contaminated sediments were statistically compared to those of the control group (Bedard et al., 1992).

4.2. Mayflies bioindication metrics to assess HM impact

The mayflies bioindication metrics for assessing the effect of HM in freshwater ecosystems are summarised in Table 2 and explained in detail in the following subsections.

4.2.1. Alteration on the level of community structure

The benthic community response to a HM contamination is examined by comparing the communities' structure in an exposure area and a reference area or along a gradient (Clements et al., 2000; Maret et al., 2003; Costas et al., 2018; www.ec.gc.ca). To compare against reference sites, Costas et al. (2018) detected drastic changes in EPT richness and abundance in Hg and Au mining areas and defined them as the most sensitive indicators of community changes metrics (abundance, richness and biodiversity) in response to mining pressures and metal concentrations in sediments, particularly As and Hg. These observations led to the qualification of EPT as 'responsive community descriptors', indicating the potential use of their metrics for identifying metal mixture pressures in field scenarios. EPT abundance depletion as a response HM pollution was also reported by several authors (Clements et al., 2000;

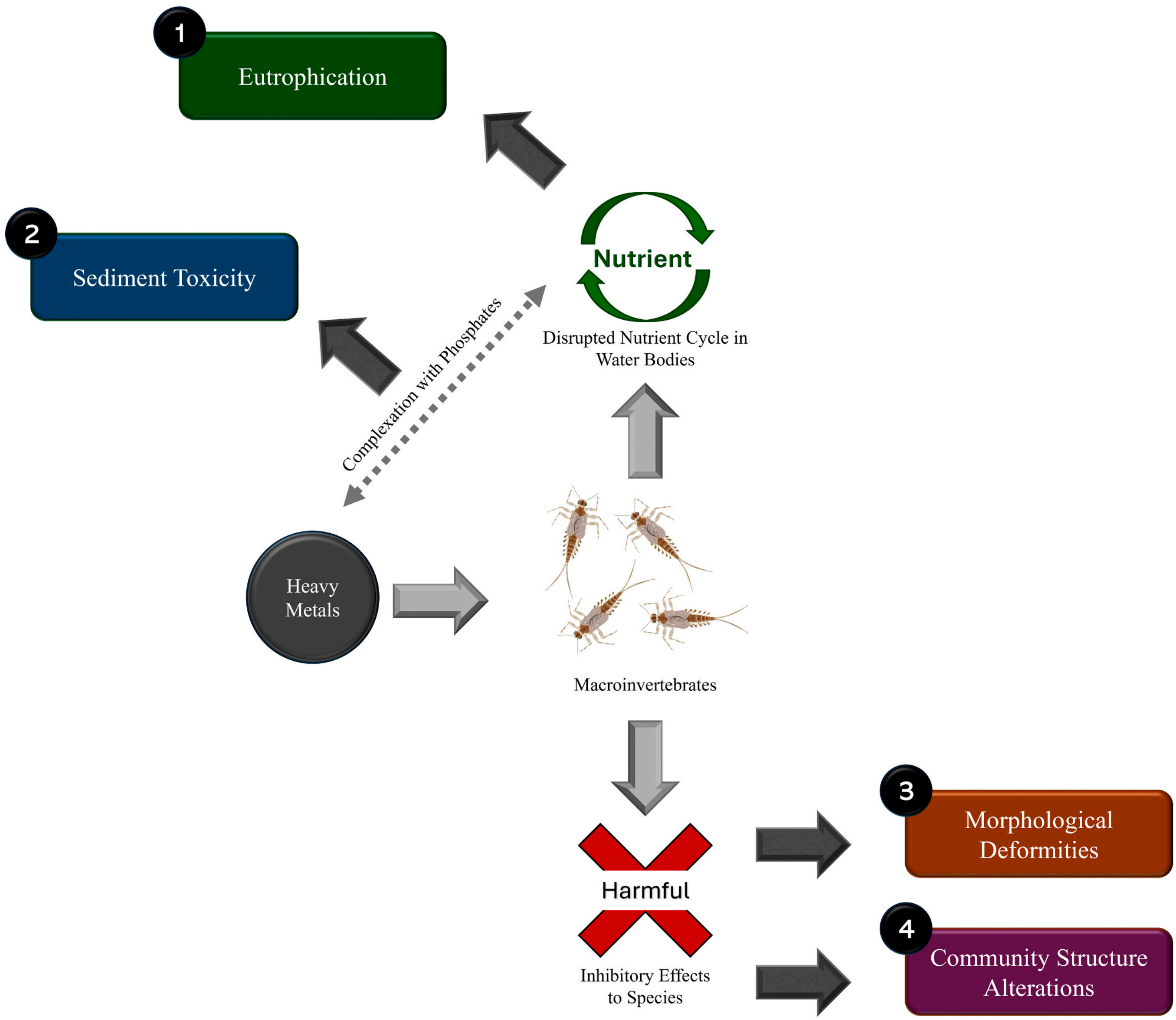

Fig. 1. Effects of heavy metals on macroinvertebrates.

Maret et al., 2003; Eden, 2016; Bae et al., 2021). By contrast, Kiffney and Clements (1996) found no differences in metal-exposed (Cd, Cu and Zn) macroinvertebrate densities between field surveys and experimental results; the densities measured in stream microcosms were consistent with those observed at reference and metal-polluted sites across different altitudes. The macroinvertebrate communities included in their study encompassed sensitive EPT taxa, specifically the families Baetidae (such as *Baetis* sp.), Heptageniidae and Ephemerellidae (e.g., *Drunella* sp.) with the smallest specimens (e.g. *Baetis tricaudatus*) from cold streams being the most sensitive.

In the Nalón River basin (Northern Spain), synchronous decreases follow the exposure of Ephemeroptera from the Baetidae, Heptageniidae and Leptophlebiidae taxa families to escalating As, Cd, Cu, Se and Cl levels and fine sediment fraction (Costas et al., 2018). The same trends of mayflies' responses to pollution were observed under Zn, Pb, Cu, Cd and Cr contaminations in American and Australian streams (Clements et al., 2000; Winner et al., 2011; Wright and Ryan, 2016). Not all Ephemeroptera families include ultra-sensitive representatives. The genus *Baetis* within the family Baetidae is recognised for its tolerance to highly polluted waters (Brittain, 1990; Clements, 1994; Rico-Sánchez et al., 2022). For instance, *Baetis tricaudatus* is ubiquitous and abundant in the sites contaminated with Cd, Pb and Zn from hard-rock mining in Colorado, USA (Maret et al., 2003), which contrasts with the findings of Kiffney and Clements (1996) for *Baetis* spp. and Bae et al. (2021) for *Baetis rhodani* that is highly tolerant to zinc. In the latter study, *Baetis fuscatus* and *Baetis ursinus* were selected as indicator species in the recovered sites from an abandoned mining area in Korea. Rico-Sánchez et al. (2022) revealed that Leptophlebiidae, mostly considered as a family with sensitive taxa due their filamentous gills, exhibits a high tolerance to HMs such as As and Hg in their assessment of the impact of mining on stream macroinvertebrate assemblages in Central Mexico.

