

# Assessment of biomarker-based ecotoxic effects in combating microplastic pollution - a review

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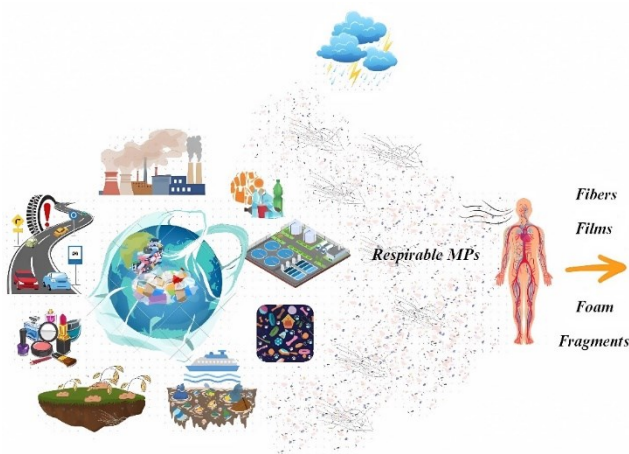
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## Graphical abstract



## Abstract

Microplastics, found in various environments oceans, freshwater systems, soil, and the atmosphere, can enter ecosystems through various pathways, including the degradation of macroplastic parts or direct release from consumer products. By polluting terrestrial, freshwater, and marine ecosystems, their formation, fate, and distribution increasingly threaten living life and ecosystems. Once found in the environment, microplastics can persist for a long time and cause toxicity by accumulating in different ecological sections. These particles can be ingested by a wide range of organisms, including species living in water, birds, and terrestrial animals, negatively affecting ecosystem functioning. Ecological risks associated with microplastics include disruption of food webs, altered nutrient cycling, and potential long-term effects on population dynamics and ecosystem stability. The accumulation of microplastics and associated toxicants in organisms can have cascading effects on higher trophic levels and ultimately affect entire ecosystems. However, biomarker studies have revealed the potential for bioaccumulating microplastics and related chemical pollutants throughout the food chain. With the analysis of biomarkers, the uptake and accumulation of

microplastics in the gastrointestinal tract, tissues, and organs of organisms can be determined. Biomarkers help assess the impact of pollutants on individual organisms and provide insight into potential risks to entire ecosystems. Therefore, it is crucial to develop effective mitigation strategies, environmental monitoring programs, and regulatory measures to minimize the harmful effects of microplastics on ecosystems and human health. This study specifies the toxicity effects of microplastic detection on living organisms in the receiving environment through biomarker-based monitoring studies and also emphasizes the need for a multidisciplinary approach.

**Keywords:** Biomarker, ecosystem, environmental impact, living organisms, microplastic, toxicity

## 1. Introduction

The environmental impact of microplastics (MPs) is a growing concern and has significant implications for ecosystems worldwide (de Souza Machado *et al.* 2018). In recent years, microplastics have become an important issue regarding environmental problems and human health (Sol *et al.* 2020; Lamichhane *et al.* 2023). Typically, smaller than 5 mm, these plastic particles have become common in a variety of environments, including oceans, rivers, lakes, and even terrestrial ecosystems (Eerkes-Medrano *et al.* 2015; Horton *et al.* 2017; Priya *et al.* 2022). The potential toxicity of microplastics to living organisms, which has emerged as an important environmental problem due to their widespread presence in various ecosystems, raises concerns about their effects on ecological health (Dong *et al.* 2021). Therefore, their presence in the environment can harm aquatic and terrestrial organisms (Jiang *et al.* 2020). One of the main concerns is the ingestion of microplastics by various organisms. Marine species such as fish, seabirds, and marine mammals often mistake microplastics for food, causing potential harm (Bajt 2021). When ingested by organisms as prey, microplastics can physically damage their digestive tract, causing blockages, malnutrition, and reduced nutritional efficiency, compromising growth, reduced reproductive success, and increased mortality in affected populations (Kalaiselvan *et al.* 2022). Another

environmental impact of microplastics is their ability to accumulate in organisms and bioaccumulate through the food chain (McIlwraith *et al.* 2021). Microplastics accumulate in the tissues of microorganisms and adversely affect the growth and development of these organisms (Maghsodian *et al.* 2022). This means that many organisms, including humans, could be exposed to higher levels of microplastics and associated pollutants. In a different scenario, microplastics catalyze the inception of potential risks, primarily driven by their intrinsic capacity to serve as vectors for various pollutants. This intricate interaction underscores the multifaceted role of microplastics as potential agents for the transport and dispersion of a wide range of contaminants, thereby amplifying the complexity of environmental risk dynamics (Baho *et al.* 2021). The other concern is that these emerging pollutants can adsorb and accumulate toxic chemicals such as heavy metals and persistent organic pollutants (POPs). For example, in the study conducted by Akhbarizadeh *et al.* (2021), it was reported that microplastics can absorb various pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxin-like chemicals, polybrominated diphenyl ethers (PBDEs), toxic metals, hydrophilic organic compounds (such as ciprofloxacin), and pharmaceuticals (antibiotics and antidepressants).

When organisms ingest toxic microplastics containing these chemicals (Lamichhane *et al.* 2023), the release of these substances can occur, leading to further negative impacts on health and the overall functioning of ecosystems. This situation can cause habitat change and deterioration of ecological interactions (Talukdar *et al.* 2023). For example, the accumulation of microplastics on the seafloor or in river beds can alter sediment properties by affecting benthic organisms and sediment-dwelling communities (Coppock *et al.* 2021). Microplastics can also cause population declines and ecosystem imbalances by interfering with feeding behavior, reproduction, and immune responses in various species (Ma *et al.* 2020; Bostan *et al.* 2023). This situation poses increasing risks and dangers to ecosystems, and its persistence, especially on land and in water, negatively impacts human health (Afreen *et al.* 2023). Therefore, addressing the environmental impacts of emerging microplastics requires a multifaceted approach (Nikiema and Asiedu 2022).

In this context, it is very important to evaluate the toxicity of microplastics on living organisms and to understand the biomarkers that contribute to a better understanding of the physiological and biochemical responses and toxic effects that occur when living organisms are exposed to microplastics. In this approach, the presence and severity of toxicity caused by microplastics can be determined, and their overall environmental impact potential can be evaluated. Current studies showed that Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy are commonly employed to determine the chemical composition of microplastics (Xu *et al.* 2019; Kumar *et al.* 2021). Microscopy is used for visual inspection and examination of the morphological characteristics of microplastics (Kalaronis *et al.* 2022), while polymerase

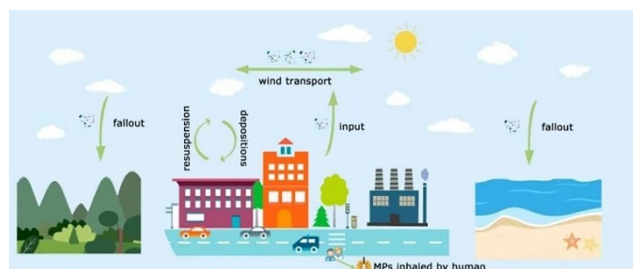
chain reaction (PCR) (Liu *et al.* 2019; Sun *et al.* 2021) and immunological tests (Nakanishi *et al.* 2023) can assist in identifying the type and source of microplastics. DNA-based identification (barcoding) is used to determine the species of microplastics through genetic analysis (Nelms *et al.* 2019; Rsondoni *et al.* 2021). Additionally, special filters and membranes can also be used to physically isolate and collect microplastics from samples, increasing the concentration of microplastics and making them more sensitive for analysis (Shen *et al.* 2021; Jiao *et al.* 2022; Guan *et al.* 2023).

This study aims to highlight the microplastic pollution in terrestrial and aquatic ecosystems and determine the ecotoxicological effects observed in these ecosystems. In addition to microplastics' presence and potential impact on air, waters, and soil ecosystems, the focus will also be on the effects on human food security, animals, and human health. The study also aims to investigate the damage caused by microplastics on living organisms and commonly observed biomarkers to address the challenges and address perspectives and challenges for future research by revealing the alarming effects of microplastics.

## 2. The adverse effects of microplastics on the environment and biota

### 2.1. The effects of microplastics in the atmosphere

Although research has predominantly focused on the effects of microplastics in aquatic ecosystems, the presence and potential effects of microplastics in the atmosphere have also become noteworthy (Kacprzak and Tijing 2022). Microplastics released into the atmosphere can occur through various pathways, including air pollution sources, the degradation of plastic particles, and erosion (Lwanga *et al.* 2022). Microplastics found in the atmosphere, particularly in conjunction with natural processes such as wind and erosion, have the potential to disperse and be transported over long distances (Yang *et al.* 2023). Consequently, microplastics can be inhaled by humans and other organisms through respiration, suggesting that microplastics may adversely affect respiratory health. Moreover, the ability of microplastic particles to spread and be transported in the atmosphere can contribute to their widespread presence in different regions and their dispersion into ecosystems (Amato-Lourenço *et al.* 2020) (Figure 1).



