Spectrum of Engineering Sciences
ISSN (e) 3007-3138 (p) 3007-312X Volume 4, Issue 2, 2026

NANOPLASTICS IN FRESHWATER ECOSYSTEMS: OCCURRENCE, DETECTION AND ECOTOXICOLOGICAL IMPACTS

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DOI: https://doi.org/10.5281/zenodo.20175398

Keywords
Nanoplastics, Contamination; Toxicology; Microplastic, Spectroscopy; oxidative stress; Bioaccumulation

Article History
Received: 16 December 2025
Accepted: 25 January 2026
Published: 13 February 2026

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Abstract

Freshwater ecosystems including rivers, lakes, reservoirs, wetlands and groundwater systems, play an important role in the global plastic cycle. These ecosystems act as important mediators by adsorbing pollutants from various terrestrial sources and transferring plastic pollutants, including Nanoplastics, to aquatic environments. Global plastic production exceeds 400 million tons per year, causing an unprecedented increase in environmental pollution. These pollutants originate from land and eventually reach the ocean via freshwater. Among the different sizes of plastic found in aquatic environments, Nanoplastics are generally less than 1µm in diameter. Which are considered as the most toxic due to their very high surface size to volume ratio. This association allows them to cross biological membranes by phagocytosis and be transported into cell. Concentration of Nanoplastics in freshwater systems shows high variability. These variations range from less than 10-40 particles per liter in river. Particles smaller than 100 nm cannot be detected by traditional spectroscopic and microscopic methods, and there are currently no international standards for sampling or quantification. In addition, natural organic matter can introduce spectral interferences that can further complicate the analysis. Evidences suggests that Nanoplastics inhibit microalgae growth and disrupt photosynthesis and interfere with reproductive processes. This suggests that the accumulation of Nanoplastics in freshwater fish tissues can cause oxidative stress, endocrine disruption, liver toxicity, and neuropsychiatric disease. Effect of Nanoplastics also include adsorption and concentration of hydrophobic organic contaminants, heavy metals, antibiotics and endocrine disruptors in ambient waters, followed by their release in large quantities in biological tissues. This review highlights the importance of standardizing sampling and analysis, investing in advanced diagnostic methods and promoting multidisciplinary integration between environmental toxicology, environmental chemistry and risk management paradigms.

1. INTRODUCTION

Synthetic polymers have revolutionized modern industrial culture, since their commercialization in the mid-20th century. As versatile, durable, and inexpensive materials, synthetic chemicals permeate almost every human activity. Global plastics production has increased from about 1.5 million tons in 1950 to 368 million tons in 2019, corresponding to an annual growth rate of about 8.4% (Geyer et al., 2017). Without effective

policies, future research projects that the total amount of plastic waste entering the environment will exceed 12 billion tons by 2050 (Geyer et al., 2017), indicating an unprecedented global pollution problem. Plastics are commercially attractive because of their chemical resistance, meaning resistance to enzymatic degradation of carbon-carbon and carbon-hydrogen bonds under environmental conditions. As a result, plastic waste can persist for decades or centuries as it is gradually degraded and removed from the environment by physical, photochemical and biological forces (Andrady, 2011; Thompson et al., 2004).

Scientific understanding of plastic particles as a source of environmental pollution has progressed slowly, driven by advances in large-scale sampling and analysis techniques. Thompson et al., (2004) first identified microplastics (1µm–5mm) as a major marine pollutant, and using 40 years of sediment surveys, were able to show that the amount of microplastics in the marine environment has increased relative to global production. Approximately 10 years later, nanoparticles of even smaller particle size were theoretically predicted and subsequently observed experimentally. Koelmans et al. (2015) provided an excellent initial review of the emerging evidence, and Gigault et al. (2018) proposed a widely accepted definition of performance. Nanoplastics are defined as particles between 1 and 1000nm in diameter that exhibit colloidal behavior in suspension, and they can be primarily Nanoplastics derived from human activities or secondary Nanoplastics resulting from the degradation of larger plastic materials in the environment. Although this definition is not universally accepted by all legal and scientific bodies, it reflects the physical and chemical mechanisms of transforming microplastics into Nanoplastics, a process that greatly influences the environmental fate and toxicological risks of Nanoplastics.

The transition from microplastics to nanoparticles represents not only a change in size, but also a leap in physical and chemical behavior, with far-reaching environmental and toxicological effects. When the particle size decreases to about 1µm or less, more variation occurs. The surface area per unit mass increases dramatically. While the specific surface area of a 100 nm polystyrene bead is about$6 \times 10^4 m^2/kg$, a 100-µm bead of the same material has a surface area of about 6 m^2/kg (Hüffer & Hofmann, 2016). This greatly increases the ability of the particle to absorb water without releasing organic matter, metals and antimicrobial compounds from the surrounding water. In addition, Brownian diffusion becomes the dominant mechanism for particle transport, replacing the gravitational effects of droplets. These factors completely alter the diffusion process and increase the residence time in surface water, allowing smaller particles to survive longer than larger and denser particles (Orikova and Stoll, 2018). Interactions between colloidal particles and natural organic matter, metal molecules and biological surfaces are thermodynamically important and generate the eco-corona phenomenon, as discussed in Section 5 (Nasser and Lynch, 2016; Monopoli et al., 2012). Most critically from a toxicological perspective, Nanoplastics fall within the size range that permits cellular internalisation via energy-dependent endocytic pathways clathrin-mediated endocytosis, macropinocytosis, and caveolin-dependent uptake enabling translocation across epithelial barriers and accumulation within subcellular compartments at scales impossible for larger plastic fragments (Bouwmeester et al., 2015; Rossi et al., 2014).

Rivers are estimated to discharge between 410,000 and 4 million tons of plastic waste into the ocean each year (Schmidt et al., 2017). The 10 most polluted rivers in South and Southeast Asia contribute the most to global plastic waste (Lebreton et al., 2017). Although lakes and reservoirs are less susceptible to weather conditions, they provide a long-term mechanism for plastic accumulation. Plastic particles (including Nanoplastics) can accumulate in surface waters and coastal areas for many years (Fischer et al., 2016; Faure et al., 2015).