Each taxon can characterise ecological conditions within an ecoregion and provide the necessary background information for the evaluation of future changes in water quality. In several studies, identifying macroinvertebrate taxa—Ephemeroptera included—to family taxonomic level was a sufficient and reliable method to detect and assess ecological impairments (Clements et al., 2000; Costas et al., 2018; Marqués et al., 2003; Pond et al., 2008; Wright and Ryan, 2016). However, Clements et al. (2000) suggested that the family level is a sufficient taxonomic resolution in relatively large spatial scale (basin) but not for unique stream where a coarse taxonomic level is needed (genus or species) to perceive ecological impairments inside this narrow

Table 2
Mayflies' bioindication metrics for assessing heavy metal impact.

Level	Metric	Description	Pollution indicator
Community structure	Taxa richness	Number of taxa in the community	Lower number of taxa by the increasing of pollution severity
	Abundance	Number of individuals in sampling area	Low number of individuals with the increasing pollution severity
	Ephemeroptera, Plecoptera and Trichoptera (EPT) index	Relative composition of the sensitive taxa	Low ratio indicated poor water quality
	Presence of sensitive species	Presence or absence of highly sensitive species	Absence of highly specific species indicated HM stress
Functional feeding behaviour	Functional Feeding Groups	Composition of grazers/scrapers, collector–gatherers, shredders, filter-feeders and predators within the community	Alteration following the decline of sensitive species
	Metal tissue accumulation	Metal concentration in tissue based on feeding behaviour	High concentration in collectors and scrapers
Molecular level	Metallothionein expression	Protein involved in heavy metal detoxification	High concentration in polluted environment
	Biomarker	Specific protein or enzymes	Presence indicating heavy metal stress

scale. For instance, the mayfly family Leptophlebiidae has been stated by Wright and Ryan (2016) as one of the most intolerant and sensitive macroinvertebrate families to pollution in the world. Meanwhile, Clements et al. (2000) observed the highest sensitivity for Heptageniidae species (*Rhithrogena* sp.) to HM in the Southern Rocky Mountain ecoregion (Colorado, USA), where the loss of their abundance was even significant in moderately polluted sites (−70 %).

According to Pond et al. (2008), ionically overload waters can induce organism stress or lethality due to disrupted ion exchanges and water balance. As water-dwelling insects, mayflies' larvae possess permeable cuticles (skin) that manage ion movements across the biological membrane; this process is performed by external chloride cells found in gills and integument and by excretory organs called the Malpighian tubules (Gaino and Rebora, 2000; Komnick, 1977; Pond et al., 2008). Moreover, the Mg/Ca ratio, calcium ions Ca^{2+}, chromium ions Cr^{3+} and potassium ions K^{+} significantly influence the composition of benthic macroinvertebrate communities in metal-contaminated streams from the Manyame River system in Zimbabwe (Bere et al., 2016). This physiological sensibility is increasingly detrimental in the presence of highly toxic forms of ionizable HM as Hg^{2+}, Pb^{2+} or Cd^{2+}.

Mayflies are one of the most, if not the most, sensitive taxa to HM pollution (Albutra et al., 2017; Clements et al., 2000; Jacobus et al., 2019). Their ethology and relationship to the substrate can predict the way they will be affected by HM because the bioavailability and toxicity of metals might depend on their different chemical forms (Rodriguez et al., 2018; Simpson and Batley, 2007). HM exacerbation alters the different compartments of a freshwater ecosystem (water column, sediments and interstitial water) because they can be present in different forms. Thus, the impact of HM concentration and its bioavailability are inherent to the habitat in which mayflies dwell. For instance, Marqués et al. (2003) studied the effects of mining activities on running waters in the Basque (Spain) and found an increase in HM levels in the water column and sediments and a high accumulation in the sediments, with the crawler–swimmer families Ephemeridae, Leptophlebiidae, Baetidae and Heptageniidae being sensitive to the water column concentrations and the burrower Ephemeridae family being sensitive to both. Similarly, Waterways and Eden (2016) found that sediment-bound metals were stronger indicators of macroinvertebrate community variation than metals in water, serving as the primary factor in community structuring. Bae et al. (2021) reported that high concentrations of Cd and Zn deteriorate food resource quality and chemically alter the streambed, resulting in a decrease in the ratio of scraper groups, such as the Heptageniidae family (namely, *Ecdyonurus levis* and *Epeorus pellucidus*).

Metal concentration in sediments is significantly associated with the community structure metrics, including abundance, richness, biodiversity and viability (Costas et al., 2018; Maret et al., 2003; Opfer et al., 2011; Pollard and Yuan, 2006), and trophic transfer (Hug Peter et al., 2018; Rodriguez et al., 2018). To determine the effects of pollutants on organisms, we must understand the physiology and ecology (e.g. habitat requirements), level of sensitivity or resistance and trophic and ecological behaviour of the species.

4.2.2. *Alteration on the level of functional feeding behaviour*

HM concentrations in the tissues of organisms are generally assessed at the family level because the ecological requirements for the following taxonomic levels (genus, species) are supposed to be expressed at this level. However, Rodriguez et al. (2018) drew attention to the importance of the species level in analysing metal bioaccumulation and the variations of ethological and feeding behaviours that influence the levels of metal bioaccumulation.

The extent of bioaccumulation differs according to the chosen biomonitor taxa and their feeding habits (Cain et al., 1992; De Jonge et al., 2013, 2010; Rodriguez et al., 2018). Nevertheless, Pond et al. (2008) reported no differences between taxonomic levels (order, family and genus) with regards to the impact of coal mining in Central Appalachian Mountains on Ephemeroptera metrics. In all cases, the decline of relative abundance and total richness was negatively correlated to the mining disturbances.