**Figure 1.** Schematic representation of the distribution of microplastics in the atmosphere. Reproduced with permission from (Chen *et al.* 2020)

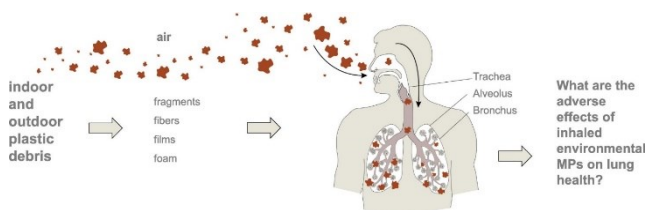
The release of these particles into the atmosphere from various sources encompasses a wide spectrum. Primarily,

textile products play a significant role in the release of microplastic fibers. These fibers, generated during washing, drying, wearing, and use, can escape into the atmosphere by evading wastewater treatment plants or household washing machines (Henry *et al.* 2019). Similarly, cosmetic products, especially personal care items like peeling agents, toothpaste, shower gels, shampoos, and makeup, can contribute to air pollution due to their microplastic content after use (Bashir *et al.* 2021). The breakdown of plastic waste is another crucial source. Plastic products in the sea or on land can transform into microplastics under the influence of factors such as sunlight, temperature, humidity, oxygen, microorganisms, and mechanical forces, and can be transported into the atmosphere by factors like wind or rain (Liu *et al.* 2022). The melting of glaciers, linked to global warming, is another factor leading to the release of microplastics into the atmosphere. This occurs by liberating microplastics stored within glaciers for extended periods (Zhang *et al.* 2022). Additionally, the increased use of disposable materials and masks during the COVID-19 pandemic has emerged as a new source of microplastic pollution. These materials, if improperly disposed due to inadequate waste management or lack of awareness, can transform into microplastics in the environment. Moreover, during the use of masks, the moisture and heat generated from respiration can disrupt the structure of the masks, leading to the airborne release of microplastic fibers. The convergence of these factors highlights the significance of understanding the impact of microplastic pollution on air quality (Torres-Agullo *et al.* 2021; Lee and Kim 2022).

The long-distance transport of microplastics occurs through atmospheric air movements, primarily driven by the force of wind. During this process, microplastics can spread among different regions. Notably, plastic pollution in coastal areas can be transported inland and even to remote mountainous regions, plateaus, polar regions, and troposphere through the influence of wind (Huang *et al.* 2022). In a study aimed at understanding the types and forms of microplastics in the atmosphere and developing targeted solutions to minimize their impact on ecosystems and human health, Mbachu *et al.* (2020) conducted research on various sampling techniques for collecting samples from street dust (< 100-5000  $\mu\text{m}$ ), indoor air ( $\leq$  3250-4850  $\mu\text{m}$ ) and outdoor air (2-9555  $\mu\text{m}$ ), as well as examined sample preparation, pre-treatment and physical characterization techniques. The findings revealed that microplastics were ubiquitously detected in diverse atmospheric environments, encompassing street dust and indoor and outdoor air. The concentrations of these particles exhibited variations attributed to personal preferences, human-induced actions, and weather patterns. The study showed that the most common polymers, including spheres, films, fragments, fibers, foam (Figure 2), and granules, were polyamide (PA), polyethylene (PE), polypropylene (PP) and polyester.

The results also showed that the majority of the identified shapes were fibrous microplastics, representing various forms of microplastics. Studies on the characterization and

density of microplastics in the atmosphere are listed in Table 1.



**Figure 2.** Effects of dispersal of microplastic forms in the atmosphere. Reproduced with permission from (Amato-Lourenço *et al.* 2020)

The distance transport of microplastics raises concerns about their environmental impact. Evidence of long-distance transport of microplastics in the troposphere indicates that the effects of these plastics may exceed the boundaries of regions where sources of pollution exist (Allen *et al.* 2021; Zhang *et al.* 2021). During long-distance transportation, microplastics' shape, size, omnipresence, and lightweight nature play a significant role (Zhang *et al.* 2021). However, small-sized microplastics can remain in the atmosphere for longer periods and can be transported to more distant locations. Additionally, interacting with other particles in the atmosphere, microplastics can form agglomerates that facilitate their long-distance transport. (Allen *et al.* 2021). These particles can reach ecosystems and water resources in remote areas and adversely affect plant health, pollution in water resources, food chain in ecosystem, and soil fertility (Singh *et al.* 2023) and can block the stomata of plants and affect photosynthesis and respiration processes (Wang *et al.* 2023). In addition, Napper *et al.* (2020) have reported that microplastics in the atmosphere can fall to the earth through rain and snow and reach water resources, leading to increased microplastic pollution in aquatic ecosystems and adverse effects on aquatic and terrestrial organisms.

Thus, microplastics can spread among organisms in the ecosystem and create biological effects (Wang *et al.* 2022). For example, as a result of a wide range of transport of microplastics influenced by various factors, the particles can settle into the soil, which can limit fertility, accumulate in the soil, disrupt the soil structure, reduce water-holding capacity, and hinder the development of plant roots (Rose *et al.* 2023). Additionally, supported by a body of evidence indicating significant effects on environmental and biological systems, the toxicity of microplastics is clear. In a study of microplastic pollution on the ecological environment, Mao *et al.* (2022) reported that the transport of microplastics in the atmosphere can also adsorb toxic pollutants, heavy metals, and organic pollutants in the atmosphere to their surfaces, which may cause the pollutants to combine with microplastics to be transported in the atmosphere and then spread to different regions. Zhang *et al.* (2022) highlighted the risks of biopollution and the introduction of pollutants into ecosystems through microplastics may increase unpredictably. In addition to these, De-la-Torre *et al.* (2021) pointed out that the effects of microplastics on human health through respiration are also being investigated. According to another study on the properties and toxic effects of microplastics in the

atmosphere, Yang *et al.* (2021) examined the dispersion, origins, and destiny of these particles, along with the factors influencing their presence. The researchers emphasized that inhalation is the primary route through which atmospheric microplastics enter the body, leading to

systemic exposure and toxic reactions (Figure 3) and impairments in different organs and systems. Furthermore, the researchers highlighted the potential cancer risk these microplastics pose to animals and humans.

**Table 1.** Types and concentrations of microplastics in different locations

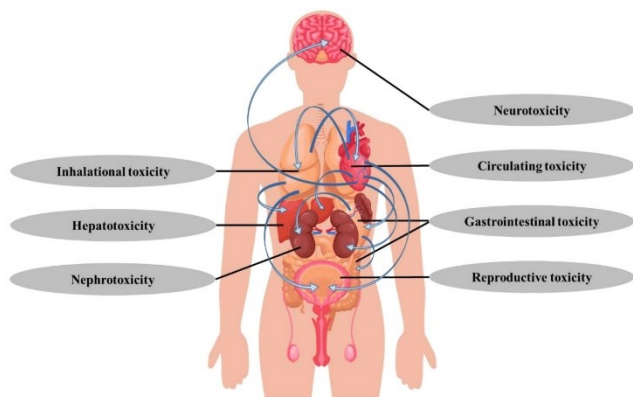
Location	Particle Type	Particle Concentration	Particle Dimension	References
Greater Paris region	Synthetic fibres	29–280 particles/m <sup>2</sup> /day or 3.5–7.6 × 10 <sup>10</sup> MPs/year	Around 50% of the fibers were over 1000 µm, while the remaining particles were evenly split between the 100–500 and 501–1000 µm categories.	(Dris <i>et al.</i> 2015)
Dongguan, China	Fibrous and non-fibrous/MPs from atmospheric deposition	175–313 particles/m <sup>2</sup> /day	Colored fibers: 400 µm, PS foam: 2 mm, PP fragments: 1 mm, PE films: 1 mm	(Cai <i>et al.</i> 2017)
Shanghai	Fibres (67%), Fragments (30%), Granules (30%), Synthetic compounds (54%)	Various types, not specified	The observed microplastics varied in size from 23.07 to 9555 µm, averaging 582.2 µm.	(Liu <i>et al.</i> 2019)
University of Nottingham, UK	Natural textile fibres (97.7%)	Average from 7,810,000 to 197,000,000 fibers/day	-	(Stanton <i>et al.</i> 2019)
London	Various polymers, 92% fibres	575–1008 particles/m <sup>2</sup> /day	Fibre dimensions varied between 20 and 25 µm, with some as thin as 5 µm and others as thick as 75 µm. The predominant lengths fell within the range of 400 to 500 µm. The predominant non-fibrous microplastics had lengths ranging from 75 µm to 100 µm. With the exception of a single microplastic (low-density PE film, 1080 µm), all other non-fibrous microplastics were below 350 µm in size. The smallest identified particle (high-density PE) measured 25 µm, and the average size of non-fibrous microplastics was 164 ± 167 µm.	(Wright <i>et al.</i> 2020)

Table 1 represents microplastic samples from specific locations. The data reflects air pollution levels in different regions.

For example, according to Chen *et al.* (2020), When entering the body through breathing, microplastics have the potential to settle in the lungs, leading to various health problems. Therefore, monitoring and understanding microplastic distribution and distant transport is important for the health of the transport process and respiratory system. Similarly, Amato-Lourenço *et al.* (2020) figured out that the primary concern is that microplastics can penetrate the lungs through inhalation, leading to chronic inflammation. The accumulation of microplastic particles in the lungs can contribute to the chronic inflammation process and affect lung function, which can cause respiratory problems and an increase in asthma, bronchitis, and other respiratory diseases. However, other potential effects of microplastics on the respiratory system are also being studied. Lamichhane *et al.* (2023) showed that toxic effects may occur upon the release of the chemicals contained in microplastics. These chemicals may be additives added to the structure of the microplastic or attached to the surfaces of environmental pollutants.

According to Wright and Kelly (2017), releasing these chemicals by inhaled microplastics can damage lung cells and lead to health problems. Prata (2018) focused on the impact of exposure to airborne microplastics on human health, highlighting that excessive dust loading, oxidative stress, and translocation could lead to diseases. The study also reported that chronic exposure to microplastics could result in injury or even death. Synthetic fibers were found in human lung biopsies, indicating their presence due to microplastic exposure.