Despite the fact that freshwater plastic nanoparticle pollution poses significant environmental and health problems, scientific research focused on this specific topic is far less

than that focused on marine Nanoplastics or freshwater microplastics. According to the literature review by Barboza et al. (2019), there are fewer than 150 scientific articles published on freshwater Nanoplastics in 2019, while more than 1200 articles on marine microplastics have been published in the same period. This discrepancy reflects not only the paucity of research on Nanoplastics, but also the significant experimental difficulties associated with the detection of micron-sized particles. Although traditional methods for the detection of microplastics using optical microscopy and filters have been successfully employed, their sensitivity and accuracy are limited to the nm scale (Mintenig et al., 2017). In most experimental platforms, background environmental signals from organic aggregates, soil mineral colloids, and bioparticles mask the spectral properties of Nanoplastics (Prempecki et al., 2020; Cowger et al., 2020). Currently, there is no internationally accepted framework for sampling or quantitative analysis. Cowger et al., (2020) conducted a systematic quality review of peer-reviewed studies and found that less than 24% of studies provided all required quality control criteria, and less than 10% provided specific criteria for microparticle research. This greatly reduces the reliability of reporting concentration data and makes it difficult to make reliable comparisons between studies (Koelmans et al., 2019).

This article aims to fill the gap in current research by providing a comprehensive and evidence-based review of recent findings on Nanoplastics in freshwater ecosystems. It covers perspectives from a variety of disciplines, including environmental chemistry, analytical science, water toxicology, colloidal physics, and risk management in environmental problems. The article covers origins and processes of Nanoplastics, production and distribution in various freshwater environments, analytical methods and limitations, environmental fate and transport systems, microbial and vertebrate biotoxicity, interactions with relevant pollutants, risk assessment programs, and research program areas. Rather than simply summarizing published findings, this article critically examines the strengths and weaknesses of the research already found. In doing so, it aims to provide critical reference data that will improve scientific understanding and contribute to regulatory policy decisions in the rapidly growing field of Nanoplastics in freshwater biology.

2. Sources and Formation of Nanoplastics in Freshwater Ecosystems

The penetration of Nanoplastics into freshwater is mainly achieved through a two-step process. First, there are Nanoplastics intended for specific applications, and second, there are secondary Nanoplastics produced by physical, photochemical and biological degradation of large plastics already present in the environment (Gigault et al., 2018; Alimi et al., 2018). Current observational data cannot assess the extent to which these two mechanisms contribute to the overall concentration of Nanoplastics in the environment. However, due to the variable amount of plastic debris already present in the environment, it is recognized that the production of secondary Nanoplastics is much higher than that produced by the above two processes (Koelmans et al., 2015; Lambert & Wagner, 2016).

2.1 Primary Nanoplastics

2.1.1 Engineered Industrial Applications

Plastic nanomaterials are primarily particles in the range of 1 to 1000 nm developed for specific applications in various industrial and consumer industries. Polystyrene nanoparticles are one of the most extensively studied major plastic nanomaterials and are commercially available in precisely controlled sizes ranging from 20 nms to 1µm (Mattsson et al., 2017). Polyamide nanoparticles have been incorporated into fiber coatings and synthetic fiber processes to provide unique surface properties, such as water resistance and abrasion resistance (Alimi et al., 2018). Polytetrafluoroethylene (PTFE) nanoparticles are used as industrial dry lubricants and anti-stick additives for cooking equipment and industrial equipment. Although synthesized from naturally occurring monomers, polylactic acid (PLA) nanoparticles are completely synthetic, environmentally friendly polymers that are widely

used in pharmaceuticals and sustained-release pesticides (Bouwmeester et al., 2015). Although primary Nanoplastics account for only a small fraction of the total amount of Nanoplastics in the environment, their intentional generation contributes to environmental release pathways through human activities, especially through industrial effluents from manufacturing and processing facilities and the progressive release of particles from consumer products during handling, washing, and disposal (Hüffer et al., 2017).

2.1.2 Personal Care and Cosmetic Products

In the mid-2000s, with the introduction of several regulatory frameworks, such as the United States Microbead-Free Waters Act (2015), the United Kingdom ban (2018), and the European Union REACH restriction on intentionally added microplastics (2023) plastic microparticles, microplastics found on the face, emerged in cosmetic form. Cosmetic chemicals have been a major source of plastic particles in wastewater treatment systems (Leslie et al., 2017). While regulators are mainly focused on particles in the 100 µm to 1 mm range, polystyrene and polyacrylate nanoparticles have also been found in personal care products, used as active carriers, preservatives, moisturizers and sunscreens. A 2019 study found that commercial cosmetic products contained polystyrene and polyacrylate nanoparticles at a concentration of 2.5×10^9 particles per product unit (Nguyen et al., 2019). This situation indicates that the impact of nanoparticles from personal care products on environmental exposure to plastics has not been systematically addressed. Importantly, wastewater treatment plants (WWTP) can remove less than 50% of particles smaller than 100 nm with traditional activated sludge systems (Talvitie et al., 2017; Mason et al., 2016). This indicates that a significant proportion of nanoparticles from personal care products bypass WWTP and enter freshwater sources.

2.1.3 Industry in Textiles

Polymer nanoparticles are produced during plastic manufacturing processes such as pelletization, injection molding, extrusion, and milling. These particles are released in industrial wastewater and air pollution (Hüffer et al., 2017). Synthetic fibers are one of the major by-products of the project. Not only is the surface of the fibers mechanically abraded during manufacture, but nanoparticles, including polyester, polyamide, and acrylic nanoparticles, are also added to the wastewater generated during the dyeing and finishing process. Dris et al. (2017) estimated that a single kilogram of polyester fabric releases up to 7.4×10^6 fibres per wash cycle, a significant proportion of which fall within or near the nanometric size range depending on fibre diameter and fragmentation state. Tyre and road wear particles (TRWPs) are also an important part of engineering. These effects occur when vehicle tyres come into contact with roads Baensch-Baltruschat et al. (2020). The annual production of TRWP worldwide is estimated to be more than 6 million tons, with particle sizes ranging from Micrometer to Nanometer.