In the selection of candidates for bioaccumulation research analysis, the taxonomic identity and level, size and stage of development and feeding behaviour of the chosen specimen must be considered. Here, the notion of trophic guilds or functional feeding groups is literally significant. The feeding behaviour of Palearctic mayflies has been well investigated. Nevertheless, Rodriguez et al. (2018) stated that the feeding behaviour of mayflies can be difficult to determine because it can vary according to their stage of development. Goodyear and McNeill (1999) described all mayflies as collectors–gatherers, and Rodriguez et al. (2018) following the characters selected by Mattson (1996) for North American mayflies, attributed the scrapping feeding style to Baetidae and Heptageniidae as representatives that feed on algae and other small particles. The diet flexibility of *Seratella ignita* (Ephemerellidae) has been acknowledged by several authors (López-Rodríguez et al., 2009; Riaño et al., 1997; Rodriguez et al., 2018; Tachet et al., 2000). Unfortunately, the feeding behaviour of mayflies in other biogeographic regions is less known because in the Afrotropical and Oriental realms, the families and genera or species compositions differ from a realm to another; for example, an entire family can be absent from one to three realms (Barber-James et al., 2008). As endobenthic elements, mayflies in the first stages of their life (eggs, first small instars) are highly influenced by the substrate metal content. Ephemeridae experience these pressures all throughout their life as their feeding style consists in drilling U-shaped tubes in the substrate and filter-feeding (Bauernfeind and Soldán, 2012).

Rodriguez et al. (2018) reported no significant differences in tissue metal levels among predators, scrapers and filterers. However, differentiations were noted in the kinds of highly bioaccumulated metals. For instance, the generalist Ephemerellidae usually accumulated more (higher levels) Cu and Zn, scrapers (Baetidae and Heptageniidae) accumulated more Se and deposit-feeders accumulated more As, Hg and Ni than the other taxa in this study.

The impact of metal mixture on mayflies and macroinvertebrates was studied by Rodríguez-Olague et al. (2021), Rodriguez et al. (2018) and Moreno-Ocio et al. (2022). The only work cited in these papers was De Jonge et al.'s (2013) study that observed substantial relations among Cu, Zn and Pb burdens in *Rhithrogena* sp. (Heptageniidae). Rodriguez et al. (2021) performed one of the rarest modern studies that proposed an integrative score for bioaccumulation risk assessment based on the average ratio of the actual metal tissue residues in each macroinvertebrate taxon to the corresponding ecological threshold tissue concentration. Baetidae, Ephemerellidae, Ephemeridae and Heptageniidae were the mayfly biomonitors selected according to their feeding style for metal bioaccumulation risk assessment in mine-impacted rivers. The tissue residues in Baetidae can be considered as the early warning signals for metal impairment or toxicity on macroinvertebrate community in the Nalon River (Northern Spain). Bioaccumulation risk assessment showed high bioaccumulation in Hg and Au mining areas but not Cu mining areas. For instance, Baetidae was assessed as high in the Hg mining sites, and Ephemeridae was moderate in the Au mining area. The aim of this latter study was to propose integrative scores for tissue residues that are suitable for surveillance programs and readily interpreted in terms of risk assessment in mining impacted rivers. In the same investigated area, Moreno-Ocio et al. (2022) subsequently developed tissue residue thresholds for As and Cu for sensitive EPT taxa that involved the EPT community metrics for the same families investigated by Rodriguez et al. (2018).

Carter et al. (2006) stated that when stressors affect the food resources, the functional feeding groups can be altered, and a representative of feeding group may be vulnerable to a defined stressor. For instance, grazers (that mostly feed on algae and FPOM) would be affected by toxic metal uptake by algae. Meanwhile, Clements et al. (2000) detected high sensitivity to HM translated by decreasing abundance for scrapers and predators when the shredders and collectors were seen as or less tolerant to metals and being less abundant only in the sites with the highest metal contamination; however, no taxa composition of each feeding group was given in this study. Around 25 years ago, Goodyear and McNeill (1999) established a comprehensive review on HM bioaccumulation by feeding guilds of macroinvertebrates, whether from lakes or streams. Basing on combined data form almost 20 years of literature, they pointed out the importance (relevance) of analysing each metal source (water and sediments) to evaluate the relationships of feeding guilds with each medium. The Zn, Cu, Pb and Cd levels in the sediments or waters were specific to each feeding guild. For example, the collector–gatherer guild to which Ephemeroptera was attributed was strongly correlated to the levels of Zn, Cu and Pb in the sediments. However, they were unable to detect the mechanisms behind the interactions of each metal and feeding guild in each medium.

In Goodyear and McNeill (1999), the whole Ephemeroptera order was considered as collectors–gatherers, even Heptageniidae that are now known as scrapers–grazers and Ephemeridae that are mainly defined as filters or filter-feeders (REF). Although they feed on small organic particles when they are not predators, e.g. generalist, their feeding style is completely different due to different arrangement and morphologies of mouthparts. The most investigated families are Ephemeridae (*Hexagenia* sp.; *Hexagenia limbata*; *Ephemera danica*; *Ephemera vulgata*); Heptageniidae (*Ecdyonurus venosus*; *Stenonema* sp.; *Rithrogena* sp.); Baetidae (*Baetis* sp.; *Baetis vernus*; *Baetis rhodani*); Ephemerellidae (*Drunella grandis*; *Ephemerella ignita*); Leptophlebidae (*Leptophlebia vespertina*) and Potamanthidae (*Potamanthus* sp.).

The metal levels (Zn, Cu, Pb and Cd) in the feeding guild of collectors–gatherers directly reflect the metal concentrations in sediments and waters. Nevertheless, the low concentrations of aqueous metals in organisms when a high correlation exist between particulate metals and organisms' metal concentrations infer the great impact of sediment metal contamination on these organisms. The concentration gradient between organism and sediment/water metal levels suggests close associations between sediment and water metal concentrations, implying that organisms interact with both metal sources. Metal concentration gradients exhibit the same pattern following the order Cd < Pb ≤ Cu ≤ Zn in sediments and waters, implying a close association between sediment and water metal concentrations and the active engagement of organisms with both metal sources. Liber et al. (2011) evaluated the hypothesis that the metal concentrations in sediment pore are better correlated with sediment toxicity to benthic organisms than whole-sediment metal concentrations; thus, this compartment should have better weight of evidence for the quantification of HM bioaccumulation and toxicity gradient.

The ability of an organism to avert inappropriate metal binding to sensitive sites is connected to its metal tolerance or resistance (www.ec.gc.ca). Toxicity is directly correlated with metal accumulation, specifically for non-essential, non-regulated metals such as cadmium and thallium and only in invertebrates (Borgmann, 2000; Borgmann and Norwood, 1999, 1997a, 1997b; McGeer et al., 2012). The bioaccumulation of copper and zinc in invertebrate tissues is not as informative due to the varying degrees of regulation for these elements, regardless if their background concentrations are high (McGeer et al., 2012).

As mayflies are considered mainly as primary consumers and preys to predators, they can transfer bioaccumulated metals to the next trophic levels of trophic chain and participate in biomagnification as nymph in the aquatic ecosystem (ingested by other predator insects or fish) and as adults when they hatch when they emerge (as praise for birds). Some adult female *Baetis* Baetidae can even return to the aquatic ecosystem and enter into the water column to lay eggs in the bottom sediments onto/under the stones (Sartori and Brittain, 2015). However, the fate of accumulated HM after mayflies have emerged remains unknown.