In this context, microplastics are recognized as a significant component of environmental pollution, and their presence in the atmosphere raises concerns for environmental health. Therefore, it is essential to prioritize key goals such as reducing pollution sources, promoting sustainability efforts, establishing environmental policies, and developing measures to mitigate the effects of microplastics. These actions are crucial for minimizing the impacts of microplastics and safeguarding the environment.



**Figure 3.** The detrimental repercussions of microplastic pollution in the atmosphere on various organ systems. Reproduced with permission from (Yang *et al.* 2021)

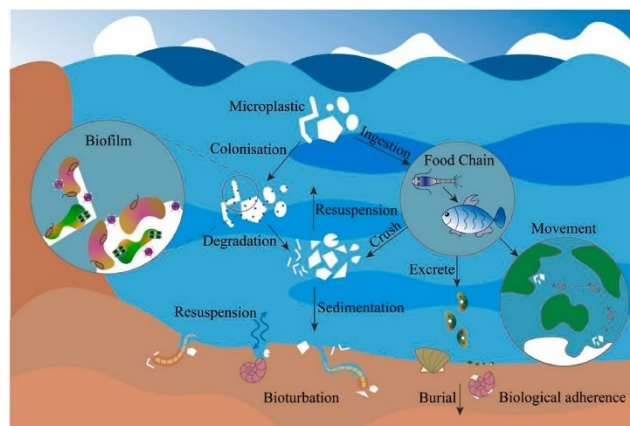
## 2.2. The effects of microplastics on the aquatic environment

Microplastics have been identified as a cause of substantial detrimental effects on the environment and various forms of biota. Two major factors influence microplastics' presence and dispersion in aquatic ecosystems. The first set comprises the intrinsic characteristics of microplastics, such as their hydrophobicity, specific gravity, and size. The second set encompasses environmental factors, including biological interactions within aquatic environments, meteorological phenomena, and the proximity of industrial facilities to water systems (Kye *et al.* 2023).

The tiny plastic particles, ubiquitous in various ecosystems, pose a growing threat to the ecological balance. Microplastics contribute to the deterioration of ecosystem functions and structures, increasing pollution and ecosystem degradation raising concerns about potential environmental consequences (Judy *et al.* 2019). Many different researchers have explained the degradation and environmental effects of microplastics. According to Fan *et al.* (2023), the migration and degradation of microplastics are influenced by crucial biological factors, including organisms consuming and breaking down microplastics in their digestive systems, the accumulation of microorganisms and algae on the surface of microplastics, organisms transporting microplastics vertically or horizontally in the water column or sediments, and the ability of microplastics to adhere to the body surfaces of organisms through mucus secretion. A study by Klun *et al.* (2022) demonstrated that water fleas *Daphnia magna* interacted differently with bakelite fragments, measuring  $7.6 \pm 3.5 \mu\text{m}$  and present at 100 mg/L concentration. According to the findings, 84% of the microplastics were found inside the water fleas through ingestion, whereas only 16% were observed sticking to their surface. Bivalves have also been investigated for their potential adhesion properties, as demonstrated by Kolandhasamy *et al.* (2018). The study focused on *Mytilus edulis*, which was exposed to 2000 fibers/L (with unspecified material). The research revealed that adhered microplastics constituted 42-59% of the total uptake in these organisms. Similarly, as indicated by Costa *et al.* (2020), *Jellyfish Aurelia sp.* species have been found to absorb microplastics (PE particles measuring 1-4  $\mu\text{m}$ , present at concentrations ranging from

0.01 to 10 mg/L) through adhesion, with a particular affinity for microplastics adhering to their oral arms.

The density of microplastics can be altered by forming feces, residues, or biofilms, potentially causing them to sink (Figure 4). Also, digestion and colonization processes promote the degradation of microplastics (Kowalski *et al.* 2016). In contrast, dynamic processes, photochemical oxidation, and biological activities have diverse and intricate effects on microplastics, shaping their ultimate fate in the environment (Boldrini *et al.* 2021).

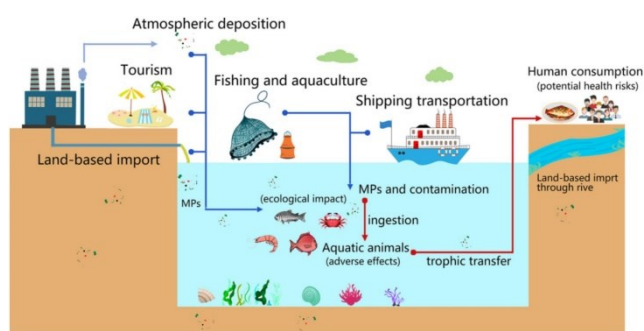


**Figure 4.** The movement and alteration of microplastics in the marine environment result from biological factors. Reproduced with permission from (Fan *et al.* 2023)

While research on the environmental impact of microplastics continues, according to Birch *et al.* (2020), there is still a lack of knowledge about their presence and impact, particularly in freshwater and soil environments. In particular, the effects of microplastics on human food safety, animals, ecosystems, and human health need to be examined in depth, as the accumulation and transport of microplastics alongside other environmental pollutants can contribute to the occurrence of combined effects, causes the combined effects of microplastics to manifest in various ways. In some instances, the combined effects of microplastics and other pollutants can lead to synergistic toxicity, potentially exceeding the cumulative impact of individual effects. However, this scenario may vary depending on the specific pollutants and organisms involved (Xu *et al.* 2020). For example, according to Cao *et al.* (2021), the presence of different-sized microplastics together can lead to synergistic effects, and the toxicity of one microplastic may be enhanced or diminished when interacting with the presence of another chemical substance. These effects can lead to overall health issues, immune system disorders, reproductive problems, and other biological impacts on living organisms. Factors such as the chemical composition, toxicity, and environmental conditions of microplastics can influence their combined effects (Cao *et al.* 2021). However, biomarkers associated with microplastics, such as those indicating oxidative stress and damage to microalgae, should also be explored to understand the extent of their ecological impact better.

In the studies on the sources of microplastics in the aquatic and terrestrial environment, according to the research findings of Duis and Coors (2016), microplastics can function as carriers of toxic chemicals. These particles have

the potential to enter water bodies from various sources, such as the fragmentation of larger plastic products, the release of microbeads from personal care products, and the shedding of microfibers from synthetic textiles, which increase negative effects on both organisms and the overall ecosystem. One of the primary concerns is the ingestion of microplastics by aquatic organisms (Freixa *et al.* 2018), including fish (Garcia *et al.* 2020), shellfish (Ding *et al.* 2020), zooplankton (Wieczorek *et al.* 2019), and other marine life (Li *et al.* 2021), as aquatic organisms may perceive microplastic particles as prey, leading to their accumulation in the digestive system (Silva *et al.* 2023). The presence of microplastics in the tissues of aquatic organisms can lead to their accumulation, causing bioaccumulation and biomagnification processes within the food chain (Waring *et al.* 2018). This can potentially impact higher trophic levels, including fish and marine mammals (Figure 5).



**Figure 5.** The origins and behavior of microplastics within aquaculture systems. Reproduced with permission from (Chen *et al.* 2021)

In a study aiming at observing the effect of nanoplastics after individual and combined exposure to the active substance, Brandts *et al.* (2018) conducted a study in which the *Mediterranean mussel* and *Mytilus galloprovincialis* were exposed to polystyrene nanoplastics and the substance carbamazepine, either separately or in combination. It was observed that these exposures led to significant changes in gene expression associated with the digestive glands and gills of the mussels. Changes were observed in genes related to biotransformation, DNA repair, cellular stress response, and innate immunity. Furthermore, exposure to the combination of polystyrene and carbamazepine significantly reduced the expression of genes involved in heat shock proteins (e.g., hsp70) compared to exposure to the chemicals individually. Genotoxicity in hemocytes increased due to exposure to the combination of polystyrene and carbamazepine or exposure to them individually. These findings indicate that the combination of microplastics and chemical pollutants (e.g., heavy metals, pesticides, organic solvents, hormone disruptors, and other harmful compounds) can have interactive effects that enhance the adverse impacts of microplastics, which can increase toxicity, have adverse effects on the immune system, cause hormonal imbalances and affect the health of native populations. In addition, Pannetier *et al.* (2020) explained that the physical presence of microplastics in living organisms negatively affects growth, development, and overall health by causing

