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Systematic Review of Microplastic Characterization Methods and Associated Toxicological Outcomes in Fish

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ABSTRACT: Microplastics (MPs) have emerged as significant aquatic pollutants, yet standardized protocols for their detection and biological impact assessment remain limited. This study systematically evaluated current analytical methods used for microplastic identification and synthesized existing evidence on their reported health effects in fish. Following the PRISMA framework, a comprehensive literature search identified eight eligible studies encompassing both laboratory and field investigations. Results indicated that Raman and Fourier Transform Infrared (FTIR) spectroscopy were the most frequently employed analytical techniques. Raman spectroscopy demonstrated greater sensitivity for MPs smaller than 20 µm, whereas FTIR provided reliable identification of larger particles. Stereomicroscopy and Scanning Electron Microscopy (SEM) were also utilized, although they offered limited chemical specificity. The reviewed studies revealed species- and condition-dependent toxicological outcomes. Smaller MPs induced more pronounced oxidative stress, apoptosis, and genotoxicity, particularly in liver and muscle tissues, with perch appearing more sensitive than zebrafish. Additionally, polymer type, particle size, exposure duration, exposure route, and concentration were key determinants of toxicity. Overall, polystyrene and polypropylene were consistently linked to stronger biochemical disruptions, whereas polyethylene vinyl acetate (PEVA) and high-density polyethylene (HDPE) exhibited site-specific effects in wild-caught fish. These findings underscore the need for multi-analytical approaches and integrated biomarker assays to improve MP detection and ecological risk assessment in aquatic organisms.

KEYWORDS: Microplastic; FTIR spectroscopy; Raman spectroscopy; toxicity; analytical methods; fish

1. Introduction

Environmental degradation had intensified in recent decades, with pollution emerging as a critical threat to the health of ecosystems and biodiversity. Among various pollutants, plastic waste had become a dominant and persistent environmental problem due to its durability, widespread use, and slow degradation rate. Over time, larger plastic debris underwent physical, chemical, and biological breakdown, resulting in the formation of microplastics. Microplastic

(MP) was defined as plastic smaller than five micrometers (5 μm), and its proliferation often originated from the fragmentation of larger plastics due to industrial activities. Because of their extremely small size, these particles became pervasive in marine environments as a result of inadequate waste management and surface water runoff. Tracking of microplastic contamination had revealed that MP pollution was influenced by urban activities [1]. For instance, rubber-based microplastics originating from tire wear were reported as dominant in areas with intense road traffic [2]. Such widespread environmental presence indicated that aquatic organisms, particularly fish, were continuously exposed to varying levels of microplastic contamination. Fish, being important components of aquatic food webs, were especially vulnerable to MP uptake through ingestion or respiration. Studies consistently reported MP accumulation in the gills, liver, gastrointestinal tract, and muscle tissues of various fish species [3]. For example, [3] found that the gastrointestinal tract contained the highest proportion of MPs, accounting for about 40% of the total particles detected. This bioaccumulation raised concern not only for fish but also for human consumers, as fish served as a major dietary protein source. Estimates suggested that adults might ingest up to 842 microplastic particles annually through seafood consumption, with higher exposure in regions where fish exhibited elevated contamination levels [4, 5].

Given these findings, accurately assessing MP contamination in fish tissues required robust and standardized analytical procedures. Variability in detection results often stemmed from differences in sample processing and analysis protocols. Common extraction methods, including alkaline or enzymatic digestion and density separation, differed in recovery efficiency and could alter polymer structure. For instance, strong acid treatments degraded certain polymers such as nylon, polyethylene terephthalate (PET), and polycarbonate (PC), while hydrogen peroxide digestion achieved only about 70% recovery [6]. These inconsistencies during extraction directly affected subsequent identification and quantification steps. Despite numerous studies documenting microplastic presence in fish, inconsistencies remained due to variations in extraction and identification methods. Differences in digestion agents, density separation techniques, and instrumental approaches could alter polymer integrity and affect recovery efficiency. Such methodological variations complicated interstudy comparisons and hindered the establishment of standardized monitoring frameworks. In response to these knowledge gaps, this systematic review aimed to critically evaluate current findings regarding microplastics and their reported health impacts on fish. Specifically, the study sought to (1) synthesize the analytical techniques used to extract and characterize microplastics from fish tissues, and (2) examine potential associations between microplastic characteristics and specific health outcomes in fish across both laboratory and field studies..