4.2.3. Alteration on the molecular level

McGeer et al. (2004) and Fairbrother et al. (2007) define bioaccumulation as the net accumulation of metals in tissues or whole organisms due to exposure to an environment or even through diet. For this phenomenon to happen, the metal must be bioaccessible (called bioavailability) to be absorbed into the biological membranes of an organism. Various organisms accumulate metals to different extents (Adams et al., 2011); thus, bioaccumulation toxicity may differ according to a given organism and its physiology, which plays a key role in trapping or detoxifying toxic metals. However, the metals must firstly interact with the cell membranes to generate a biological response and then are transported (facilitated) across cell membranes; they do not cross by simple diffusion (Campbell, 2012, 1995). Once within the intracellular environment, the metals generally undergo detoxification by binding to inducible metal-binding proteins as metallothionein and thus are considered as biologically detoxified, in contrast to metal-sensitive fractions. Ultimately, the metal tolerance or resistance depends on an organism's ability to hinder metal binding to sensitive sites and metal-induced stress that can follow due to spillover (i.e. incomplete detoxification) (Campbell and Hare, 2015).

Metallothionein (MT) is considered a biomarker because it can reflect the biochemical changes that occur in an organism in contact with a stressor (Carter et al., 2006). According to the environment and the zoological group, MT can adapt various isoforms and thus engage in different physiological roles, especially during detoxification (Amiard et al., 2006). In biomonitoring studies, biomarkers help assess metal-specific exposure as their significant increases in response could translate metal bioavailability in a given environment, e.g. for trace metal such as Cd, Cu, Zn and Ag but not metals such as As, Ni, Pb and Hg (www.ec.gc.ca). Nevertheless, Amiard et al. (2006) mentioned that MTs are involved in the metabolic regulation of Cu and Zn and in the intracellular detoxification of excessive metals and non-essential metals Cd, Ag and Hg. Moreover, high MT concentrations may harm organisms and populations by exceeding their capacity and leading to non-specific metal binding to cellular targets of toxicity. Despite the profusion of literature on MT in aquatic macroinvertebrates such as molluscs, crustaceans and annelid worms (Amiard et al., 2006), studies involving mayflies are lacking. Rodriguez et al. (2018) stated that when macroinvertebrates (mayflies included) exhibit high tissue metal concentrations without showing toxicity afterwards, they have entered an acclimation stage by developing a coping mechanism, such as detoxification for processing MT proteins.

Mo et al. (2013) performed one of the rarest studies to identify biomarkers in mayflies by using freshly laid eggs of *Ephemera orientalis*. They observed changes in protein expression in the hatching after exposure to inhibiting concentrations of trace/HMs (i.e. Cd, Cr, Cu, Pb and Hg) and succeeded to isolate three biomarker candidate proteins to detect HM toxicity. The rate of egg hatching over a 14-day exposure period was determined as a toxicity endpoint. Hg exhibited the highest toxicity, with a concentration of 0.11 mg/L, followed by Cu (0.32 mg/L) and Pb (4.39 mg/L). The *E. orientalis* eggs demonstrated significant tolerance to Cd and Cr, surpassing concentrations of 120 mg/L.

5. General impacts of MPs on freshwaters and macroinvertebrates

Research on MPs has historically been more extensive in marine environments than in freshwater environments (Baztan, 2018; Expósito Lorenzo, 2023; Mushtaq Reshi et al., 2023) due to the high public awareness and concerns about the impact of MP pollution and hazards on marine biota raised largely by mediatisation. Meanwhile, data are lacking on the fate of MPs in freshwater ecosystems and biota where they are highly pervasive. Research efforts have started gradually in the last decade, with noticeable emphasis in the last 4–5 years by various environmental scientists that are developing standardised sampling methods and study protocols for assessing MPs in freshwater environments. They explored the origin and role of urban and agricultural runoff and evaluated the potential ecological, physiological, behavioural (diet, locomotion) and biological impairments on aquatic organisms (Carvalho, 2021; Hidayati et al., 2023; Jakubowska et al., 2020; Khedre et al., 2023; Silva et al., 2022; Windsor et al., 2019). These impairments can be bioaccumulation and biomagnification (transfer of contaminants through various levels of the food web), biodiversity threat (populations and community structures shifts), habitat alterations (MPs accumulate and mix with sediments), chemical contamination and ecotoxicity (MPs adsorb other contaminants) and adsorption and combination with HM.

Liro et al. (2023) stated that MPs' occurrence in rivers' downstream can be reinforced by macroplastic degradation from upstream mountain rivers, which act as a MP factory, through mechanical fragmentation induced by high flow and velocities. MP are transported and stored in freshwater ecosystems. Owing to their different forms (fragments, fibres) and densities, their bioavailability differs (Jâms et al., 2020), and their weight will determine the level of their vertical distribution within a cross section of a waterbody. Their concentrations seem to increase generally from the surface water to the water column down to the benthic sediments surface and inside (Mushtaq Reshi et al., 2023). Thus, they affect organisms in different forms, ecosystem layers and habitats. Several different reviews or case reports have addressed the issue of MPs in freshwater environment and its biota (Bhatt and Chauhan, 2023; Castro et al., 2018; Gao et al., 2022; Mushtaq Reshi et al., 2023). However, due to different focus and angle of view (mostly general), the impact on small macroinvertebrates, such as insects and Ephemeroptera, lacks full understanding.

Some recent studies mainly tackled the issue of MP use and ingestion by macroinvertebrates and its presence and/or obstruction of the digestive tube, including Odonata and Trichoptera from Gua Musang Tributaries in Kelantan, Malaysia with cellophane and chipboard (Zain et al., 2022); for *Odontocerum albicorne* (Odontoceridae, Trichoptera) and *Ephemera danica* (Ephemeridae, Ephemeroptera) from River Licenza (Latium, Italy) (Gallitelli et al., 2021). The latter study showed that all caddisflies exclusively reconstructed their cases using the supplied MP polymers, foregoing the use of natural materials. This work provided the first evidence that mayflies predominantly burrow in MP substrates rather than natural ones. D'Souza et al. (2020) were the first to provide evidence of the transfer of plastic through freshwater food webs. Some effects of MP on macroinvertebrates are summarised in Fig. 2 and detailed in the following paragraphs.