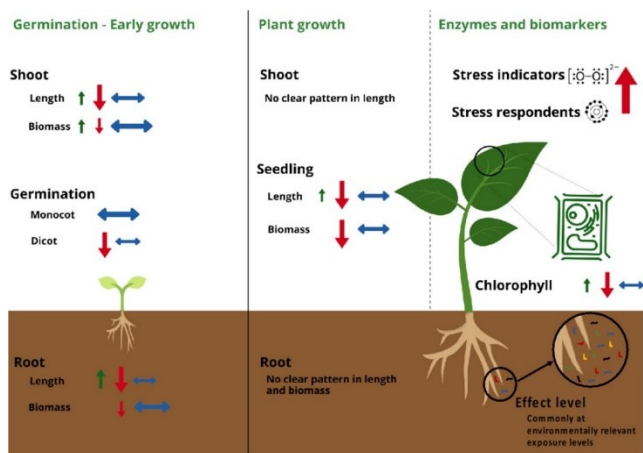
internal injuries, blockages, and a decrease in feeding efficiency. Another factor that negatively affects aquatic organisms is the role of microplastics as carriers of toxic chemicals (Duis and Coors 2016; Baho *et al.* 2021). In the work on microplastic-toxic chemical interactions, Verla *et al.* (2019) stated that microplastics, due to their high surface area-to-volume ratio, possess the capacity to adsorb and accumulate various heavy metals, including iron (Fe), manganese (Mn), aluminum (Al), lead (Pb), copper (Cu), silver (Ag), zinc (Zn), as well as hydrophobic organic contaminants (HOCs) commonly known as POPs, such as PAHs, organochlorine pesticides (OCPs) and PCBs found in aquatic environments. These toxic substances can adhere to the surface of microplastics or be adsorbed into their polymer matrices (Wang *et al.* 2020). When organisms ingest microplastics, the organisms can also ingest associated toxic pollutants, which can lead to a variety of toxic effects on the organism (McIlwraith *et al.* 2021), such as oxidative stress, DNA damage, endocrine system disruption, and impaired immune function. As a result, these effects can strongly impact aquatic organisms' feeding behavior, reproductive rate, and survival (Pérez-Albaladejo *et al.* 2020). To evaluate the prevalence and effects of microplastic pollution in the study of microplastic biomarkers, in a study conducted by Prokić *et al.* (2019), the researchers conducted a study to investigate the detrimental impacts of microplastics varying in size, concentration, and type on the antioxidant system, energy metabolism, and nervous system of animals. The findings revealed that microplastics have the potential to induce oxidative damage, as evidenced by increased lipid peroxidation (LPO) and DNA chain breaks. Additionally, the study highlighted significant changes in the antioxidant system, particularly in the activities of superoxide dismutase, catalase, and glutathione peroxidase. Microplastics also affected metabolism, as indicated by alterations in isocitrate dehydrogenase and lactate dehydrogenase activities, and suggested potential neurotoxic effects of microplastics, including inhibiting acetylcholinesterase activity. These findings also imply potential ecological implications at the ecosystem level.

The presence of microplastics in the structure and functioning of aquatic ecosystems can be also triggered in the opposite direction. It can affect the feeding behavior of zooplankton, which plays an important role in maintaining water clarity and controlling algal blooms (Reid *et al.* 2019). For example, this can alter the nutrient cycle, reduce primary productivity, and destabilize interspecies interactions (Ma *et al.* 2020). Changes in the abundance and distribution of major species can significantly affect aquatic ecosystems' overall biodiversity and stability (Galloway *et al.* 2017; Baho *et al.* 2021; Dong *et al.* 2021). Similarly, Sharma *et al.* (2023) declared that microplastics can negatively affect the step of providing concrete parameters for living life and a sustainable ecosystem, such as water's physical and chemical properties. For example, microplastics can adversely affect tangible parameters crucial for the survival of living organisms and sustainable ecosystems, such as water's physical and chemical properties (Pandey *et al.* 2022). By contributing to the

formation of marine sediments, microplastics can negatively impact light transmission and precipitation processes, altering the compliance of sustainable water parameters (Nuelle *et al.* 2014). Additionally, microplastics can serve as substrates for the formation and colonization of microorganisms' biofilms, which can significantly disrupt microbial communities and nutrient-cycling dynamics in water environments (Chen *et al.* 2020). Furthermore, the accumulation and transport of microplastics in aquatic environments can diminish water quality and threaten the health of aquatic ecosystems (Du *et al.* 2021). Therefore, contamination of water resources represents a significant risk for both ecosystems and human health. It is crucial to take measures to understand and mitigate the adverse effects caused by microplastics in water environments. Actions such as reducing the sources of microplastic pollution, promoting recycling, limiting plastic usage, and improving waste management processes play a significant role in reducing the entry of microplastics into aquatic environments.

### 2.3. The effects of microplastics on the terrestrial environment

Another growing concern in recent years is the toxic effects of microplastics in the soil environment. In particular, macroplastics decompose under different generations and turn into microplastics which threaten the terrestrial environment from many different sources, such as waste disposal, agricultural practices, and decomposition of plastic materials. This worrying pollutant can accumulate in the soil and adversely affect the growth and development of plant roots (Huang *et al.* 2020) (Figure 6).



**Figure 6.** The effects of micro and nanoplastics on seeds' germination and early growth. Reproduced with permission from (Zantis *et al.* 2023)

↓ show effects on plant roots, ↑ show effects on plant leaves, ↔ show the effects on plant bodies.

According to Kim *et al.* (2022), the accumulation of microplastic particles around the roots of plants can have detrimental effects on their growth and development. It can impede root growth and reduce root length, thereby inhibiting the absorption of water and nutrients by the plants. This accumulation also has the potential to disrupt the nutritional balance within plants, which can negatively impact their overall growth. Wang *et al.* (2023) aimed to conduct physiological and biochemical tests on *Mirabilis*

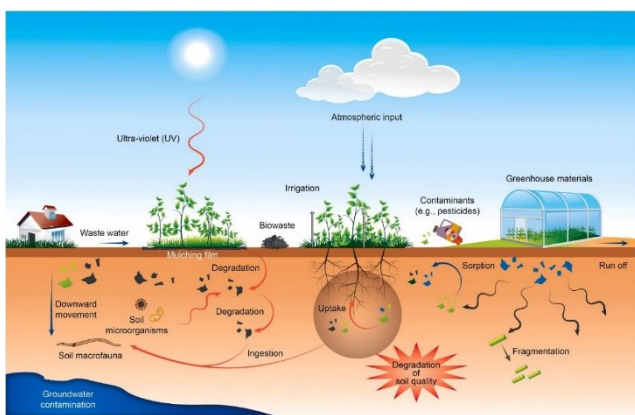
*jalapa L.* plants using galaxolide (HHCB) and PS. The research revealed that HHCB increased the antioxidant enzyme activity in plants. However, it found that superoxide dismutase (SOD) activity increased by only 206.85% when exposed to 0.5 mg/L HHCB alone, whereas exposure to 0.5 mg/L HHCB and 500 nm PS together increased SOD activity by 93.82%. Additionally, it reported that 500 nm PS could be absorbed by the roots and transported to shoots, while 5 μm PS could be transferred to shoots under the influence of HHCB transport. In addition, in a study conducted by Lian *et al.* (2020), higher plants, such as *Triticum aestivum L.*, exhibited noteworthy alterations in their growth and metabolic processes when exposed to MPs. It was observed that polystyrene microplastics (PS-MP), even at a small size of 5 μm, had inhibitory effects on root growth and led to impairments in the plants' photosynthetic machinery and antioxidative metabolism. Similarly, Jiang *et al.* (2019), when *Vicia faba L.* was subjected to exposure levels of 50–100 mg/L of PS-MPs, noted substantial alterations in the plant's physiology. These changes showed signs of oxidative stress, a reduction in biomass, and a decrease in mineral nutrition, as indicated by the study's findings. Therefore, microplastics can create physical barriers that hinder important physiological processes in plants, such as photosynthesis and respiration. These disruptions can ultimately reduce soil fertility and affect plant ecosystems' overall health and productivity. It highlights the need to understand and mitigate the effects of microplastics on plant physiology and ecosystem functioning. Therefore, it is crucial to address the impact of plastic pollution on plants, as it can have far-reaching implications for ecosystem functioning and agricultural productivity.

The adherence of plastic particles to plant leaves can pose significant risks to plant health and overall development, as emphasized by Wang *et al.* (2022). One of the primary consequences of this adherence is the obstruction of light transmission, which plays a vital role in efficient photosynthesis. Covering the leaf surface, plastic particles impede sunlight penetration and subsequently reduce the plant's ability to produce energy through photosynthesis. In addition to light obstruction, plastic particles can trigger various detrimental effects on plants. These effects include the induction of oxidative stress (Baihetiyaer *et al.* 2023), cytotoxicity (Wu *et al.* 2019), and genotoxicity (Jiang *et al.* 2019). Plastic particles can generate reactive oxygen species, causing oxidative damage to plant cells and disrupting normal cellular processes, leading to impaired growth and development. The interaction between plastic particles and plants can alter various aspects of plant physiology, including disturbances in mineral nutrition, hampered photosynthetic activity, and the accumulation of toxic substances within plant tissues (Wang *et al.* 2022). So, plastic particles can interfere with the uptake and assimilation of essential minerals, leading to nutrient imbalances and deficiencies. Additionally, plastic particles can disrupt the normal functioning of chloroplasts and other cellular components involved in photosynthesis (Nava and Leoni 2021), compromising the plant's energy production. It can contribute to changes in the metabolite

profiles within plant tissues, which can have cascading effects on plant metabolism and overall physiological processes (Liu *et al.* 2023). The accumulation of toxic substances derived from plastic particles can also disrupt metabolic pathways and potentially impact the quality and safety of harvested crops (Wang *et al.* 2021).

In addition, microplastics' inhibition of gas exchange due to their potential to obstruct the respiratory tract may adversely affect plants' respiratory process and hormonal balance (Liao *et al.* 2023). The work by Arikan *et al.* (2022), plastic pollution, specifically the presence of plastic particles PS, has been found to have detrimental effects on plant growth. PS applications have been found to interfere with photosynthesis and quantum efficiency in plants. However, the work showed that the negative impacts of PS can be mitigated by the use of functionalized graphene oxide (FGO). When FGO is applied, it effectively eliminates the adverse effects of PS on growth, relative water content, and gas exchange in plants. This is due to FGO's high antioxidant capacity, allowing it to efficiently scavenge reactive oxygen species (ROS) generated by PS exposure (Arikan *et al.* 2022).