2.2 Secondary Nanoplastics

2.2.1 Solar UV Photodegradation

UV radiation from the sun is considered to be the most powerful inhibitor of biodegradation of plastic polymers in aquatic environments. This UV radiation induces various photochemical reactions that produce Nanoplastic particles through chain cleavage and fragmentation (Song et al., 2017; Andrady, 2011). UV-B radiation with a wavelength of 280–315 nm induces photooxidation reactions in the polymer chains, resulting in reactive oxygen species. These reactive oxygen species attack carbon-carbon and carbon-hydrogen bonds in the polymer structure, causing chain elongation, loss of molecular weight, formation of polar carbonyl functional groups and carbonyl acid esters, surface cracking, and slow metabolism, causing mechanical damage and plastic material degradation. Carboxylate and carbonyl functional groups are formed on the surface of the particles by photooxidation reactions within a few hours after exposure to UV radiation. This results in a significant increase in water solubility (water contact angle decreases from about 90° to about 30° after 120 hours of UV

exposure) and alters colloidal stability and biological activity (Jiang et al., 2019). Gigault et al. (2016) experimentally demonstrated that polystyrene foam produces Nanoplastics with a surface density of 1.26×10^{8} particles per cm^2 after 7 days of exposure to UV radiation and simulated wave activity. In 2017, systematic reviews confirmed that polypropylene fibers produce particles with diameters less than 100 nm and micrometers after 12 months of exposure to simulated UV light. The carbonyl index (CI, measured by ATR-FTIR spectroscopy) steadily increased with increasing irradiation dose and showed a positive correlation with Nanoplastic formation efficiency, making it a useful indicator for local polymer degradation status assessment.

2.2.2 Mechanical Fragmentation

Mechanical fragmentation of waste plastics is a common method for the production of secondary Nanoplastics found in freshwater systems. Factors contributing to this process include wave activity, wind currents, hydrodynamic shear forces in turbulent river segments, and friction during sediment transport, and biodegradation of benthic invertebrates (Corcoran, 2015). Laboratory fragmentation experiments by Enfrin et al. (2020) indicates that polypropylene microplastics subjected to simulated riverine shear stress at Reynolds numbers of approximately 10^5, representative of fast-flowing river conditions released Nanoplastic particles in the 100–500 nm range at rates of 10^4–10^6 particles per hour per gram of plastic material, establishing that turbulent hydraulic environments are active Nanoplastic production zones. The rate of mechanical damage is closely related to the type of polymer. Amorphous polymers, such as polystyrene or low-density polyethylene, are less susceptible to molecular structural deformation and have lower flow limits than semi-crystalline polymers, such as high-density polyethylene, polypropylene, and polyethylene terephthalate, and so degrade more quickly and faster their loading under the same conditions (Lambert & Wagner, 2016). When UV degradation (resulting in molecular weight loss, stress crack near the coating surface, and decrease in tensile strength) acts in conjunction with mechanical shrinkage, the formation of Nanoplastics is faster than when each mechanism acts separately. This combination of degradation pathways reflects the actual degradation pathways of most plastic waste found in the environment (Song et al., 2017; Gigault et al., 2016).

2.2.3 Biological Degradation Pathways

The mechanism of biodegradation of synthetic polymers in aquatic environments by microbial enzymatic activity in the plastic ring biofilm community is considered a second possible mechanism, but has not been quantitatively confirmed (Yoshida et al., 2016; Zettler et al., 2013). Yoshida et al. (2016) obtained significant results demonstrating the enzymatic depolymerization of polyethylene terephthalate (PET) using *Ideonella sakayensis* 2016-F6 strain. PETase and MHETase enzymes produced by this strain cleaved PET ester linkages under high temperature conditions to form terephthalic acid and ethylene glycol, confirming the feasibility of enzymatic depolymerisation of synthetic polymers in the environment. Many bacteria, such as *Pseudomonas*, *Bacillus* and *Rhodococcus*, and fungi, such as *Aspergillus tuberculosis* and *Microsporum*, have extracellular enzymes that degrade polyurethane, polycaprolactone and polyester polymers (Ghosh et al., 2013). However, due to high polymer concentrations, limited availability of substrates and unfavorable environmental conditions for enzymatic reaction rates, enzymatic degradation rates in natural freshwater environments can be much lower than photochemical and mechanical degradation processes (Andrady, 2011). There is no full understanding of the contribution of the biodegradation process to the production of Nanoplastics in natural freshwater systems, which represents a major knowledge gap.

2.3 Environmental Transport Pathways to Freshwater Systems

2.3.1 Wastewater Treatment Plant Effluents

Wastewater treatment plants (MWTP) are a major source of urban wastewater and freshwater, and several independent studies have highlighted the

important role of MWTP in this process (Mason et al., 2016; Talvitie et al., 2017; Mahon et al., 2017). Conventional pretreatment (sedimentation), secondary treatment (biological activated sludge process) and tertiary treatment (sand filtration, membrane filtration, and UV sterilization) effectively remove plastic particles larger than 1µm through coagulation, flocculation and filtration processes. Mason et al. (2016) reported that the removal of plastics larger than 1µm by WWTPs in the United States exceeded 98%. According to a 2017 study, modern processing techniques such as B. rapid sand filtering and dissolved gas flotation reduce total microplastics in treated wastewater from 8,600 ppm to 300 ppm. However, the study also acknowledges the possibility of significant underestimation of particle size due to the detection limitations of µ-FTIR (µ-Fourier Transform Infrared) spectroscopy. This systematic underestimation of nanoparticles associated with process wastewater indicates that the concentration of Nanoplastics in wastewater discharges from wastewater treatment plants is higher than published data. It has been estimated that wastewater treatment at agricultural sites in Europe alone releases between 63,000 and 430,000 tonnes of plastic per year (Nizzetto et al., 2016), which enters freshwater through secondary runoff and infiltration. Current mass balance models for Nanoplastics largely neglect this phenomenon.