MPs have cascading effects on the structure of aquatic ecosystems and the macroinvertebrate communities they house. The spatial occurrence of MPs in waterbodies compartments is determined by their density, i.e. MPs with high densities naturally sink to the sediments, and those with low densities are either suspended in the water column or float on the water surface. Therefore, macroinvertebrates come in contact with MPs depending on their habitat preference or the taxa ecology in a cross section of the aquatic ecosystem (de Sá et al., 2018; Mushtaq Reshi et al., 2023; Szymańska and Obolewski, 2020).

Bioavailability is the core problem of MP occurrence in freshwater environments (Wdowczyk and Szymańska-Pulikowska, 2021). The extent to which these small plastic particles can be absorbed and assimilated by macroinvertebrates is called bioavailability. Depending on this extent, macroinvertebrates can accumulate MPs through ingestion and the MPs remain trapped until the organism dies or is ingested by a predator because they are immune to digestive enzymes (Szymańska and Obolewski, 2020). Eventually, the MPs are transferred to another trophic level. The physical blockage of MPs in the digestive systems can affect nutrient absorption and potentially cause internal damage, though their impact differs according to the species and its feeding behaviours (Moyo, 2022). MP bioaccumulation can also occur in macroinvertebrate tissues (by ingestion or adherence to body) and can induce high concentrations of MPs in consumers higher up the food chain. For, instance, Davidson and Dudas (2016) found no significant differences in MP consumption between free living and reared bivalves (from shellfish farms).

Under laboratory conditions, the concentrations and sizes of MPs used often do not align with those observed in natural conditions, highlighting a gap between field and laboratory conditions (Phuong et al., 2016). Indeed, Ward et al. (2019) highlighted these discrepancies while examining the differences in the size of MP particles ingested by bivalves in natural versus artificial environments. Furthermore, Liza et al. (2024) showed the limitations of various approaches for the detection and extraction of MPs in laboratory conditions. Lusher et al. (2017) highlighted the lack of laboratory conditions on MP contamination and the inconsistency of MP concentrations between experimental and natural conditions, which often include confounding environmental factors. This inconsistency complicates the accurate assessment of organism responses and baseline contamination levels and emphasises the need for standardised methodologies to improve data comparability and avoid overestimating plastic contamination.

MPs can be transferred between trophic levels (Carvalho, 2021; Lourenço et al., 2017) and even between small organisms such as macroinvertebrates of different trophic levels (García-Bueno and Marín, 2021), with the latter seemingly passive (Roch et al., 2020). Mateos-Cárdenas et al. (2019) demonstrated that various types and sizes of MPs enter the freshwater food chain as they move from duckweed to shredders amphipods through the feeding process without any avoidance behaviour; once in the guts, the MPs can be fragmented into small particles, such as in *Gammarus duebeni* (Amphipoda, Gammaridae). Parker et al. (2022) did not detect any bioaccumulation or biomagnification patterns in the egested guts of sampled macroinvertebrates and fish community in Dorset Stour River (SW England) and any relationship–predictability between the ecological, functional (i.e. feeding guild or trophic position) or morphological characteristics (body length) and the quantification of MP levels ingested by these

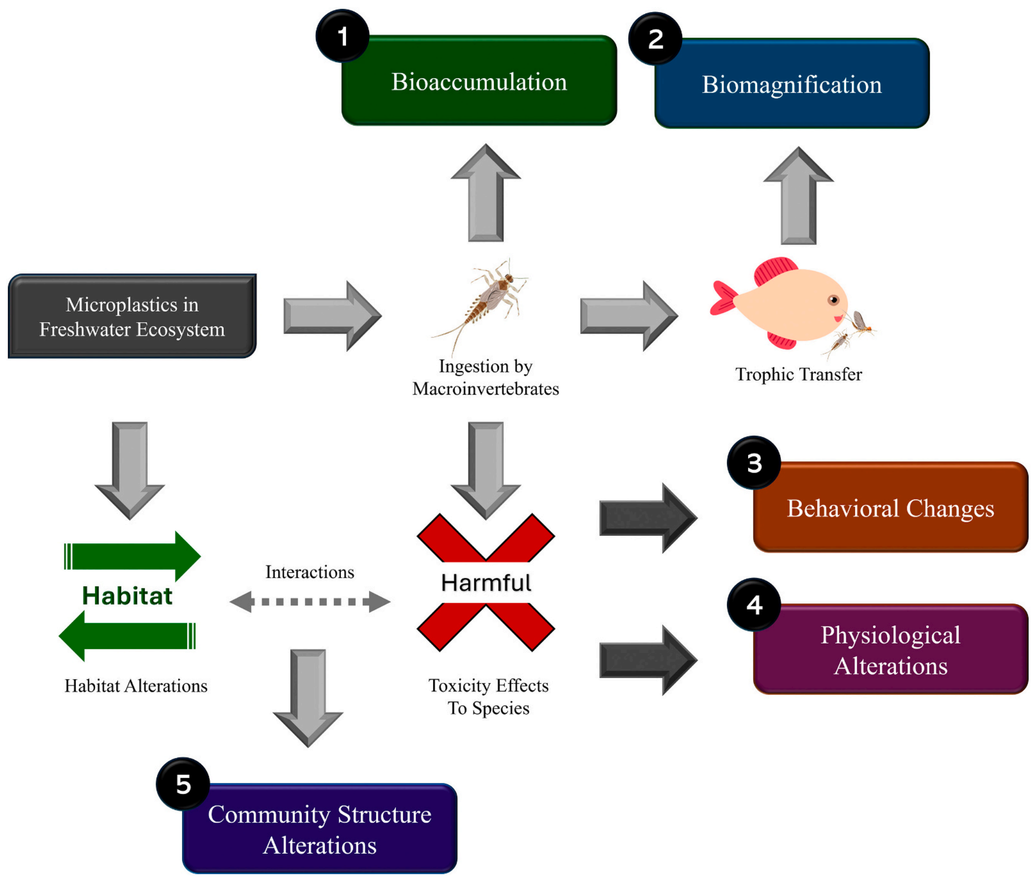

Fig. 2. MP effects on macroinvertebrates.

organisms.

The ubiquity of MPs in sediments deteriorates the structure and composition of natural habitat for macroinvertebrates that may impact their diet (reduced feeding activity) and locomotion (changes in movement patterns). In Windsor et al.'s (2019) case study, MP abundance seems to be linked to macroinvertebrate biomass and taxonomic family, and MP ingestion by macroinvertebrates is independent from the feeding guild/behaviour or biological traits such as habitat affinity and ecological niche. According to Silva et al. (2022), the behavioural and physiological alterations induced by MPs in sediments can contribute to the observed differences in macroinvertebrate abundance.