The chemicals in some plastics, such as phthalates, polybrominated diphenyl ethers, and bisphenol (Mao *et al.* 2022), can also disrupt the hormonal regulation of plants and cause negative effects on growth, which in turn, by disrupting the herbal defense mechanism, can suppress the natural defense responses of plants (Pflugmacher *et al.* 2021) and significantly reduce the resistance of plants against diseases or pests. Microplastics can also alter soil structure, reducing its water-holding capacity and affecting soil porosity, making it difficult for plant roots to access nutrients and water, negatively impacting plant growth, and limiting water retention in the soil. This can reduce the plant's access to water and its ability to cope with water stress, leading to soil blockages and compaction, hindering the passage of air and water through the soil, restricting the access of plant roots to air and water, negatively affecting root development and plant productivity (Roy *et al.* 2023) (Figure 7).



**Figure 7.** The origins and pathways of microplastics in the terrestrial environment and their subsequent fate and behavior.

Reproduced with permission from (Dissanayake *et al.* 2022)

It can be concluded that microplastics can directly or indirectly threaten plants in terrestrial systems. Directly, microplastic particles can settle on the roots, leaves, or

stems of plants. This can affect the normal physiological functions of plants, such as reducing photosynthetic activity or hindering water and nutrient uptake (Dissanayake *et al.* 2022). Additionally, microplastics can enter plant cells and cause damage to the cellular structure (Khalid *et al.* 2020). Indirectly, microplastics can accumulate in the soil, altering soil structure and impeding plant root development. Furthermore, microplastics can affect the activities of microorganisms in the soil, making it difficult for plants to obtain necessary nutrients (Ya *et al.* 2021). Microplastics can also facilitate the transport of toxic chemicals, exposing plants to harmful substances (Huang *et al.* 2021). Therefore, it is important to understand the effects of microplastics on plants in terrestrial systems and take measures to mitigate this threat. In line with these goals, Khalid *et al.* (2020) reported in their study that microplastics can potentially disrupt the nutrient cycle within soil ecosystems by altering the carbon-to-nitrogen (C:N) ratio. Additionally, the physicochemical properties of soil can be modified by the presence of microplastics, leading to changes in plant community composition. Microplastics can directly cause toxicity in plants through uptake via their roots. Furthermore, the researchers emphasized that microplastics can introduce an additional hazard to plants and soil biota by releasing toxic environmental pollutants. It was also noted that microplastics present in the soil can directly impact plants by obstructing seed pores, hindering water and nutrient uptake through the roots, and accumulating in various plant parts such as roots, shoots, and leaves. Therefore, these findings showed that the dissolution rate of nutrients in the soil can be adversely affected, which may affect the nutritional balance of plants and negatively affect their productivity by restricting the access of plants to nutrients. So, it can reduce the microbial diversity in the soil and adversely affect the activities and populations of microorganisms, which affects soil health and organic matter cycle, causing a decrease in soil fertility also (Brucker *et al.* 2020). Microplastics accumulated in the soil can negatively affect plant diversity and biological activity by disrupting the habitats and activities of soil organisms such as plants, bacteria, fungus, and pathogens, which may affect biodiversity (Ding *et al.* 2022). Thus, the ecosystem balance is likely to be affected negatively due to these reasons.

However, the effects of microplastics on biodiversity are complex and not fully realized. It can spread to natural habitats in ecosystems, disrupting natural habitats (Backhaus and Wagner 2020). Especially in aquatic environments, microplastics can enter the habitats of aquatic organisms at an alarming rate and disrupt their habitats, negatively impacting biodiversity. It may also cause effects on biodiversity by forging ahead in the food chain (Khalid *et al.* 2021). In aquatic environments, small organisms such as plankton can be exposed to microplastics and then consumed by fish and other marine life. This situation can disrupt the balance of ecosystems by causing the accumulation and spread of microplastics among organisms in the food chain (Zhao *et al.* 2023), thereby altering the physical characteristics of habitats



such as water, wastewater, freshwater, marine water ecosystems, and soil (Meng *et al.* 2020). This can affect vital ecosystem functions, such as water circulation, nutrient cycling, and plant growth, ultimately impacting biodiversity. These impacts can, therefore, have the ability to impair the health of organisms (Junaid *et al.* 2022), as microplastics can enter the digestive system and affect nutrient absorption and digestive processes. The work by Ya *et al.* (2021) provided a thorough overview of the formation and sources of microplastics in terrestrial soil, considering the combined effects of microplastics and other pollutants, including heavy metals and antibiotics. The researchers examined the ecotoxicological impacts of microplastics on soil ecosystems, focusing on soil physical and chemical properties, soil nutrient cycling, and the abundance of soil flora and fauna. The study emphasized that microplastics, particularly nanoplastics with smaller particle sizes, harm soil organisms even more. The study also reported that microplastics can adsorb antibiotic-resistance genes, potentially serving as a pathway for disseminating such genes into deep soil layers and groundwater, highlighting the potential long-term implications of microplastic pollution in soil environments.

The absorption of pollutants by plants and water sources can lead to toxic effects and biological contamination in ecosystems, resulting in living organisms undergoing biological changes or harm, usually under the influence of pollutants. The ability of microplastics to absorb pollutants can lead these pollutants to reach biodiversity-sensitive areas and damage ecosystems, which can lead to increased toxicity, as microplastics can act as a vector in transporting contaminants that can bind to their surface and be toxic and accumulate and transport contaminants that adhere to microplastic surfaces. This accumulation can cause concentrations of pollutants to increase with microplastics (Kim *et al.* 2023). Alengebawy *et al.* (2021) figured out that microplastics can absorb pesticides, heavy metals, and other pollutants present in the soil, causing these pollutants to have toxic effects on plants and soil organisms, which results in contaminants being carried by microplastics and exposed to organisms. In other words, it can cause microplastics to release pollutants from the soil into water sources and plants. According to the study conducted by Yu *et al.* (2021), it was proposed that microplastics in terrestrial systems can have diverse impacts on plant communities, with root uptake being identified as the main pathway for microplastic entry into plants. The study also suggested that soil pollution plays a significant role in disseminating microplastics within plant communities.

Living organisms may experience toxic effects when exposed to pollutant concentrations, which may pave the way for many biological effects. For example, combining pollutants with microplastics can produce various biological effects on organisms (Issac and Kandasubramanian 2021). Contaminants deposited on microplastic surfaces can affect organisms' respiratory, nutrient absorption, and circulatory systems (Prata *et al.* 2020). These impacts can adversely affect organisms' growth, development, reproduction, and immune systems,

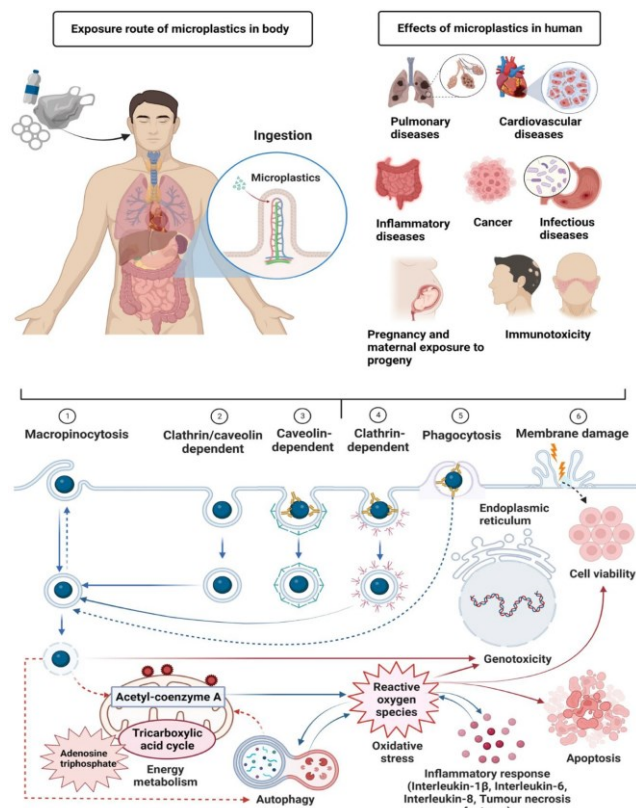
disrupting the dynamics of ecosystems, affecting populations, and leading to biodiversity loss. When exposed to high concentrations of pollutants, organisms can experience toxic effects with various biological consequences. For example, combining pollutants with microplastics can lead to various biological effects on organisms (Prinz and Korez 2020), leading to population-level impacts and biodiversity loss. Disruptions in the normal functioning of organisms can disrupt ecological interactions, reduce reproductive success, and impair the ability of species to adapt and thrive in their environments, cascading effects on the structure and stability of ecosystems and the overall health and diversity of populations (Cao *et al.* 2021). Therefore, combining pollutants with microplastics can exacerbate the negative biological effects on organisms, potentially leading to widespread ecological consequences. The findings highlight the need to address the potential risks associated with plastic particle contamination in agricultural and natural ecosystems. Therefore, strategies to mitigate the adherence and accumulation of plastic particles on plant surfaces and to reduce their negative impacts on plant health are crucial for sustainable plant production and environmental conservation (Wang *et al.* 2022). Nevertheless, besides understanding and addressing the potential risks associated with the interaction between microplastics and pollutants, effective environmental management and conservation strategies are still sorely needed to reduce these impacts and preserve biodiversity.