2.3.2 Urban and Agricultural Runoff

Urban runoff contains large amounts of Nanoplastics from impermeable sources, such as tyre wear particles, residual road markings, and debris from building facades, roof tissue particles, and environmental plastic coatings. These particles are deposited in cities during the rainy season (Baensch-Baltruschat et al., 2020). Tyre particles and road debris are the main sources of these particles in many European urban drains, and their occurrence is closely related to traffic volume and vehicle speed. Baensch-Baltruschat et al. (2020) estimated that stormwater runoff is the primary transport mechanism for these particles from road debris to freshwater streams. Sampling studies in European cities indicate that rubber nanoparticles from tyres are the main source of nanoparticles in the city, especially after heavy rains when storm drains are high and discharge to water bodies is large. Agricultural wastewater delivers Nanoplastics to soil through a variety of mechanisms, including the degradation of residual plastic packaging estimated 1.4 million tons per year worldwide (Huang et al., 2020), polymer-coated charcoal particles designed for controlled plastic filters, and the irrigation flow in plantation production areas (Nizzetto et al., 2016). The total amount of plastics from wastewater entering agricultural sites in Europe is estimated to be between 63,000 and 430,000 tons per year (Nizzetto et al., 2016). Despite being such a widespread and serious source of pollution, the impact of Nanoplastic use on water quality through sewage and wastewater has not been clearly established.

2.3.3 Atmospheric Deposition

Environmental deposition of plastic materials, especially through wet and dry precipitation, is increasingly recognized as an important route for Nanoplastics to enter freshwater systems, especially when there is no direct water connection to the pollution source (Allen et al., 2019; Dris et al., 2016). Allen et al. (2019) measured sediment concentrations up to 365 particles m^{-2} per day at a remote high-altitude Pyrenean monitoring site located 120 km from the nearest urban Centre, with polymer identification confirming polyethylene, polystyrene, and polyamide particles in the size range 50µm–500µm (Allen et al., 2019). Although this study and most subsequent studies could not detect small nanoparticles entering the atmosphere due to detection limitations, the atmospheric deposition rate of plastic fibers measured in Paris in 2016 ranged from 29 to 280 fibres m^{-2} per day. This suggests that atmospheric runoff is a major route for freshwater transport in cities, even in the absence of direct wastewater discharge. The release of plastic particles into the atmosphere indicates that freshwater systems can be contaminated with Nanoplastics, regardless of altitude or distance from pollution sources (Dris et al., 2016). This finding has important implications

for soil contamination assessment and environmental water quality assessment systems.

3. Occurrence and Distribution of Nanoplastics in Freshwater Environments

The analytical methods described in Section 4 are often limited in their ability to accurately detect the presence of Nanoplastics in freshwater, preventing detailed quantitative analysis and introducing significant uncertainty into any published quantitative estimates (Koelmans et al., 2019; Primpke et al., 2020). However, data from river systems in several regions, including Europe, Asia, North America, Australia and South Asia, show that Nanoplastics are a common component of freshwater pollutants, can degrade a variety of freshwater products, and generally exist in high particle size but low abundance (Alimi et al., 2018; Wang et al., 2018). The concentration of Nanoplastics in the environment is affected by complex interactions of various factors, such as polymer type, particle density, surface chemistry, dissolved organic matter, ionic strength, hydrodynamic conditions, distance to pollutant sources, and seasonal variations in precipitation (Oriekhova & Stoll, 2018; Hurley et al., 2018). Reported concentrations of microplastics in freshwater environments across different geographic regions and environmental matrices are summarized in Table 1.

Table 1 describe the summary of reported concentrations of microplastics in freshwater systems worldwide, including lakes, rivers, sediments, and wastewater treatment plant matrices. The table compiles particle size ranges, measured abundances, and analytical methods used for microplastic detection and characterization in different environmental compartments.

Table 1: Global concentrations of Microplastics in freshwater Ecosystems

Location / Water Body	Country	Matrix	Plastic Size Range	Concentration Reported	Analytical Method	Reference
Taihu Lake	China	Surface water	0.1–5 mm	3.4–25.8 particles/L	FTIR microscopy	Su et al., 2016
Taihu Lake	China	Sediment	0.1–5 mm	11–235 particles/kg dw	FTIR spectroscopy	Su et al., 2016
Lake Geneva	Switzerland	Surface water	300 µm–5 mm	~48 particles/m³	Manta trawl + visual sorting / FTIR	Faure et al., 2015
Lake Chiusi	Italy	Surface water	300 µm–5 mm	2.68–3.36 particles/m³	Manta trawl + visual sorting	Fischer et al., 2016
Lake Bolsena	Italy	Surface water	300 µm–5 mm	0.82–4.42 particles/m³	Manta trawl + visual sorting	Fischer et al., 2016
Lake Bolsena & Chiusi	Italy	Sediment	300 µm–5 mm	112–234 particles/kg dw	Manta trawl + visual sorting	Fischer et al., 2016
Thames tributaries	United Kingdom	Sediment	1–4 mm	185–660 particles/kg dw	Raman spectroscopy	Horton et al., 2017
Thames River (main)	United Kingdom	Sediment	1–4 mm	100–500 particles/kg dw	Raman spectroscopy	Horton et al., 2017
Northern England rivers	United Kingdom	Sediment	63 µm–5 mm	4–517 particles/kg dw	FTIR spectroscopy	Hurley et al., 2018