2. Materials and Methods

2.1. Inclusion and exclusion criteria.

This systematic review followed the PRISMA Guidelines and was registered with the Open Science Framework. The studies were selected based on the following inclusion criteria: (a) studies involving wild fish from aquatic environments such as freshwater, marine, and estuarine systems; (b) studies that extracted microplastics from fish tissues; (c) studies reporting both the analytical methods applied and the health impacts of microplastics on fish; (d) studies with experimental or field observational designs; (e) articles written in English; and

(f) studies published between 2020 and 2025. Studies were excluded if they involved animals other than fish, analyzed only water or other sources of microplastics, were review articles, did not report any biological or health impacts, were not written in English, or lacked full-text availability.

2.2. Literature search strategy.

A systematic search was conducted across the following databases: (a) Google Scholar, (b) PubMed, (c) ResearchGate, (d) Elsevier, and (e) the Directory of Open Access Journals. A database-specific search strategy was employed using Boolean operators and keyword combinations such as ("microplastic") AND ("fish tissues") AND ("extraction methods") AND ("health impacts"). All identified papers were managed using Zotero reference management software for organization and duplicate removal. Microsoft Excel was subsequently used to organize papers and data matrices. The selection process was conducted in three stages: (1) title screening, (2) abstract screening, and (3) full-text review. One author was contacted via email to request access to a full-text article, which was later provided and included in the review. The selection process was summarized in a PRISMA flow diagram (Figure 1).