### 3. Biomarkers for microplastic toxicity

Environmental research is currently focused on the growing impact of microplastics, identified as ecotoxic micropollutants, on biomarker formation in living organisms (Rai *et al.* 2021). Biomarkers serve as measurable biological indicators (Suman *et al.* 2021), reflecting changes at the cellular, biochemical, molecular (Singh *et al.* 2019), and physiological levels (Depledge 2020). Known as valuable tools for assessing the toxic effects, microplastics involve the evaluation of the presence, accumulation, and effects of these pollutants at various levels, including cellular, body fluid, tissue, or organ (Prokić *et al.* 2019). While the direct toxic effects of micro and nanoplastics on the human body are limited (Bouwmeester *et al.* 2015), numerous studies highlighted on animals and cell lines have demonstrated that these substances can induce oxidative stress (Solomando *et al.* 2020), immune responses (Kim *et al.* 2021), genotoxicity (Li *et al.* 2021), DNA damage (Chen *et al.* 2022), endocrine system disorders (Wang *et al.* 2023), neurotoxicity (Xiong *et al.* 2022), embryotoxicity (Capolupo *et al.* 2021) and reproductive abnormalities (Alimba and Faggio 2019) (Figure 8).

Both in vitro and in vivo studies have demonstrated the harmful effects of plastic particles on various organs and systems. The extent of these effects depends on factors such as the plastic particles' dose, size, and chemical properties. These findings underscore the need for a comprehensive understanding of the potential risks

associated with plastic particle exposure (González-Acedo *et al.* 2021).



**Figure 8.** Exposure of the human body to plastic particles.

Reproduced with permission from (Osman *et al.* 2023)

Moreover, biomarkers offer insight into the exposure and effects of xenobiotics foreign substances in the environment (Malchi *et al.* 2022). Studying biomarkers allows researchers to comprehend the impact of xenobiotics on organisms, enabling assessments of environmental pollution and potential risks to ecosystems and human health (Provenza *et al.* 2022). For instance, assessing microplastic toxicity involves using biochemical indicators such as antioxidant enzyme activity, DNA damage, and stress response protein expression (Baihetiayer *et al.* 2023). Techniques like histopathology analysis, biochemical analysis, and gene expression analysis are employed as biomarkers to evaluate microplastic toxicity (Abarghouei *et al.* 2021), which plays a crucial role in determining microplastic toxicity by measuring effects like oxidative stress, inflammation, and immune system disorders induced by microplastics. Therefore, these versatile indicators serve as research tools to monitor microplastic pollution, evaluate its environmental consequences, and gauge potential health risks. The information derived from biomarkers in assessing microplastic toxicity contributes significantly to environmental risk assessments and enhances understanding of the effects on living organisms (Atamanalp *et al.* 2023).

Exposure to microplastics and nanoplastics has been found to significantly impact the secretion of cytokines and chemokines by inflammatory cells, which play crucial roles in immunomodulation and cell signaling processes (Chen *et al.* 2023). Organisms exposed to microplastics can have

various effects on the immune system. These effects may occur in the form of activation of immune cells, cytokine production, antibody response, and alteration of other immunological markers (Fackelmann and Sommer 2019; Li *et al.* 2020). Researches on living things has shown that micro and nanoplastics can reach organisms through nutrition, respiration, and epidermis (Yin *et al.* 2019). Reported effects include changes in the microbiota composition, alterations in the production of digestive enzymes, and respiratory inflammatory processes (Table 2). Plastic particles have been associated with circulatory and reproductive system disorders and neurotoxicity, leading to behavioral changes (Yin *et al.* 2021). Increased cytokine levels, changes in immunoglobulins, and altered immune cell reactivity are observed in organisms exposed to microplastics (Hirt and Body-Malapel 2020). Ingested microplastics pose health risks due to their ability to act as carriers for toxic chemicals, potentially leading to exposure to harmful substances. One of the primary concerns is the ability of microplastics to act as carriers for toxic chemicals (Campanale *et al.* 2020), which can adsorb onto the surface of microplastics. These chemicals can be released into the gastrointestinal tract upon ingestion, exposing the body to harmful substances, including additives, heavy metals, and other pollutants (Campanale *et al.* 2020). In addition, the surface properties and size of microplastics can affect the functions of immune cells, such as adhesion, migration, and phagocytosis (Hwang *et al.* 2020). Disruptions to the microbiota composition and function can have wide-ranging consequences for living organisms, including immune system dysregulation, impaired nutrient absorption, and increased susceptibility to infections and diseases (Conlon and Bird 2014). Therefore, understanding how microplastic biomarkers have changed is important to fully evaluate the potential effects of microplastics on the environment and human health.

### 3.1. Detection of antioxidant activity with biomarkers

Oxidative stress signifies an imbalance in the biochemical equilibrium of cells, leading to the excessive production of ROS and inadequate antioxidant defence systems. Antioxidants render ROS and other free radicals ineffective or neutralize them (Lone *et al.* 2013). ROS encompass molecules with oxidative properties, such as superoxide radicals, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and free radicals, and are often produced during normal cellular activities within mitochondria or other cellular compartments (Singh *et al.* 2019). Under normal conditions, a delicate balance is maintained within cells by antioxidant enzymes (e.g., superoxide dismutase, catalase) (Nandi *et al.* 2019) and non-enzymatic antioxidants (e.g., glutathione, vitamins C and E) (George and Abrahamse 2020). However, various factors (e.g., environmental stress, smoking, inadequate nutrition, and inflammation) can increase oxidative stress (Seyedsadjadi and Grant 2020; Caliri *et al.* 2021). Oxidative stress arises from an increase in ROS production or a decrease in antioxidant defence. In this scenario, ROS levels within cells rapidly rise, and antioxidant defence mechanisms are unable to counteract this increase effectively (Pisoschi *et al.* 2021), leading to oxidative

damage, mainly through interactions with biological molecules such as lipids, proteins, and nucleic acids (DNA and RNA) (Singh *et al.* 2019). Products of lipid peroxidation, especially malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), can damage cell membranes and disrupt cellular functions. Protein carbonylation can adversely affect the structure and function of proteins (Gallo *et al.* 2020). Oxidative damage to nucleic acids can disrupt normal cellular functions and contribute to developing diseases such as cancer. The potential effects of oxidative stress on individual health can be diverse (Poetsch 2020). Chronic oxidative stress plays a role in various diseases' pathogenesis (García-Sánchez *et al.* 2020). Cardiovascular diseases, neurodegenerative disorders, inflammatory conditions, and cancer are among the health issues associated with oxidative stress (Zuo *et al.* 2019). Therefore, assessing oxidative stress, examining potential biomarkers, and understanding antioxidant defence strategies are important for comprehending its effects on health and mitigating these effects.

Xenobiotics are substances foreign to the organism's normal biochemistry, including drugs, pesticides, industrial chemicals, heavy metals, and microplastics (Curpan *et al.* 2022). Exposure to xenobiotics can create oxidative stress by increasing the production of ROS in the body (Silvestre 2020). ROS can cause damage by oxidizing lipids, proteins, and DNA in cells. This damage plays a role in the

pathogenesis of various diseases, such as inflammation, cancer, diabetes, cardiovascular disease, and neurodegenerative disease (Liu *et al.* 2020; Teleanu *et al.* 2022). Oxidative stress represents an imbalance between ROS and antioxidants in the body (Adwas *et al.* 2019). Antioxidants protect cells from oxidative damage by neutralizing ROS and other free radicals (Adwas *et al.* 2019). Various methods exist to measure oxidative stress, including the direct or indirect measurement of ROS and free radicals, the measurement of antioxidants, and oxidative damage products (Katerji *et al.* 2019). Studies examining biomarkers induced by microplastics typically involve assessing different components of biomarkers to evaluate microplastics' environmental and biological effects (Table 2).

Some studies have investigated how microplastics affect the production of ROS (Zhu *et al.* 2020), antioxidant enzyme activity (Jaikumar *et al.* 2021), lipid peroxidation (Xu *et al.* 2023), and DNA damage (Jiang *et al.* 2020). These studies have shown that microplastics increase oxidative stress, leading to toxic effects in various living organisms such as fish (Kim *et al.* 2021), crustaceans (D'Costa 2022), worms (Jiang *et al.* 2020), plants (Yu *et al.* 2021), human (Xie *et al.* 2020), etc. Cellular and animal model studies have suggested that exposure to microplastics can increase cellular oxidative stress, potentially resulting in various health effects (Prata *et al.* 2020)

**Table 2.** Critical indicators of redox homeostasis, responses of the antioxidant system, and manifestations of oxidative stress

Biomarkers	Definition	Reference
Carbonyls	The result of the oxidation of lipids is the formation of aldehyde and ketone groups.	(Faisal <i>et al.</i> 2019)
Catalase (CAT)	CAT is an antioxidant enzyme that converts H <sub>2</sub> O <sub>2</sub> into water and oxygen.	(Nandi <i>et al.</i> 2019)
DNA micronuclei	DNA micronuclei are small nuclei formed due to chromosome damage or loss during cell division.	(Kwon <i>et al.</i> 2020)
DNA olive tail moment (OTM)	OTM is a parameter of DNA damage measured by single cell gel electrophoresis (comet assay) method. Cells with DNA damage appear as comets as a result of electrophoresis. DNA olive tail moment is related to the length and density of the tail.	(Gajski <i>et al.</i> 2021)
DNA strand breaks	It represents breaks or cuts in the DNA chain.	(Ensminger and Löbrich 2020)
Glutathione peroxidase (GPx)	GPx is an antioxidant enzyme that converts H <sub>2</sub> O <sub>2</sub> and organic hydroperoxides (ROOH) into water and alcohol using glutathione (GSH).	(Andrés <i>et al.</i> 2022)
Glutathione reductase (GR)	GR is an antioxidant enzyme that converts oxidized glutathione (GSSG) into reduced GSH.	(Narayanankutty <i>et al.</i> 2019)
Glutathione-s-transferase (GST)	GST is an antioxidant enzyme that catalyzes the conjugation reaction between GSH and xenobiotics.	(Bocedi <i>et al.</i> 2019)
Malondialdehyde	MDA is an aldehyde, an end product of lipid peroxidation.	(Mas-Bargues <i>et al.</i> 2021)
Protein carbonyls	Protein carbonyls refer to carbonyl groups formed from the oxidation of proteins.	(Akagawa 2021)
Reactive oxygen species concentration	ROS is the concentration of chemical substances that gain and lose electrons, which are the primary factors of oxidative stress.	(Singh <i>et al.</i> 2019)