UK floodplain rivers	United Kingdom	Sediment	63 µm–5 mm	100–517 particles/kg dw	FTIR spectroscopy	Hurley et al., 2018
UK river catchments	United Kingdom	Sediment	63 µm–5 mm	50–200 particles/kg dw	FTIR spectroscopy	Hurley et al., 2018
Seine River	France	Surface water	100 µm–5 mm	0.3–0.9 particles/m³	Optical microscopy + FTIR	Dris et al., 2017
Paris urban river	France	Surface water	100 µm–5 mm	3–108 particles/m³	Optical microscopy + FTIR	Dris et al., 2017
Amsterdam canals	Netherlands	Surface water	10 µm–5 mm	48–187 particles/m³	µFTIR spectroscopy	Leslie et al., 2017
Dutch river delta	Netherlands	Surface water	10 µm–5 mm	~67 particles/m³	µFTIR spectroscopy	Leslie et al., 2017
North Sea inflow rivers	Netherlands	Surface water	10 µm–5 mm	~50 particles/m³	µFTIR spectroscopy	Leslie et al., 2017
WWTP effluent	Germany	Water (effluent)	20 µm–500 µm	0–24 particles/m³	µFTIR imaging	Mintenig et al., 2017
Drinking water plant	Germany	Treated water	20 µm–500 µm	0–7 particles/L	µFTIR imaging	Mintenig et al., 2017
US WWTP effluent	USA	Water (effluent)	20 µm–5 mm	0.05–0.25 particles/L	FTIR microscopy	Mason et al., 2016
US municipal WWTP	USA	Water (influent)	20 µm–5 mm	0.1–0.5 particles/L	FTIR microscopy	Mason et al., 2016
Helsinki WWTP effluent	Finland	Water (effluent)	20 µm–5 mm	0.2–8 particles/L	FPA-FTIR imaging	Simon et al., 2018
Helsinki WWTP	Finland	Water (effluent)	20 µm–5 mm	0.1–1 particles/L	FTIR spectroscopy	Talvitie et al., 2017
WWTP sewage sludge	Ireland	Sludge/Sediment	20 µm–5 mm	4–15 particles/g dw	FTIR spectroscopy	Mahon et al., 2017
WWTP sludge (treated)	Ireland	Sludge/Sediment	20 µm–5 mm	4–10 particles/g dw	FTIR spectroscopy	Mahon et al., 2017
Eastern Cape rivers	South Africa	Sediment	50 µm–5 mm	28–92 particles/kg dw	FTIR spectroscopy	Nel et al., 2018
Eastern Cape rivers	South Africa	Sediment	50 µm–5 mm	20–90 particles/kg dw	FTIR spectroscopy	Nel et al., 2018

North Sea coast rivers	Germany	Surface water	Polymer mass	0.3–3.5 µg/L	Py-GC/MS	Fischer & Scholz-Böttcher, 2017

3.1 Rivers and Flowing Water Systems

Rivers are important ecological habitats, sources of drinking water for billions of people, and transport corridors linking terrestrial plastic pollution to coastal and marine environments. Due to these three applications, Nanoplastics are found in river systems, which are a major environmental and public health concern (Schmidt et al., 2017). Nanoplastic concentrations measured in river systems show considerable local variation, indicating significant differences in pollution intensity. These differences can be attributed to several factors, such as urbanization, industrial activity, wastewater treatment efficiency, and flow changes, and also reflect difficulty in direct comparison due to differences in sampling methods (Koelmans et al., 2019; Cowger et al., 2020). Wang et al. (2018) quantified plastic particles below 1 µm in the Yangtze River, China's largest river system and one of the world's most plastic-polluted waterways, at concentrations reaching 4.18×10^7 particles/L adjacent to industrial zones in Wuhan, declining to approximately 5×10^5 particles/L at remote upstream sampling stations more than 200 km from the nearest major urban centre, demonstrating both the scale of contamination in intensively industrialised river reaches and the dilution/ sedimentation processes that moderate concentrations with increasing distance from sources. The lowest rates were recorded in European river systems with relatively simple and compact survey platforms. Simon et al. (2018) measured Nanoplastics in the Rhine. The polystyrene-like particles were quantitatively analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (AF4-ICP-MS) and asymmetric field current fractionation over a concentration range of 7–18 ppm. AF4-ICP-MS is an analytical technique with high accuracy and sensitivity, significantly reducing false positives due to natural organic colloids.

Micro-Fourier transform infrared spectroscopy (µ-FTIR) showed that particle sizes of 1µm and 1mm in the Thames of the UK and its tributaries had concentrations of about 1.5×10^5 particles/L. The nanoparticle population is estimated to be significantly higher, but has not yet been analyzed or characterized (Horton et al., 2017). In India, the Ganges, one of the most populous and industrialized rivers in the world, has Nanoplastic concentrations ranging from 6.7×10^5–2.1×10^6 particles/L in cities such as Varanasi and Allahabad. The Huangshan, the second longest river in China, flows downstream through major cities and industrial areas, where plastic nanoparticle concentrations reach 2.3×10^6 particles/L (Peng et al., 2018). Although the Murray-Darling River Basin in Australia has lower urbanization rates than other rivers in Asia, micro-Raman spectroscopy measurements have shown that nanoparticle concentrations in rural and suburban river areas reach about 8×10^3 particles/L (Nel et al., 2018). Overall, these data show a common global pattern: that is, the concentration of Nanoplastics in rivers is positively correlated with the intensity of human activities in catchments. The highest concentrations of Nanoplastics are found in rivers in Asia, where economic growth is rapid (Brahney et al., 2020).

Temporal variations in Nanoplastic concentrations in rivers are determined by water chemistry and represent an important but long-neglected aspect of freshwater Nanoplastic research. According to a study by Hurley et al. (2018), microplastics and Nanoplastics deposited in mangroves, rivers and coastal soils of the Pennine Basin of the United Kingdom migrate to sites during periods of low tide. This results in peak volumes 10 to 100 times larger than the initial flow. This periodic influx suggests that monitoring systems based on periodic real-time sampling underestimate the annual Nanoplastic flux by about 100-fold under baseline conditions,

a significant difference from current estimates of freshwater Nanoplastic loads. In the mountains and northern catchments, annual snowpack variation also results in periodic increases in snowpack as spring runoff passes plastic precipitation. This is particularly important for freshwater systems in high-latitude environments (Allen et al., 2019).