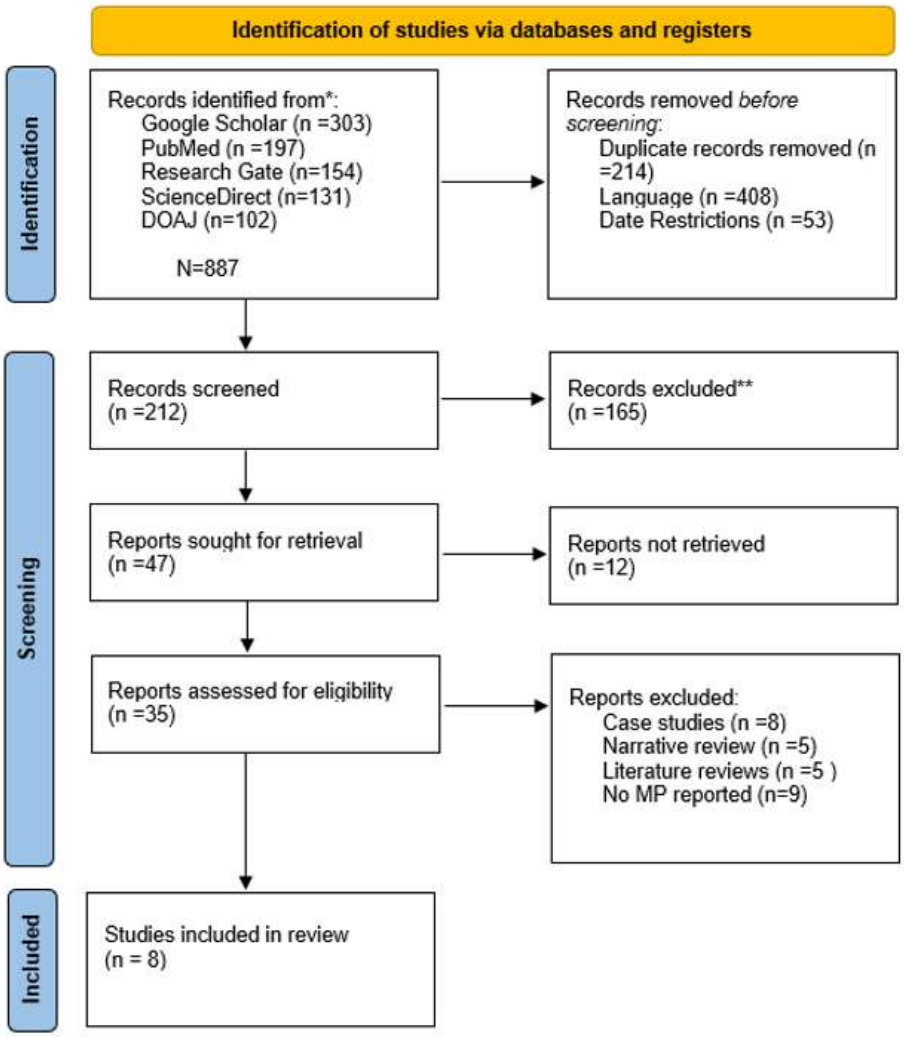

Figure 1. Adapted the PRISMA 2020 Flow diagram in screening and selection of studies via databases and registers.

2.2. Search results and data extraction.

A total of 887 articles were initially identified across the five online databases. Before screening, 675 studies were excluded based on the following criteria: (1) publication date outside the range of 2020–2025, (2) non-English language without available translation, and (3) duplication across databases. After the initial screening, 212 studies remained for title and abstract review. Following this stage, 165 studies were excluded, primarily because they did not report the biological or health impacts of microplastic exposure in fish, despite involving fish samples. The remaining 47 studies were retrieved for full-text assessment; however, 12 studies could not be accessed due to paywall restrictions or unavailability, leaving 35 full-text articles for eligibility evaluation. After full-text evaluation, 27 studies were excluded for the following reasons: 8 were case studies, 5 were narrative reviews, 5 were literature reviews, and 9 did not address microplastics. Ultimately, 8 studies met all inclusion criteria and were included in this systematic review. Four studies were obtained from open-access journals, while the other four were retrieved from ScienceDirect.

3. Results and Discussion

3.1. Analytical techniques in characterization of microplastic.

Table 1 presents the characterization method used in the reviewed studies and the type of microplastic detected while Table 2 presents the characterization methods and the size of microplatic detected. From the eight studies reviewed, only seven studies indicated the size of microplastics discovered.

Table 1. Summary of synthesized studies and the analytical methods utilized in identifying the type microplastics from fish tissues.

Analytical Methods	Type of Microplastic (polymer)	Reference
Raman and FTIR spectroscopy	Polystyrene (PS-MP)	[7]
stereo light microscope and Senterra II Compact Raman Microscope	polyester (PES), polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS).	[8]
Fourier transform infrared (ATR-FTIR) spectroscopy	Polypropylene (PP-MP)	[9]
scanning electron microscopy (SEM) and FTIR	polystyrene (PS-MP)	[10]
FTIR spectroscopy	Polyethylene (PE-MP)	[11]
Raman micro-spectroscopy	Polystyrene, Polyethylene vinyl acetate, Polyamide, High density polyethylene, Low density polyethylene, polyamide; Poly ethylene-co-methyl acrylate, polyethylene wax; Organic PVC: organic poly-vinyl-chloride)	[12]
stereomicroscope and Raman spectroscopy	Polypropylene (PP-MP) Polyester (PES), Polyethylene Terephthalate (PET)	[13]
FTIR analysis	Polypropylene (PP-MP)	[14]

Table 2. Summary of synthesized articles and the analytical methods utilized in identifying the size of microplastics in fish tissues.

Analytical Methods	Size of Microplastic	Reference
Raman and FTIR spectroscopy	5–12 μm	[7]
stereo light microscope and Senterra II Compact Raman Microscope	0.3–0.5 mm, 0.5–1.0 mm, 1.0–2.5 mm, and 2.5–5.0 mm.	[8]
Fourier transform infrared (ATR-FTIR) spectroscopy	8–10 μm	[9]
scanning electron microscopy (SEM) and FTIR	5 μm	[10]
FTIR spectroscopy	10–45 μm and 106–125 μm	[11]
Raman micro-spectroscopy	3–1.2 μm and 1.2–0.45 μm	[12]
stereomicroscope and Raman spectroscopy	Not specified	[13]
FTIR analysis	11.86–44.62 μm.	[14]