**Table 3.** Biomarkers of microplastics in biota

Type	Size	Concentration	Target Organism	Tissue	Biomarkers	Response	References
Spherical microplastics made of PS material	5-5.9 µm	On each plate, 8 µL of cDNA sample was utilized, and primers were introduced into each well at a concentration of 250 nM per primer.	Chironomus riparius	mRNA levels of 80 genes	Endocrine response, detoxification mechanisms, stress response, DNA repair mechanisms, hypoxia, oxidative stress, apoptosis, immunity, cholesterol metabolism, energy metabolism, circadian rhythm, signaling, regulation of piRNAs	Temporal changes in gene expression were observed, with stress response genes most affected initially.	(Kalman <i>et al.</i> 2023)
PS	20 µm	1 mg/L	Scrobicularia plana	Gilts and digestive gland	SOD, CAT, GPx, GST, genotoxicity, neurotoxicity, oxidative damage	Significant effects on antioxidant capacity, DNA damage, neurotoxicity, and oxidative stress levels, with a progressive increase in the genotoxicity of polystyrene microplastics over time	(Ribeiro <i>et al.</i> 2017)
PS-MPs	6 µm	102 particles/L, 104 particles/L and 106 particles/L	Zebrafish larvae	Accumulated in the gastrointestinal tract and varied depending on exposure time and concentration	Developmental and behavioral indices, Transcriptional profiles of genes	Environmentally significant concentrations of PS-MP caused an inflammatory response, inhibiting the immune functions of larvae and slowing their growth	(Chen <i>et al.</i> 2022)
Commercial synthetic polymer microspheres	1–5 µm	300 µg MP in 20 µL saline	Normal and HDM-induced allergic asthmatic mice	Lung tissue	Pulmonary inflammatory cell infiltration, aggregation of bronchoalveolar macrophages, increased levels of tnf-α in bronchoalveolar lavage fluid (balf), elevated production of plasma igg1 in normal mice, increased mucus production in asthmatic mice, inflammatory cell infiltration, and notable aggregation of macrophages	Microplastic exposure caused changes in gene expressions associated with the immune response, cellular stress response, and programmed cell death in mice with asthma.	(Lu <i>et al.</i> 2021)
Polyurethane microplastics (PU-MP)	7-9 µm	375 mg PU-MP/kg	Girardia tigrina planarians	Physiological and biochemical changes	LPO, energy reserves and allocation, aerobic energy production (ETS), electron transport system (ETS) activity	Exposure to 375 mg PU-MP/kg induced behavioral changes in contaminated prey ( <i>C. riparius</i> ), enhancing	(Silva <i>et al.</i> 2023)

						detoxification and antioxidant processes.	
PS, PE, PP	5 µm and 50 µm	ranging from 0.01 to 10 mg/L	Amaranth plants	Amaranth seed germination and growth, particularly oxidative damage in amaranth roots	ROS	MPs were found to negatively impact seed germination, root and shoot growth, and the physio-biochemical activity of amaranth.	(Wang <i>et al.</i> 2023)
Fluorescence-labelled PS-MPs	0.2 µm	ranging from 0 to 50 mg/L	Lettuce	Roots and leaves	In lettuce roots, the activities of SOD, CAT, and GR, as well as the content of MDA, showed specific patterns. In lettuce leaves, the activities of SOD, ascorbate peroxidase (APX), and GR, along with the MDA content, displayed different trends.	Exposure to microplastic stress resulted in the upregulation of genes associated with diverse antioxidant systems at distinct time points in both roots and leaves. The extracted root exudates exhibited elevated levels of ascorbic acid, terpenoids, flavonoids, and sphingolipids, coinciding with the subsequent downregulation of genes related to ion homeostasis.	(Wang <i>et al.</i> 2023)
MPs-PE	PE: mean 246 ± 98 µm, Min: 46 µm, Max: 548 µm	Different concentrations	Oncorhynchus mykiss	Gills, Gastrointestinal System, Brain, Liver, Muscle	Growth parameters, hematological indices, oxidative stress markers, MDA, ROS, DNA damage, apoptosis, neurotransmission	Exposure to different concentrations of MPs-PE resulted in changes in growth parameters and hematological indices in the fish, and oxidative stress was observed in all targeted tissues, with decreased levels of GSH and antioxidant enzyme activities.	(Atamanalp <i>et al.</i> 2023)
Spherical PS-MPs+Cd	80 nm diameter	50 µg/L and 500 µg/L	Hybrid snakehead	Gill and liver	MT gene mRNA levels to determine antioxidant enzyme activities (HSP70, SOD), inflammation-related gene	Adverse effects of nano-microplastics on gill morphology, liver oxidative	(Wang <i>et al.</i> 2022)

					expressions (IL-1 $\beta$ , TNF- $\alpha$ ), and heavy metal accumulation	stress, and gene expressions related to inflammation were observed in hybrid snakeheads.	
Plastic microfibers, natural microparticles (non-plastic), and nylon microfibers	PS particles: 26.8 $\mu$ m, spartina particles: 39.2 $\mu$ m, undyed nylon 6'6 fibers: nearly 10 $\mu$ m	PS: 1 mg/L and nylon fiber dosages: 10 mg/L	Salmon species	Histopathology of gills and aspects of the immune response	Histopathology and immune response	Virus-related deaths increased when a species was exposed to microplastics, plastic microfibers, and natural (non-plastic) microparticles, mostly nylon fiber.	(Seeley <i>et al.</i> 2023)
PE-MPs + PFOS (perfluorooctane sulfonate)	ranging from 150 to 500 $\mu$ m	PE-MPs:100 mg/kg, and MP-PFOS; ultimate levels of 4.83 $\mu$ g and 100 mg PFOS and MP per kg of diet	Dicentrarchus labrax L.	Liver and gut tissues	Inflammation, oxidative stress, ROD activity, acetylcholinesterase (AChE) and cholinesterase (ChE) activities	High levels of PFOS have been detected in the liver of fish fed with PFOS. However, these levels decreased significantly when PFOS was adsorbed onto microplastics.	(Espinosa <i>et al.</i> 2023)
MPs-PET	<1 $\mu$ m and <2.6 $\mu$ m	-	Human	Human bone marrow mesenchymal stromal cells (BMMSCs) and adipose mesenchymal stromal cells (AMSCs)	Intracellular ROS levels, stressed cell percentage (Ki67, p-RPS6, $\beta$ -Gal), persistent DNA damage (pHA2.X, pATM), neoplastic transformation (soft agar colony formation)	Exposure to MPs-PET revealed that it altered the fate of mesenchymal stromal cells in vitro and triggered aging with the loss of various stem cell properties.	(Najahi <i>et al.</i> 2022)
PS	Specifically, PS particles with sizes of 3 $\mu$ m and 10 $\mu$ m.	Concentrations ranging from 100–1600 particles/mL	Human intestinal epithelial cell	Intestinal cells	DNA damage and ROS	Exposure to PS-MPs appeared to cause moderate acute effects and, to a lesser extent, subchronic effects.	(Visalli <i>et al.</i> 2021)

As seen in Table 3, detecting and monitoring microplastic contamination in ecosystems such as oceans (Kurtela and Antolović 2019), rivers (Han *et al.* 2020), and soil organisms (Ding *et al.* 2022), is possible. The utilization of biomarkers for microplastics emerges as a crucial instrument for tracking and mitigating microplastic pollution, enabling the assessment of its impact and intervention at the earliest stages (Palmer and Herat 2021; Suman *et al.* 2021). These valuable tools also assist in evaluating the ecological consequences of microplastic pollution. For example, studying the responses of organisms and ecosystems to microplastic exposure allows to understand the ecological effects and potential risks associated with microplastics (Teng *et al.* 2021).

By examining changes in behavior, physiology (Costantini 2014), reproduction (Sarasamma *et al.* 2020), immune function (Abarghouei *et al.* 2021), and overall health as biomarkers, it can detect the impacts of microplastic exposure, enabling the evaluation of human exposure to microplastics and assessing their potential health effects (Gouin *et al.* 2022), which provides valuable information on detecting the presence of microplastics in tissues (Atamanalp *et al.* 2023), organs (Guerrera *et al.* 2021), or body fluids and evaluating associated biological responses such as inflammation, oxidative stress (Baihetiyaer *et al.* 2023), genotoxicity (Li *et al.* 2021), and endocrine disruption (Prokić *et al.* 2019) and valuable insights into the potential risks and health effects of microplastic exposure for human populations, which proves instrumental in formulating strategies aimed at controlling and minimizing microplastic pollution, aiding in comprehending environmental risks and facilitating the development of effective policies and regulations (Masud *et al.* 2023). This proactive methodology not only aids in establishing early warning systems but also enhances comprehension of the impact of microplastics on human health, allowing for the assessment of potential health risks (Patra *et al.* 2022). This, in turn, furnishes valuable information for crafting reduction strategies, promoting conservation efforts, and formulating evidence-based, effective policies to prevent further pollution and address microplastic contamination.