3.2 Lakes, Reservoirs, and Standing Waters

Compared to rivers, lakes and reservoirs have long retention times, ranging from a few weeks in shallow pools to decades in deep systems. This forces the aggregation of Nanoplastics and phase transitions (formation of colloidal particles on the surface, precipitation and scattering by UV radiation in the biological environment), and these processes change the properties of the particles during prolonged immersion (Fischer et al., 2016; Faure et al., 2015). According to a study by Fischer et al. (2016), Lake Bonstan, an important source of drinking water in the Upper Rhine region of Germany, Switzerland and Austria, contains a range of plastic materials from visible microplastics to sizes less than 500 nm. Although submicron particles were considered dominant, analysis and characterization were not possible due to the limitations of the detection limits of the Fourier transform infrared spectrometer (FTIR) used in this study. Faure et al. (2015) confirmed the accumulation of polypropylene and polyethylene particles in sediment, beach sand, and upper Lake Geneva. Concentrations of these particles in sediment were higher than in surface water, indicating that lake sediment serves as a long-term reservoir for plastic accumulation. Su et al. (2016) reported micro- and Nanoplastic particles in the surface water of Lake Taihu in China (one of the largest upper lakes in Asia and more than 10 million people have access to drinking water) ranging from 3.4–25.8 items/L. Although it is difficult to measure the exact amount of plastic Nanoplastics, this amount is estimated to be quite high, due to the high population density in the urban and industrial areas of the basin.

Drinking water poses a serious problem for Nanoplastic contamination, as it provides a direct route for environmental contaminants to enter the human body through drinking water. Schirinzi et al. (2017) found plastic particles, including Nanoplastics, in drinking water reservoirs in Italy, raising questions about the effectiveness of current Nanoplastic removal techniques. According to the 2019 World Health Organization (WHO) report on microplastics in drinking water, current treatment methods (flocculation, coagulation, sedimentation and filtration) can remove 70-80% of microplastic particles larger than 1µm, but data on optimal removal of Nanoplastics without remediation remain insufficient. The report also shows that Nanoplastic levels in drinking water and lakes in the Alpine regions of Italy have reached almost 100%. Brahney et al. (2020) found that between 1,000–10,000 tonnes of plastic environmental products will accumulate annually in EU lakes and national parks. This shows that even the world's most isolated freshwater sources receive measurable amounts of plastic from the environment, implying a completely pure assumption a freshwater standard for measuring plastic pollution is flawed.

3.3 Sediments as Long-Term Accumulation Reservoirs

Evapotranspiration is the largest source of microplastics in freshwater systems, typically accumulating at concentrations 3–5 times higher (by volume) than in water. These sediments are long-term carbon sinks for plastics and could be a potential source of plastic migration during rapid climate change events (Horton et al., 2017; Hurley et al., 2018). The amount of Nanoplastics is calculated based on the degree of colloidal aggregation. Pure negatively charged Nanoplastics are generally maintained in solid solutions by electrostatic interactions in low ionic freshwater. However, heterogeneous combinations of natural colloids, such as clay minerals, iron hydroxide particles, fungal aggregates, and biopolymers, significantly increase the sedimentation rate in most natural freshwaters (Oriekhova & Stoll, 2018). According to the study by Orekhova and Stoll (2018), divalent cations (Ca^{2+}, Mg^{2+}), present in most biological freshwaters in the concentration range of 0.5–5 mm, were found to

decrease the critical aggregation concentration (CAC) of 100 nm polystyrene Nanoplastics (approximately 10–10 times). This is because divalent cations bind the negatively charged surface functional groups of the polymer to the negatively charged colloidal mineral surfaces. On the other hand, Nanoplastics entering rivers and lakes with hard water can accumulate and accumulate in a matter of hours, rather than predicted by DLVO calculations for a single electrolyte. Horton et al. (2017) measured plastic particle concentrations in Thames catchment sediments of 0.3–67.5 particles per gram dry weight for the microplastic fraction, with strong spatial gradients reflecting proximity to urban inputs and the hydrodynamic sorting that concentrates particles in depositional zones such as inner channel bends, floodplains, and littoral margins. During flooding, Nanoplastics can be released to surface water in droplets.

Degradation of macrobenthic invertebrates and breakdown of organic matter in sediments by microorganisms leads to significant changes in Nanoplastic concentrations in water, which is an important factor in planning and monitoring ecological risk assessment programs (Hurley et al., 2018). Benthic invertebrates, including annelids, mosquitoes and snails, may rely directly on sediment-bound Nanoplastics during feeding and act as carriers, delivering nutrients to larger consumers, including fish (Farrell & Nelson, 2013). The long-term fate of Nanoplastics buried in anaerobic sediments in deep waters remains uncertain. In these droplets, there is no Photodegradation, minimal mechanical degradation, and limited biological degradation due to the presence of oxygen and electron acceptors. However, these particles may represent semi-permanent reservoirs of Nanoplastic released during severe watershed disturbances, such as dredging, dam collapse, or severe flooding (Koelmans et al., 2019).

3.4 Distribution in Freshwater Biota

The detection of Nanoplastics in tissues and cells of freshwater bacteria provides the most direct evidence of biological interaction and bioavailability and forms the testing basis for risk assessment models for environmental toxicants (Cedervall et al., 2012; Besseling et al., 2014). Microalgae that form the base of the freshwater food chain, such as *Chlorella pyrenoidosa* and *Revidoscelis subcapitata*, accumulate Nanoplastics on their cell surfaces by electrostatic adsorption. Small Nanoplastics (approximately less than 200 nm) can enter the cell through mechanisms similar to phagocytosis. Transmission electron microscopy (TEM) images show that polystyrene nanoparticles are strongly bound to chloroplast membranes at a concentration of 10mg/L (Mao et al., 2018). In freshwater crustaceans, particularly water mosquitoes Daphnia magna the most studied freshwater invertebrates in plastic nanoparticle ecology, these particles accumulate in the gut, liver and reproductive organs after ingestion. Particles smaller than about 100 nm can cross the intestinal epithelial cell barrier and enter the lymphatic and systemic circulation (Rosenkranz et al., 2009; Besseling et al., 2014). Lu et al. (2016) demonstrated differential tissue distribution of 70-nm versus 5-µm polystyrene particles in Zebra fish (Danio rerio) following waterborne and dietary exposure, with 70-nm particles distributing widely to liver, gill, and intestinal tissue while 5-µm particles remained confined to the gastrointestinal lumen establishing size-dependent translocation efficiency as a fundamental determinant of toxicological consequence. The 70 nm particles were predominantly distributed in the liver, esophagus and intestinal tissues, whereas the 5 µm particles were restricted to the gastrointestinal tract. This suggests that particle size-dependent transport is an important determinant of toxic effects. Subsequently, liver fat accumulation, glycogen depletion, and leukocyte infiltration were observed in Zebra fish exposed to 70 nm nanoparticles (Lu et al., 2016), suggesting a clear mechanistic link between tissue distribution and response to nanoparticles.