3.1.1. Limitations of spectroscopic methods and the complementarity of RAMAN and FTIR.

All of the studies utilized either Fourier Transform Inrared (FTIR) and Raman spectroscopy; these characterization techniques have been widely used and standardized in microplastic research. However, their applicability in detecting the size of certain polymers is limited and varies between the two. For instance, the Raman spectroscopy can be used to identify microplastics less than 20 μm [15], offering an insight into the chemical structure of the polymer. Since Raman Spectroscopy may create a unique fingerprint through the use of light with vibrations, this allows a precise identification and distinction among polymer types. However, in using Raman spectroscopy, samples may require pre-treatment because biological tissues and even dyes present in some microplastics may distort Raman signals [16]. On the other hand, FTIR can be used to detect microplastics above 20 μm only [17], thus giving a lower resolution. FTIR measures the absorption of infrared light by a substance, which provides information about the functional group in a sample, because each polymer or microplastic has a set of chemical bonds. However, the applicability of FTIR is challenged in detecting all types of microplastics, since it can only detect the dominant functional group. Despite the difference between Raman Spectroscopy and FTIR, both can still complement each other. Combining the two methods is helpful in complex biological and environmental matrices, where one method may miss particles to give incomplete spectra.

Although each method applied in the reviewed studies was able to detect certain types of microplastics, their generalizability is intrinsically subject to method-specific constraints like detection limit, interference threshold (e.g., fluorescence in Raman), and particle shape. This implies that no single analytical technique would be complete enough in characterizing microplastics, which further supports the importance of complementary and multi-technique analyses. Among the reviewed studies that employed laboratory-controlled exposure designs, it is expected that the type of microplastic would be identified since the fish were intentionally exposed to specific particles. However, this does not necessarily mean only the introduced microplastic is present, especially when the fish were sourced from the wild. Therefore, analyzing microplastics requires comprehensive and robust approaches. For instance, the analysis of water samples using both Raman and FTIR, revealed that Raman detected 23% more microplastics than FTIR. This finding highlights how the two methods may perform differently on different samples, thus denoting a specific method based on the sample. Additionally, their detection sensitivity is also affected in complex matrices. This corroborates

the findings and observation of [7] that Raman spectroscopy provided the confirmation and visualization of microplastics in fish tissues, while FTIR was used to confirm the presence of microplastics in fish tissue..

3.1.2. Morphological imaging techniques.

Aside from FTIR and Raman spectroscopy, other analytical methods have been used, such as scanning stereomicroscopy, stereo light microscopy, and scanning electron microscopy. According to [18], stereomicroscopy is applicable only in identifying the shape, color, and size of microplastic, but it is impossible to determine chemical composition and magnification is only limited to 100x, thus unable to detect particles smaller than 100 micrometers as supported by [8]. Additionally, SEM may provide images with high resolution and does not necessarily require pre-treatment of the sample, but, like stereomicroscopy, it cannot detect chemical composition. These limitations may lead to misclassifications of microplastic thus, an analytical method is required, such as Raman spectroscopy and FTIR. On the other hand, the stereo light microscopy has been known to be accessible and cost effective and capable to assess morphology of greater than 100 micrometers, yet it has high error rate of 20% to 70% especially in detecting Microplastic that are transparent and smaller than 100 micrometer.

3.2. Biochemical and histopathological effects.

Table 3 presents the eight studies investigating the effect of microplastics on various fish species. This includes information about the fish studied, exposure, routes, types of tissue analyzed, duration, concentration, characteristics of microplastics, and the reported histopathological and biochemical effects.

Table 3. Summary of synthesized studies, the exposure route, duration of exposure, microplastic concentration, type, size, biomarkers, and reported health impacts on fish.