In this manner, biomarkers play an important role by supplying scientific data on the effects and risks associated with microplastics, which guides policymakers in making well-informed decisions and supporting implementing measures that reduce microplastic release, encourage recycling, and improve waste management practices, implying biomarkers facilitate the acquisition of crucial information for risk assessment and the creation of sustainable solutions, as well as the implementation of effective management strategies to alleviate the adverse impacts of microplastics on ecosystems and well-being (Prokić *et al.* 2019). To sum up, using biomarkers is a significant approach to combating microplastic pollution, providing valuable insights into its effects, evaluating environmental and health risks, and fostering the development of sustainable solutions. Therefore, it should be a goal to contribute to scientists' and policymakers' assessment of the extent and distribution of the problem

by providing measurable indicators of the presence and abundance of microplastics (Lusher *et al.* 2021).

### 3.2. Decision-making strategies in biomarker selection in living organisms and microplastic studies

Using microplastics as biomarkers is crucial for thoroughly understanding microplastic pollution and determining its effects (Trestrail *et al.* 2020). In this context, it is essential to understand processes such as organisms' uptake, distribution, accumulation, and excretion of microplastics (Franzellitti *et al.* 2019). Determining microplastics' physical, chemical, and biological effects on various organisms is important. Biomarkers, defined as biological parameters used to measure the effects of environmental stressors on organisms, can effectively assess microplastic exposure in various organisms. Nonetheless, for biomarkers to be practical, specific attributes are essential (Rai *et al.* 2021). For example, these indicators should correlate with environmental stressors, illustrating a proportional shift with the presence or intensity of the stressor (van Dammen *et al.* 2022). Biomarkers must be measurable, repeatable, and standardizable, allowing consistent measurement across different times, locations, and conditions. Tools for assessment also should be sensitive, specific, and reliable, capable of distinguishing the impact of the stressor from other factors and avoiding false positive or false negative results (Khoo *et al.* 2021). In addition to these characteristics, biomarkers should be economical, practical, and ethical, ensuring that the measurement process does not require excessive time, money, or resources and does not violate living organisms' welfare.

### 3.3. Some advantages and disadvantages in the evaluation of biomarkers

The biomarkers specified in Table 3 are observed to be evaluated within the scope of microplastic studies. As a result of these evaluations, the effects of biomarkers are noted to indicate oxidative stress (Adwas *et al.* 2019), measure antioxidant enzyme activity (Patra *et al.* 2022), and assess DNA damage (Chen *et al.* 2022), lipid peroxidation (Mas-Bargues *et al.* 2021), and protein oxidation (Prokić *et al.* 2019). These biomarkers are generally used together for a more comprehensive evaluation. However, it is important to emphasize that each biomarker has its limitations and may not be reliable in determining specific microplastic types. Biomarkers may not be specific to a certain polymer type (Trestrail *et al.* 2020). The isolated use of specific enzymes for identifying and breaking down various types of microplastics may not be sufficient on its own. Evaluating these enzymes in conjunction with others may be necessary to determine whether they provide a comprehensive solution to address plastic pollution. It should be noted that certain enzymes may not be specific to particular types of microplastics (Liu *et al.* 2022). Therefore, adopting a comprehensive approach to the analysis and resolution processes of microplastics is important.

On the other hand, algae/microalgae, employing a different approach, can be advantageous in microplastic

studies, as playing a crucial role in aquatic ecosystems, algae/microalgae serve as the primary receptors of microplastics (Guzzetti *et al.* 2018). Depending on microplastics' physical, chemical, and biological characteristics, algae/microalgae may exhibit different responses, influencing their transfer and bioaccumulation in the food chain (Mahana *et al.* 2021). Thus, algae/microalgae can reflect the biological effects of microplastics (Song *et al.* 2020). However, algae/microalgae may also be subject to certain biological limitations in microplastic studies, as different responses can be exhibited by these organisms under various types, strains, and conditions of microplastic exposure (Priya *et al.* 2022). Due to the lack of standardized and reliable methods for assessing the effects of microplastics, comparing and interpreting results may be challenging. Therefore, monitoring the effects of microplastics may require long-term studies under multiple stress factors (Parsai *et al.* 2022). Additionally, for algae/microalgae to determine the effects of microplastics, controlling various parameters such as the size, shape, density, surface properties, chemical composition, concentration, and distribution of microplastics may be necessary.

#### 4. Future perspectives and challenges

There is no universally accepted method or criterion for the selection, measurement, and interpretation of biomarkers of microplastics. Different studies use different biomarkers, different types of microplastics, varying exposure durations, and different living species, making comparison and generalization of results challenging. The mechanisms behind physiological, biochemical, molecular, cellular, or whole-organism changes leading to biomarkers of microplastics have not yet been fully explained. It is known that microplastics cause adverse effects in organisms, such as oxidative stress, inflammation, endocrine disruption, growth and reproductive inhibition, behavior changes, nutritional disorders, bioaccumulation, and biomagnification. How the biomarkers of microplastics change depending on environmental and biological variables is not fully understood, limiting the use of microplastic biomarkers for ecosystem-level risk assessment and management. However, further studies are needed to understand how these effects vary based on the size, shape, chemical composition, surface properties, concentration, exposure duration, and living species of the microplastics.

The development and application of biomarkers for microplastics represent a future research area. It is crucial for these biomarkers to be more sensitive, specific, reliable, repeatable, easy, and cost-effective, catering to both laboratory and field studies. Standardizing the biomarkers for different types of microplastics, exposure durations, and species will facilitate the comparison and generalization of results. The ability of microplastic biomarkers to elucidate the mechanisms of physiological, biochemical, molecular, cellular, or whole-organism changes induced by microplastics is important for understanding their toxicity. The adaptability of microplastic biomarkers from laboratory conditions to

natural environments, from single-species to multi-species ecosystems, and from acute to chronic exposures, as well as their extrapolation from local to global scales, is essential for their use in ecosystem-level risk assessment and management. Optimizing and integrating synergistic methods working together to eliminate or reduce microplastic pollution is a future research area. Still, there is currently insufficient information about the effectiveness and compatibility of these methods. Further research is needed to understand how these methods affect the sources, toxicity, and biodegradation of microplastic pollution, which methods can be used together, and which methods may have conflicting or opposing effects. Ensuring that these methods are both environmentally, economically effective, and efficient is crucial for preventing and reducing microplastic pollution. Additionally, integrating them with existing waste management systems, cleanup efforts, policies, and regulations is important for a global solution to microplastic pollution.

However, there are still some challenges. The measurement and analysis of microplastic biomarkers pose a difficulty in the literature. Detecting biomarkers of microplastics in the environment at low concentrations in complex matrices with different sizes, shapes, and chemical compositions affects the sensitivity, accuracy, and reliability of measurement and analysis methods. Measuring microplastic biomarkers in different species, tissues, exposure durations, and stress responses complicates the standardization and comparison of measurement and analysis methods. Distinguishing whether biomarkers of microplastics are triggered by the microplastics themselves, pollutants adsorbed to microplastics, or microorganisms associated with microplastics affects the specificity of measurement and analysis methods. The applicability and sustainability of methods targeting the sources, toxicity, and biodegradation of microplastic pollution are challenging in the literature. The application of these methods in different geographical regions, climate conditions, socio-economic situations, and cultural values creates variations in terms of applicability and sustainability. Being effective and efficient from both environmental and economic perspectives is important for applicability and sustainability. Additionally, the compatibility of these methods with existing waste management systems, cleanup efforts, policies, and regulations is crucial for applicability and sustainability.

#### 5. Conclusion

Microplastics pose a significant threat to organisms due to their widespread presence in various ecosystems and can cause a range of biological responses. In living organisms, microplastic exposure can alter essential life functions, including nutrient intake, respiration, reproduction, and behavior, which can affect population dynamics and disrupt interactions between different species. Furthermore, the impact of microplastics on the ecosystem's food chain can extend to organisms at higher trophic levels. The various lines of evidence have



demonstrated that microplastics can disrupt normal metabolism, induce oxidative stress, and trigger neurotoxic, genotoxic, and inflammatory effects, as evidenced by the presence of biomarkers such as enzymatic, genetic, histological, reproductive, and developmental indicators. These effects vary depending on size, shape, chemical composition, surface characteristics, concentration, exposure duration, and depending on the species of the living organisms. The limited understanding of the toxicity of microplastics poses a challenge to the genetic and physiological translocation of ingested microplastics into the bloodstream and target tissues. There is no universally accepted method or criterion for the selection, measurement, and interpretation of biomarkers for microplastics. Various studies employ different biomarkers, different types of microplastics, varying exposure durations, and different species, which complicates the comparison and generalization of results. Research findings play a vital role in a comprehensive characterization of hazard potential and risk assessment. In conclusion, constant critical evaluation, knowledge dissemination, and collaborative efforts are paramount for devising effective strategies to combat microplastic pollution. The approaches, informed by biomarker-based assessments, can play a pivotal role in shaping sustainable policies and practices to safeguard our ecosystems and the organisms within them.

#### Authors contributions

**Enes Ozgenc:** Investigation, Writing-Original Draft, Formal Analysis, Methodology, Conceptualization. **Emine Keles:** Writing-Review, Validation, Conceptualization, Resources. **Gunay Yildiz Tore:** Writing-Review & Editing, Conceptualization, Supervision and Data Curation.

#### 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 review.

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