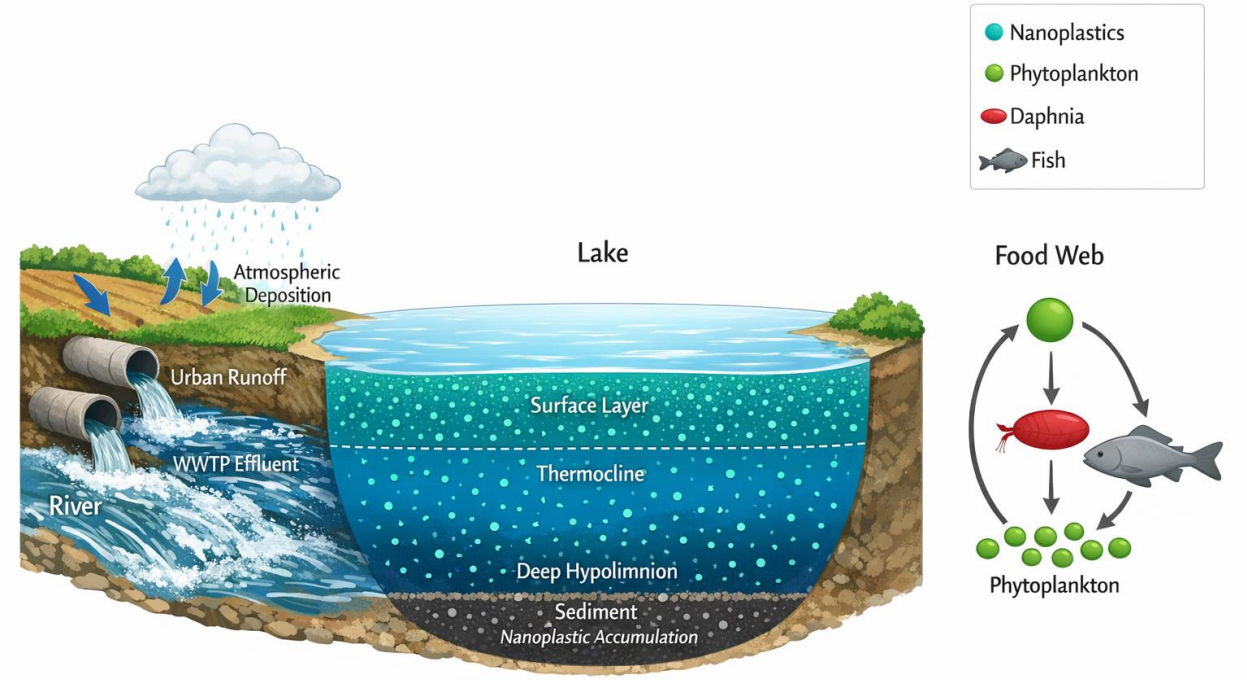

Figure 1: Spatial distribution of Nanoplastics in freshwater ecosystems.

4. Detection and Analytical Challenges

The detection, identification and quantification of Nanoplastics in various environments is a major technical challenge that hinders the progress in all aspects of freshwater plastic nanoparticle science. These challenges range from basic nanoparticle occurrence assessment to exposure modeling and hazard identification (Primpke et al., 2020; Cowger et al., 2020; Hartmann et al., 2019). Unlike microplastics, which can be visualized by conventional optical microscopy and characterized by Fourier transform infrared spectroscopy (μ-FTIR) at a resolution of 3–5 μm, Nanoplastics require mechanical performance beyond the fundamental diffraction limits of visible and infrared light. Complex and multistep sample preparation procedures induce potential impurities and missing particles and require stringent statistical controls to exclude various false positives, from biological and metallic nanoparticles to synthetic Nanoplastics with similar structures and spectra.

4.1 Sampling Protocol

4.1.1 Sample Collection

For liquid samples for plastic nanoparticle analysis in general, sample size, container, on-site contamination control, and particle size separation methods must be considered. Traditional sampling methods, such as direct or bulk sampling, commonly used in microplastic monitoring projects are not suitable for analysis of Nanoplastics because they lack particle size selectivity and require large volumes to collect sufficient amounts of Nanoplastics for spectroscopic analysis (Koelmans et al., 2019). Tangential flow filtration (TFF) and cross-flow ultrafiltration (CFU) systems have become preferred methods because nanoparticles can be produced from large liquid samples (50-1000 L) while maintaining their shape. Continuous filtration through membranes with progressively smaller pore sizes (1μm → 0.45μm → 0.1μm) can remove almost all fraction of Nanoplastics from aggregated microplastics and natural colloids (Gigault et al., 2017; Mintenig et al., 2017). However, the filtration efficiency of particles and fibers is significantly different from nominal pore

size specifications, and cases of small particles passing through a membrane with moderate removal rates are known (Koelmans et al., 2019). Polycarbonate, glass fiber, and aluminum membranes are excellent at reducing polymers such as nylon, polycarbonate, and PVDF. Airborne fibers should be controlled with glass Petri dish covers, clean rooms, or similar materials at each stage of filtration, but less than 50% of researchers in published studies used these materials (Cowger et al., 2020).

4.1.2 Sediment and Biota Extraction

To remove Nanoplastics from sediments, it is important to prevent polymer degradation while simultaneously sequentially breaking the interactions between the particles and the surrounding environment, performing density separation and organic matter removal. This process is technically challenging, since standard density separation methods are inefficient for Nanoplastics that exhibit colloidal behavior (Primpke et al., 2020; Lusher et al., 2017). Enzymatic degradation protocols using cellulase, protease K and lipase remove organic matter from sediment and biological tissue extracts respectively by precipitation of conventional synthetic polymers, and are currently the best methods for preparing biological samples and sediments (Lusher et al., 2017). Degradation with sodium hydroxide (10–30% NaOH) or potassium hydroxide (10–30% KOH) was easy and rapid. However, polyethylene terephthalate (PET) and polycarbonate are partially degraded under degradation conditions, resulting in a degree of alteration depending on the polymer type, which is rarely observed in the present research (Lusher et al., 2017). Although hydrogen peroxide (30% H_2O_2) degradation is widely used in marine and freshwater environments, careful optimization is required to avoid oxidative polymer surface degradation, especially for UV-degradable particles with high oxygen content (Primpke et al., 2020). Wet filtration and density separation methods are inefficient for submicron particles (Alimi et al., 2018).