According to Martínez Rodríguez et al. (2023), single species bioassays experiments are the most common for studying the aftermath of MPs on macroinvertebrates. Minimal interest is given to study communities responses. The same authors explored the impact of oil-based MPs (high-density polyethylene - HDPE) and bio-based biodegradable MPs (polylactic acid - PLA) on freshwater macroinvertebrate communities in a seminatural outdoor mesocosm experiment. Their findings reported no significant differences in total abundance and alpha and beta diversities, community composition based on MP type and concentration or nutrient enrichment between microplastic-exposed groups and a control group. However, at high concentrations of both MP types and under ambient nutrient conditions, a difference was observed in macroinvertebrate alpha diversity which was mediated by nutrient enrichment and a lower diversity in the communities exposed to HDPE compared with those exposed to PLA. Another interesting point regarding MP-induced behavioural changes is the findings of Gallitelli et al. (2021), who reported extraordinary first evidence that mayflies burrow mainly in MP substrates rather than in natural ones in an indoor experiment. The MP substrate was made of a mixture of light MP in equal proportions (polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS)) and was believed to be preferred by these mayflies because it is easily dogged.

The decomposition of MPs releases hazardous substances such as bisphenol A (Szymańska and Obolewski, 2020). MPs can adsorb and convey various toxins and pollutants such as HM (Moyo, 2022). Khdre et al. (2023) identified bioaccumulation features in bioindicator Chironomidae (*Chironomus* sp.) with high concentrations Cu, Pb, Cd and Ni adsorbed on MPs and ingested in *Chironomus* sp. larvae found in wastewater sludge in Sohag Governorate (Upper Egypt). If these kind of associations occur, the ingestion of MPs by macroinvertebrates will lead to chemical exposure and eventually, toxicity, especially, when the desorption of adsorbed substance is enhanced differently in organisms than in water (Bakir et al., 2014; Szymańska and Obolewski, 2020). Zhang et al. (2020) performed holistic ecotoxicology bioassays on *Drosophila melanogaster* (Diptera) and confirmed that MP exposure led to the aggravation of cadmium toxicity in *D. melanogaster* in addition to other exacerbated effects, i.e. gut damage in early instars (larvae). In adults, they observed locomotor dysfunction and increased epigenetic silencing through position-effect variegation in somatic tissues. Thus, this drosophila was suggested as an assessment tool for MP-mediated toxicity. Additionally, MPs cause destress in metabolic and membrane permeability; affect physiological functions in bioindicator taxa (Suman et al., 2021); induce feeding deficiencies and inhibit growth, reproduction and survival (Windsor et al., 2019). For instance, Silva et al. (2023) unveiled substantial delay in the regeneration of auricles (i.e. sensory structures that help detect food and other environment stimuli) regeneration in planarians (flatworms) that ingested MP-contaminated prey.

5.1. Mayflies' bioindication metrics for assessing MP impact

The persistence of MPs in fragile freshwater ecosystems has urged research for effective bioindicators to assess and monitor MP contamination in freshwater environments. In the previous section (Section 4.2), the legitimacy of mayflies as bioindicators of contaminants and HM were investigated. Can mayflies still be good bioindicators of MP occurrence and effects?

The MPs' impact on aquatic insects is seldom studied (Khdre et al., 2023; Stanković et al., 2020). As one of the most sensitive aquatic macroinvertebrates, mayflies are highly affected by MP exposure which alter mainly their diet and physiology. Furthermore, the MPs present in fine sediments can lessen food availability and accessibility for mayflies, cause physical harm through abrasion, lead to respiratory distress by clogging gills and trouble burrowing behaviour and alter their predator avoidance (Beermann et al., 2018; Silva et al., 2023). The mayflies' bioindication metrics for assessing the effect of MPs on freshwater ecosystems are summarised in Table 3 and explained in detail in the following subsections.

5.1.1. Dietary and toxicity impacts

MP ingestion and subsequent toxicity are largely contingent on the ecological traits of macroinvertebrates, specifically their feeding strategy and habitat (Hu et al., 2018; Hurley et al., 2017; Scherer et al., 2017; Silva et al., 2022). In indoor mesocosms (artificial stream), Silva et al. (2022) assessed the adverse ecological effects on benthic macroinvertebrate community structure exposed to polyethylene MPs (PE-MPs) commonly found in North-American and European running waters (1 and 10 g/kg) mixed in sediments during a short period (8 days). Alterations in the macroinvertebrate community structure were predominantly observed through a reduction in deposit-feeders Chironomidae. Baetidae and Ephemerellidae, which are considered as grazers, lost approximately 1/3 to 1/2 of their abundances upon exposure to 1 and 10 g/kg, respectively, implying high bioaccumulation in these two groups (in grazers mayflies ca 166 particles were assessed in a single individual of *Baetis* sp. and 415 in *Ephemerella* sp.). No alterations were detected in the other feeding functional groups (filter-feeders, shredders and predators).

The diet of grazers and detritivores depends on leaf decomposition and primary production (e.g. periphyton, microalgae), which are reduced by MP occurrence (Silva et al., 2022). For instance, PS MPs decreased the accumulation of essential fatty acids in common freshwater algae *Chlorella sorokiniana* (Guschina et al., 2020). Wu et al. (2019) reported that the exposure to MPs induced a decline in the photosynthetic activity and chlorophyll A concentrations of freshwater microalgae. Moreover, the introduction of MPs inside the trophic chain caused distress in the energy transfer through the trophic levels (Palmer et al., 2000; Silva et al., 2022). According to Parker et al. (2022), mayflies are highly susceptible to MP contamination because the MP particle load in these macroinvertebrates was higher in Ephemeroptera (mean of 0.74 particles per individual). They hypothesised that mayflies could mistake FPOM coated with blue or green fragments and fibres of various polyolefins for blue-green algae which they usually feed on as collectors–gatherers and scrapers.

Windsor et al. (2019) investigated the consumption of MPs in primary consumers from the insect families Baetidae, Heptageniidae and Hydropsychidae, helping identify the source and entry points of MPs that occur at the basal level of freshwater food webs and explore remediation solutions using these organisms in biological and ecological risk assessment in freshwater ecosystems. In this latter study, Baetidae specimens (larvae) held MP concentration from slightly over 0.01/mg to slightly over 0.04/mg (dry weight) and Heprageniidae from 0.01/mg to 0.03/mg (dry weight).

During their aquatic life, mayflies may inadvertently ingest MPs (mistaken for food) present in the water column or embedded in sediments. Ingested MP particles can damage, obstruct or at least disrupt the digestive systems and nutrient assimilation, potentially affecting growth and development. The feeding behaviour of mayflies (**filter feeder**, filters organic particles and detritus from the water; **collector–gatherer**, collects organic materials and detritus from the substrate; **or even predators)** will determine the size and number of ingested MPs and the most affected organisms. In addition to the physical impact and impairments, MPs can carry adsorbed and accumulated pollutants. If swallowed by mayflies, they may lead to toxicity and other physiological impairments. Furthermore, mayflies' locomotive behaviour and relationship with the different aquatic compartments (water or sediments) will determine the intensity of MP ingestion, e.g. the swimming or epibenthic filter feeding diet will increase the likelihood of encountering suspended MP particles in the water column. Scrapping, burrowing or endobenthic behaviour favours the ingestion of MPs in sediments.