Fish Sample and Tissue Analyzed	Exposure Route/Duration of Eposure/ Microplastic concentration /Type and Size of MPs	Biomarkers Used and the Reported Health Impact	Reference
*Zebrafish *(Danio rerio)* and Perch *(Perca fluviatilis)* *Gills and Liver	a. Oral exposure b. 21 days c. Zebrafish: 20 mg PS-MP/g of food Perch: 10 mg PS-MP/g of food d. Type:(PS-MP) e. Size: 5–12 µm	**Biomarkers:** oxidative stress biomarkers, Comet assay, Apoptosis and autophagy markers, Metabolomic profiling **Health Impact** 1. PS-MP was found in the liver and gills. 2. Exposure resulted in DNA damage, which is more visible in the liver of Perch, apoptosis, autophagy, and oxidative stress. 3. PS-Microplastic is more toxic to Perch than Zebrafish. 4. The toxic effect is species-specific.	[7]
**Field Exposure Design* **Coryphaena hippuru* (Dolphinfish) *Gills,Esophagus,Stomach,Intestinal tract,Muscle	a. Natural environmental exposure b.Sampling site: Eastern c.Pacific Ocean d. *Not laboratory-controlled* e. (PES:), (PET), (PE-PP),(PS). **0.1-0.5 µm (~25%) , 0.5-1 µm (~25%), 1-2.5 µm (36.7%), 2.5-5 µm (13.7%)* *Obtained 139 microplastics from 15 fish*	**Biomarkers:** Molecular docking by modeling Microplastic interaction with cytochrome P450 17A1 **Health Impacts** Impaired reproductive function	[8]

Fish Sample and Tissue Analyzed	Exposure Route/Duration of Eposure/ Microplastic concentration /Type and Size of MPs	Biomarkers Used and the Reported Health Impact	Reference
*Zebrafish *(Danio rerio)* and the freshwater perch (Perca fluviatilis) *Liver and Gills	a. Oral ingestion of food with PS-MP b. Not stated c. 1mg/g (low dose) 10 mg/g (high dose) d. Type: (PP-MP) e. Size: 8–10 µm	**Biomarkers:** NRRT , Lipid Peroxidation,DNA Damage assessment, Protein Ubiquitination, Apoptosis and autophagy markers, Metabolomics **Health Impact** 1. Higher concentration led to a greater effect 2. Impaired cell function in both tissues as indicated by the increased lipid peroxidation, DNA damage, protein ubiquination, apoptosis, and autophagy 3. Altered metabolomes in both tissues 4. Both tissues showed a similar level of toxic response	[9]
*Javanese medaka fish *(Oryzias javanicus Bleeker, 1854)* *Liver, Kidney, Brain, and Intestine	a.Waterborne exposure Fish were exposed to PS-MPs dispersed in tap water b. 21 days c. 100 µg/L (low), 500 µg/L (medium), 1000 µg/L (high) d. Type: (PS-MP) e. Size: 5 µm	**Biomarkers:** Hematoxylin and tissue staining, Catalase (CAT) and superoxide dismutase (SOD) for oxidative stress, Malondialdehyde (MDA) for lipid peroxidation ,Ach and Acetylcholinesterase for neurotoxicity **Health Impact** 1. Inflammation is significantly shown in the intestine, liver, and kidney. 2. Increase intestinal oxidative stress and permeability. 3. Positive for neurotoxic activity 4. Multiple organ effect (systemic toxicity)	[10]
*Zebrafish *(Danio rerio)* and European perch *(Perca fluviatilis)* *Liver and Gills	a.Oral ingestion via food b.21 days c.10 mg PE-MP/g d. (PE-MP) e. Small: 10–45 µm d. Large: 106-125 µm	**Biomarkers:** Lipid peroxidation, DNA Damage, Ubiquitination Signal transduction pathway, Metabolomic analysis **Health Impacts** 1. Under small PE-Microplastic, it resulted in more oxidative stress and apoptosis in the liver and gills compared to larger Microplastic. Additionally caused greater alterations in lipid peroxidation, DNA damage, and cell death pathways, and lastly, it accumulated more in the liver 2. The larger microplastics were found to have accumulated more in the gills, although both triggered tissue-dependent toxicity.	[11]
**Field Exposure Design* * *Serranus scriba* (Painted comb) *Liver	a. Natural environmental exposure b. Sampling site: Tunisian coast *c. Not laboratory-controlled* d. PEVA and HDPE e. Size: Ranging from 3-12 µm and 1.2 to 0.45 µm	**Bomarkers:** MDA for lipid peroxidation,CAT and GST for oxidative stress, MTs for metal exposure, AChE for neurotoxicity, MN assay for genotoxicity, NMR-based metabolomics to analyze changes in 36 key liver metabolites related to energy, amino acid, and osmolyte metabolism **Health Impacts** 1. Site-dependent toxicity in the liver *(Bizerte Channel (BC), located in Bizerte city, Tunisia, this site is reported to have the most abundant microplastics.)* 2. Significant metabolomic disorder, indicating disrupted liver function. 3. Hepatoxicity	[12]

Fish Sample and Tissue Analyzed	Exposure Route/Duration of Eposure/ Microplastic concentration /Type and Size of MPs	Biomarkers Used and the Reported Health Impact	Reference
*Field Exposure Design * Trematomus bernacchii Emerald rockcod *GI tract and muscle tissue	a.Natural environmental exposure b.Sampling Site: Road Bay (Ross Sea, Antarctica) c.*Not laboratory-controlled* d. (PP-MP),(PES), (PET) e. Size: less than 5 µm **average of 1.4 g microplastics per per specimen*	**Biomarkers:** Gas chromatography-mass spectrometry (GC–MS) ,Flame ionization detection (GC-FID) for analysis of fatty acid methyl esters (FAMEs) in muscle tissue to assess lipid metabolism **Health Impacts:** No significant impact on muscle lipid metabolism	[13]
*Oreochromis mossambicus (Mozambique tilapia) *Liver and Brain	a.Oral ingestion via dietary supplement b.Acute (96 hours) Chronic (14 days) c.100, 500, and 1000 mg polypropylene microplastic per kg of dry feed d. Type:(PP-MP) e. Size: 11. 86 µm. and 44. 62 µm.	**Biomarkers:** Reactive oxygen species (ROS), Catalase (CAT) and superoxide dismutase (SOD), glutathione-S-transferase (GST), glutathione peroxidase, Malondialdehyde (MDA) for lipid peroxidation Acetylcholinesterase for neurotoxicity,tissue and cell staining, Comet assay **Health Impact** 1. Increase ROS and oxidative stress in the liver 2. Altered antioxidant activity 3. Increased LPO 4. Inhibition and denaturation of AChE after 14 days 5. Cell and tissue necrosis 6. Longer exposure resulted in more severe toxic effects compared to 96 hours of exposure	[14]

3.2.1 Size-dependent toxicity.

Across multiple studies, smaller microplastics (5–12 µm) were consistently reported to penetrate biological membranes and accumulate in organs, leading to cellular damage, metabolic disorder, and oxidative stress. Studies by [7, 9, 10] revealed that microplastics within this size range induced apoptosis, oxidative stress, DNA damage, and neurotoxicity in fish tissues, underscoring their high mobility and large surface area–to–volume ratio. These characteristics facilitate their interaction with cellular targets and their translocation across epithelial barriers. Smaller microplastics are also more likely to be mistaken for food, facilitating trophic transfer across the aquatic food web [19]. This enhances the potential for bioaccumulation and biomagnification, posing ecological and food safety threats. The relationship between particle size and biochemical response is further evident in [20], which noted that microplastics small enough to circulate in the bloodstream tend to accumulate in diverse tissues, whereas larger particles primarily remain in the gastrointestinal tract or gills [21, 22]. Comparative findings across studies demonstrate that while smaller microplastics (< 5 µm) readily migrate across cell membranes, larger particles (> 70 µm) exert more mechanical problems. For instance, [23] reported differential accumulation patterns, with 0.3 µm particles concentrating in the gut, 5 µm in the gills, and 70–90 µm in the liver. Similarly, [24] described that large particles may cause physical obstruction or irritation (e.g., clogging of the intestine or gills) rather than molecular toxicity. Collectively, these findings suggest a dual mechanism

of size-dependent toxicity: smaller particles induce biochemical and molecular disruptions, while larger ones cause mechanical stress, both contributing to tissue damage and impaired physiological functions.