MPs at concentrations reaching up to 0.14 MP/mg tissue were identified in Baetidae and Heptageniidae, included in the trophic guild of grazers. Nevertheless, mayfly swimmers such as Baetidae have a low content of MPs, implying that organisms residing in water column are less prone to encounter high MP concentrations. Heptageniidae are epibenthic elements and mostly scrapers feeding on fine organic matter covering rocky bottoms where MPs are dense (Windsor et al., 2019). Mayflies (e.g. from Ephemeridae family) alter the composition (grain size), structure (porosity) or dynamics of sediments (sediments mixing), thus participating in bioturbation and enhancing the nutrient cycling by facilitating the decomposition of organic matter within the sediments, contributing to the release of nutrients such as nitrogen and phosphorus into the water.

5.1.2. Bioaccumulation and biomagnification

In any organism, the bioaccumulation of MPs is triggered when the threshold of excretion capacity is surpassed (Parolini et al., 2023). Mayflies are an important part of aquatic food webs as nymphs and even terrestrial ones as flying adults. They can be herbivorous, detritivore, omnivore or even predators. In all cases, they are prey to predators. However, in their terrestrial or aerial stage, they have vestigial mouthparts and are incapable of ingestion but would still have bioaccumulated MPs from their aquatic life. Nonetheless, bioaccumulated MPs in mayflies' tissues potentially affect high trophic levels; this trophic transfer is also called biomagnification.

The impact of MPs on mayflies' life cycle has not been investigated, but studies demonstrating significant effects on the biology of Diptera Chironomidae (growth, development, and emergence patterns) may

Table 3
Mayflies' bioindication metrics for assessing MP impact.

Level	Metric	Description	Pollution indicator
Dietary and toxicity impacts	Community structure	Deposit-feeders composition	Lower abundance due to ingestion and bioaccumulation
	Organ deformation	Alteration of functional organ	High deformity in polluted area indicating higher ingestion
Bioaccumulation and biomagnification	Metal tissue accumulation	Metal concentration in tissue based on feeding behaviour	Higher concentration in collectors and scrapers
Molecular level	Biomarker	Acetylcholinesterase (AChE) activity	Elevation of AChe indicated detoxification mechanisms

hint similar effects on Ephemeroptera (Scherer et al., 2020b; Silva et al., 2022, 2019; Stanković et al., 2020). Therefore, our knowledge about the flow and fate of MPs within the life cycle of mayflies, especially after their emergence, is poor. Zhang et al. (2020) analysed MP transfer and threat beyond the aquatic part of this cycle, as MPs being clogged in insects' small guts are definitely present in flying mayfly adults and are transferred to predators or other environments. Stanković et al. (2020) noticed mouthpart deformities and alterations in developmental time (longer) and length of adult wings in Diptera Chiromidae *Chironomus riparius* upon exposure to a mixture of MPs in sediments, proving the impact of MPs on all insect life stages. D'Souza et al. (2020) is the only one to observe the transfer of MPs (MPs) from mayflies (Ephemeroptera) collected in microplastic-contaminated catchments in South Wales, Great Britain, to their predators, species of dipper birds. This transfer was noted due to the considerable similarity in size and morphology of the MPs found in the prey (mayflies) and predators' (dipper birds) faecal pellets.

5.1.3. Biomarker research

According to Suman et al. (2021), biomarkers in invertebrates and vertebrates can help detect and quantify anomalies in biological processes or responses on the molecular, biochemical or histopathological level. For example, the inhibition of acetylcholinesterase (AChE) activity can indicate neurotoxic effects or changes in enzyme activity involved in detoxification. Nevertheless, the understanding of the toxicity of MPs is still constrained, which is even worse for such important aquatic bioindicator insects such as mayflies. No biomarker study for MPs' physiological effects exists.

The influence of MPs on mayflies underscores the intricate connections between pollution and freshwater ecosystems. As research in this field continues to evolve, there is a growing need for comprehensive studies to understand the mechanisms and consequences of MP exposure on mayflies and other aquatic organisms. We undeniably need further research to discern the polymer composition of MPs retrieved from mayflies or in macroinvertebrates in general, especially when plastic fibres can be really small. Karaban et al. (2023) recorded the high occurrence of 700–1900 μm fibre length and even smaller 10–200 μm in Central Poland (Lake Dziekanowskie and Reservoir Ruda). Hidayati et al. (2023) recorded several polymers found in fish guts from Indonesia, namely, PS, nylon, acrylonitrile butadiene styrene (ABS), polyurethane (PU), polypropylene (PP), high-density polyethylene (HDPE) and low-density polyethylene (LDPE). Developing sustainable practices and implementing remediation measures are imperative to mitigate plastic contamination within aquatic environments.

Simmerman and Coleman Wasik (2020) retrieved MPs in 100 % of Ephemeroptera from the Heptageniidae family, suggesting bioaccumulation (body burden) in a group of macroinvertebrates from the Kinnickinnic River (Western Wisconsin, USA). Among them, the Heptageniidae larvae with a mean length of ingested MP particle between 147 and 340 μm typically mirrored the observed pattern of MP concentrations in water samples. Using automated μFTIR Imaging, Pan et al. (2021) found taxon-specific uptake of MPs, with acrylates/polyurethane/varnish prevalent in sediments and ethylene propylene diene monomer and polyethylene-chlorinated dominant in invertebrates that included Ephemeroptera. They demonstrated that MP polymer composition varied notably between invertebrates and sediment, and the size of ingested MPs in Ephemeroptera was influenced by the MP levels and size in the sediment. Pan et al. (2021) suggested a filtering effect in trophic transfer at the food web's base, i.e. biomagnification.

To tackle the lack of standardization in ecotoxicity tests, Abdolahpur Monikh et al. (2023) formulated an exposure protocol designed for ecotoxicity bioassays, specifically tailored for testing MPs (MPs) and nanoplastics (NPs). The protocol considered the particle-specific characteristics, dynamic behaviour and toxicity of MPs and NPs within exposure systems. These identified physicochemical properties were deemed crucial and effective in evaluating the associated risks and were tested on two aquatic species *Daphnia magna* (Crustaceans, Daphnidae) and *Paracyclopina nana* (Crustaceans, Copepoda).