3.2.2 Species-specific sensitivity to microplastic.

Toxic responses varied markedly among fish species, reflecting differences in physiology, metabolic activity, and habitat. Perch, for instance, exhibited higher biochemical disruptions such as lipid peroxidation, DNA damage, and apoptosis than Zebrafish, as observed by [7]. This interspecific variation aligns with findings in juvenile largemouth bass and Jian carp, which showed intestinal and villi abnormalities at varying exposure levels [24], while grass carp juveniles showed no observable tissue difference, implying potential tolerance or lower uptake rates. Comparative evidence suggests that species sensitivity may depend on both morphology and exposure ecology. In [25], microplastic loads differed among species and locations, with Australian fish showing higher MP concentrations than Fijian species, reflecting regional pollution intensity and habitat-specific accumulation. Similarly, [12] confirmed that spatial variation in microplastic density influences exposure risk and physiological response. These patterns reveal a gap in understanding the physiological and ecological factors such as feeding behavior, habitat depth, and water flow that mediate interspecies differences in microplastic uptake and toxicity.

3.3.3 Route and duration of exposure contribute to toxic outcome.

Variation in experimental design particularly in exposure route and duration significantly influences reported toxic effects. Laboratory studies typically apply controlled, high-dose exposures, revealing clear biochemical and tissue-level responses [11, 13, 14]. In contrast, field studies reflect environmentally realistic conditions but often show less pronounced toxicity, possibly due to lower microplastic concentrations and complex pollutant interactions. For instance, [13] reported no significant lipid metabolism changes in wild fish, whereas [11] observed marked disruptions in controlled settings. Such discrepancies highlight how exposure route (oral ingestion vs. water-borne contact) and environmental context shape toxicity outcomes. Across ingestion studies [7, 11, 14], consistent patterns emerged microplastics accumulated in the liver and gills, causing oxidative and histological damage. Water-borne exposures [10] similarly produced systemic effects through gill penetration and skin absorption. Duration of exposure further modulated toxicity severity. Short-term exposures (hours to days) primarily induced oxidative stress and inflammation, whereas long-term exposures (weeks to months) led to sustained tissue accumulation and cellular degeneration [26]. For example, [14] found that 14-day exposure to PP microplastics caused stronger AChE inhibition and oxidative stress than 96-hour exposure. Collectively, these results reveal dose-, duration-, and route-dependent responses, suggesting that chronic low-level exposures may pose subtle but cumulative risks in natural environments.

3.3.4 Polymer-specific and site-dependent toxicity.

Differences in polymer composition also produced distinct toxicological outcomes. Polystyrene (PS) microplastics commonly derived from packaging and consumer products [27] were repeatedly linked to severe biochemical disturbances. Studies [7, 10] observed

pronounced PS accumulation in liver, gill, and neural tissues, likely due to their small size and aromatic hydrocarbon structure, which enhances cellular reactivity. By contrast, polyethylene (PE) and polypropylene (PP) microplastics caused comparatively milder but still measurable oxidative and genotoxic effects [28]. The lower reactivity of these saturated polymers reduces direct toxicity; however, their hydrophobic nature enhances adsorption of persistent organic pollutants, thereby acting as secondary vectors of chemical toxicity [29].

Field-based studies introduced additional complexity. [12] found that PEVA and HDPE were associated with site-specific hepatotoxicity and metabolic shifts, as revealed by NMR analysis. Environmental factors such as pollution load, pH, salinity, and microplastic aging likely influenced these outcomes. Meanwhile, PET and PES microplastics [30] produced minimal biochemical disruption in field settings, possibly due to lower exposure concentrations or inherent polymer stability. Overall, cross-study comparison suggests that polymer type, exposure site, and environmental context jointly determine toxicity profiles. The same polymer may exhibit variable effects depending on local pollution burden and degradation state—highlighting the need for standardized, cross-polymer comparative studies to clarify mechanisms of toxicity.

3.3.5. Biomarker-based interpretation of microplastic toxicity.

The reviewed studies presented a wide range of biomarkers used to assess the physicochemical responses of fish towards MP contamination and toxic effects. These biomarkers are not only tissue-specific but also used to identify pathways of damage, including oxidative stress, neurotoxicity, DNA damage, and metabolic disruptions. The following are the biomarkers utilized in assessing the toxicity of microplastics.

3.3.6. Oxidative stress biomarkers (CAT, SOD, MDA, ROS).

Oxidative stress was the commonly reported physiological response across studies. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and reactive oxygen species (ROS) were used as primary indicators for cellular activities of imbalance. Elevated malondialdehyde (MAD) is an indication of lipid peroxidation, which consistently appeared in the liver and gills of fish. Notably, [14], showed a dose and time-dependent increase in oxidative biomarkers. Small microplastics induced lipid peroxidation, consistent with their high cell penetration.

3.3.7. Genotoxicity biomarkers (comet assay and micronucleus assay).

DNA damage is reported as a key toxic effect of microplastics, especially under laboratory-controlled exposure using PS and PE. The comet assay utilized in the study of [7] and [14] reported DNA damage in liver cells, which indicates genotoxic. [31] went further by using MN or micronucleus Assay to confirm chromosomal damage, which reinforces the findings that genotoxicity does not happen only in controlled environments but also in natural ecosystems.

3.3.8 Neurotoxicity biomarkers (ache activity and ach level).

The disruption of Acetylcholinesterase, a critical enzyme in neurotransmission, denotes a brain or neural problem, which was consistently reported across the reviewed studies. For instance,

after MP ingestion, it was reported that AChE activity was suppressed [32] and [33]. This biochemical inhibition histological findings of inflammation and necrosis in neural tissues.

3.3.9.Protein and metabolite markers (ubiquitination, NMR metabolomics).

Protein level indicators such as ubiquitination and signal transduction pathway assay, pointing to protein abnormal folding and damage [34]. In addition, NMR-based Metabolomics used by [7] and [12] revealed disruptions in liver metabolites balance, thus confirming systemic metabolic stress.

4. Conclusions

Vibrational spectroscopy techniques, particularly Raman and FTIR, remain the cornerstone of MP identification, yet each presents distinct trade-offs in sensitivity and specificity. Raman spectroscopy demonstrates superior performance for particles <20 μm due to its fine spectral resolution, whereas FTIR offers more robust detection for larger polymers through functional group identification. Complementary imaging tools such as SEM and stereomicroscopy enhance morphological assessment but fall short in providing chemical specificity, underscoring the need for integrated, multi-analytical workflows rather than reliance on a single detection method. Synthesis across studies revealed that MP toxicity is multifactorial, governed by size, polymer type, exposure route, and duration. Smaller particles (<20 μm), especially PE and PP, exhibit greater membrane penetration and bioaccumulation potential, leading to oxidative stress, genotoxicity, and neurotoxicity. However, species-specific differences indicate that physiological and ecological traits modulate sensitivity, suggesting that laboratory models alone may not fully represent natural exposure dynamics. Biomarkers such as CAT, SOD, MDA, and AChE consistently served as reliable indicators of oxidative and neurotoxic stress, yet variations in their application hinder cross-study comparability. Importantly, the contrast between laboratory and field studies exposes a methodological gap: while controlled exposures yield mechanistic insights, they may overstate real-world risks, whereas field observations reflect ecological realism but lack standardized exposure quantification. This divergence highlights the urgent need for harmonized experimental frameworks that bridge both contexts. Moving forward, future research should prioritize (1) the development of standardized protocols that integrate chemical and morphological analyses to improve detection accuracy; (2) cross-validation of biomarker panels for consistent toxicity assessment; and (3) long-term, low-dose exposure studies reflecting realistic environmental conditions. Expanding taxonomic and habitat diversity in toxicity trials will also be crucial to capture species-specific and ecosystem-level effects. Ultimately, advancing methodological consistency and ecological relevance will strengthen the global understanding of microplastic risks in aquatic life and their implications for food safety and environmental health.

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Author Contribution

The author is responsible to all stages/roles in conducting this review.

Competing Interest

The author declares no known competing financial interest such as grants or personal relationships that could have appeared to influence the work reported in this paper.

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