The absence of investigations pertaining to biomarkers inherent to aquatic insects, specifically mayflies, is a matter of concern, especially given their huge importance as bioindicators and as one of the first links in the food chain. Scientifically phrased, the field of biomarker research is still in its early stages. Suman et al. (2021) reviewed the following biomarkers for MPs to detect and assess fish and several bioindicator-macroinvertebrates responses to MP contamination (mussels and other bivalves, crustaceans, nematode worm and aquatic species): antioxidant enzymes, lipid peroxidation, DNA oxidative damage and DNA adducts and AChE and lysosomal stability.

6. Current limitation and future approach

This current review sets the stage for investigating the effectiveness of mayflies as bioindicators of HM and MP. The different impacts of MPs on mayflies can vary depending on factors such as the type of MPs, their concentration and the species of mayflies involved. Understanding these impacts is crucial for assessing the overall health of freshwater ecosystems and the organisms inhabiting them. Current challenges and future approaches for the use of mayflies as bioindicator of HMs and MPs pollutions in freshwater ecosystem are summarised in Fig. 3.

The sensitivity of bioindicators may exhibit temporal variations within seasons and over extended periods (Ovaskainen et al., 2019). Seasonal variations in the concentration of HMs and MPs in freshwater aquatic environments can be attributed to several causes, such as precipitation, temperature and runoff (Hu et al., 2022; Qiu et al., 2021). These fluctuations have the potential to impact the reliability and precision of bioindicator evaluations. Additionally, freshwater aquatic ecosystems can exhibit inherent fluctuations in HM concentrations due to geological influences and the presence of metals at baseline levels within the surrounding environment. These variances might pose a challenge in discerning between alterations in bioindicators caused by natural factors and those generated by pollution. Developing such a record of the native species and invasive species existence in specific areas might be the baseline for future research to analyse the seasonal variations for bioindicators of pollutions.

The field of MPs research currently faces a dearth of universally recognised and established protocols for employing bioindicators in this context (Alimi et al., 2022; Kurniawan et al., 2021). This phenomenon might give rise to discrepancies in data gathering, hence posing difficulties in comparing findings across various investigations. The detrimental impact of MPs on aquatic creatures is evident, yet a comprehensive understanding about the long-term consequences and ecosystem-wide implications of MP contamination is lacking (Du et al., 2021). MPs pose a significant obstacle in establishing unequivocal causal connections between these particles and the ecological well-being of ecosystems. Analysing and summarizing the impact of each applied step or protocol (Kurniawan et al., 2023) on the selected MP types might be a good starting point for the development of standardised protocol for MP analysis to assess its abundance and/or toxicity.

The applicability of mayflies to be used as bioindicators may vary across different locations and ecosystems (Parmar et al., 2016) and depend on the prevailing local environmental circumstances. Hence, selecting suitable indicators that align with the unique characteristics of the studied ecosystem is crucial. Conducting preliminary studies related to mayflies in the studied area will benefit the whole research of using these taxa as bioindicators of HM and MP pollutions.

Bioindicators typically offer insights into recent or current instances of pollution and may not be appropriate for evaluating prolonged or persistent exposure to HMs and/or MPs (Khdre et al., 2023). Meanwhile, the complexity of ecosystems is heightened by the coexistence of several stressors, such as nutrient pollution and habitat loss, which can provide challenges in comprehending the reactions of bioindicators, including mayflies (Sagova-Mareckova et al., 2021). Discerning the specific

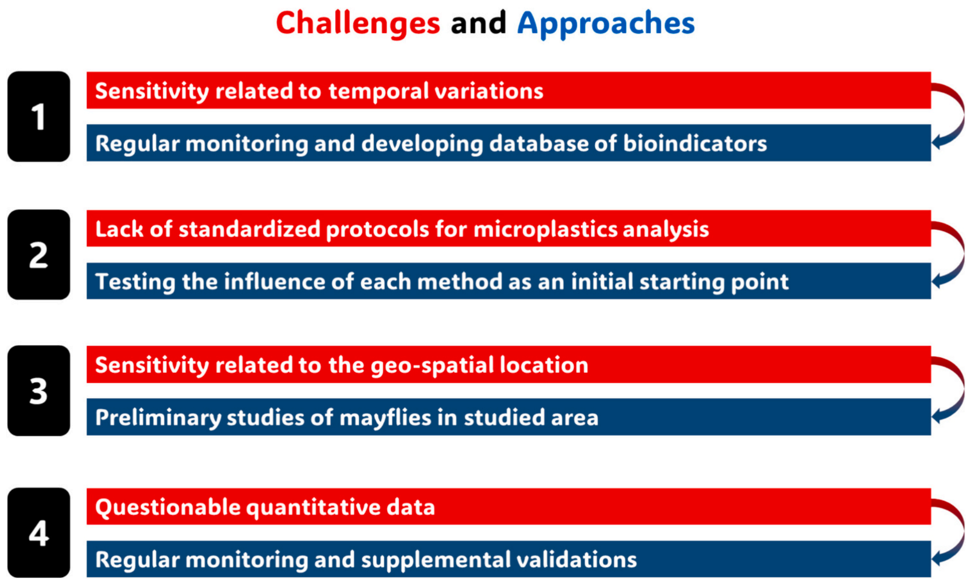

Fig. 3. Current challenges and suggested future approaches for the use of mayflies as bioindicator of heavy metals and microplastic pollutions.

impacts of HM contamination in isolation from other concurrent environmental stressors is a considerable challenge. Bioindicators can provide qualitative or semi-quantitative data on the levels of pollution (Zaghloul et al., 2020). Regular monitoring and database establishment and supplementary chemical studies for validating the reliability of mayflies as bioindicators are necessary to acquire accurate and measurable data on the amounts of HMs and MPs.

7. Conclusion

The increasing presence of HM and MP contamination in freshwater environments has become a significant cause for worry. Mayflies can function as bioindicators for HM because the HM levels can forecast the shape of benthic communities and influence the physiology and behaviour of these organisms. Mayflies exhibit a high sensitivity to metal pollution, and their behaviour can serve as an indicator for its consequences, including as eutrophication, changes in ecological structure, inhibitory effects and sediment toxicity. Exposure to MPs can result in ingestion, accumulation and magnification; modification of habitats and communities; changes in behaviour and toxicity. The challenges encompass fluctuations in the sensitivity of bioindicators with time, the absence of globally acknowledged procedures and the variable suitability of their use across different ecosystems.

CRediT authorship contribution statement

Nadhira Benhadji: Writing – original draft, Supervision, Data curation, Conceptualization. **Setyo Budi Kurniawan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Data curation, Conceptualization. **Muhammad Fauzul Imron:** Writing – review & editing, Writing – original draft, Visualization, Project administration.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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