4.2 Analytical Characterization Techniques

4.2.1 Infrared Spectroscopy and Its Limitations

Fourier transform infrared spectroscopy (FTIR) and related micro-FTIR are among the most widely used polymer identification techniques in microplastic research worldwide. This is because polymers can be identified by comparing the absorption spectra of a sample with a large spectral reference library, and are suitable for particle imaging using automated software (Primpke et al., 2020; Mintenig et al., 2017). However, the initial resolution of traditional FTIR spectroscopy is limited by a diffraction limit of 3–5 µm and 2–20 µm in the mid-infrared wavelength range. On the other hand, even the most advanced traditional FTIR systems cannot detect Nanoplastics with typical sizes (less than 1µm). Synchrotron-assisted Fourier transform infrared spectroscopy (SR-FTIR) uses the high brightness and spatial coherence of the synchrontron photon beam to reduce the spatial resolution to about 1–3 µm, thereby extending the detection limit to the upper limit of the size range of nano/micrometer. However, this technology is still not able to separate individual particles into different Nanoplastics and requires particle acceleration tools, which are only available to a few researchers worldwide (Mintenig et al., 2017). SR-FTIR with ATR (ATR-ATR) of all concentrations can be used to examine the amount of Nanoplastics recovered in membrane filters after highly selective filtration (Fischer & Scholz-Böttcher, 2017). In freshwater samples, organic compounds are present at concentrations of 1–20 mg carbon/liter and exhibit broad carbonyl and hydroxyl absorption bands in the infrared spectrum. These absorption bands clearly correspond to the diagnostic features of the absorption of many synthetic polymers. Especially in the case of oxidized and degraded Nanoplastics, carbonyl absorption features can be easily masked by signals from natural organic matter, resulting in false positives and low signal-to-noise ratios (Adeleye et al., 2018).

4.2.2 Raman Spectroscopy and Surface Enhancement

Micro-Raman spectroscopy uses passive photon scattering (Raman scattering) rather than photon

absorption and typically provides a resolution of 0.5–1 µm at laser excitation wavelengths of 532 nm or 785 nm. Because it exhibits much higher efficiency than traditional Fourier transform infrared spectroscopy (FTIR), it is a preferred technique for detecting individual particles in Nanoplastic and microparticle size (Primpke et al., 2020; Bhatt et al., 2021). In Raman microscopy, the range is limited by the focal length. Under optimal conditions, the use of high-number objective lenses enables a typical resolution of about 200 nm. This method has been successfully used to detect polystyrene nanoparticles in biological tissue components (Bhatt et al., 2021). The main disadvantage of traditional Raman spectroscopy in the analysis of Nanoplastics is that most polymers have low Raman scattering cross sections, resulting in low signal-to-noise ratios for particles smaller than 200 nm. These problems are compounded by the fluorescence effects of organic compounds (especially humic compounds) when excitable light is used. Surface-enhanced Raman spectroscopy (SERS) has revolutionized the detection of Nanoplastics by increasing the Raman signal intensity 10^6–10^8 by coupling local surface plasmon resonances of target molecules to gold or silver nanostructures. Zhu et al. (2020) demonstrated that a SERS substrate based on gold nanoparticle clusters can detect polystyrene Nanoplastics at a concentration of 10 ng/mL, which is three times lower than the detection limit of conventional Raman spectroscopy. These results were confirmed by comparison with a spectral library. SERS technology has shown effectiveness in analyzing Nanoplastics in freshwater. SERS substrates characterized by a wide range of polymers, operating in stable aqueous environments, and having sufficient operational stability for field applications are currently top research and development goals (Zhu et al., 2020; Hartmann et al., 2019).

4.2.3 Pyrolysis-Gas Chromatography/Mass Spectrometry

Py-GC/MS is a technique that can characterize polymers, regardless of their particle size, shape, color, or coating. The method was developed by thermal degradation of polymer samples at temperatures between 500 and 800°C and determination of specific polymer degradation products (Fischer & Scholz-Böttcher, 2017). Particle size independence is a major advantage for Nanoplastics research. Py-GC/MS can also be applied to nanocomposites trapped in membrane filters after size-selective filtration. By thermal removal of the total polymer content, quantitative mass data can be obtained simultaneously for the concentration of all polymers in a given size range. Fischer & Scholz-Böttcher (2017) quantitatively analyzed Nanoplastics in the range of 0.5–5 mg/kg dry weight in North Sea sediments using a TED-GC/MS method similar to thermal degradation. This provided the first reliable mass quantification data for environmental plastic samples containing nanoparticles. Major drawbacks of TED-GC/MS are its completely destructive nature (making replication or subsequent spectroscopic analysis of the same sample impossible), failure to provide individual particle count data or dispersion-specific information, and the need for large numbers of tangential water quality samples of stream (Primpke et al., 2020).

4.2.4 Electron Microscopy, Nanoparticle Tracking, and Field-Flow Fractionation

Scanning electron microscopy (SEM), coupled with energy dispersive X-ray spectroscopy (EDX), allows elemental composition data to be obtained at the level of individual particles with nonmetric resolution (<10nm). This allows the identification of key component characteristics of different polymers, such as nitrogen in polyamides or polyurethanes, chlorine in polyvinyl chloride (PVC), and pigment polymers of titanium and titanium dioxide. In addition, SEM reveals detailed surface morphology, seasonal variation, and particle size at a level other than optical microscopy (Primpke et al., 2020). Transmission electron microscopy (TEM) enables direct observation of Nanoplastics and cellular organelles in biological membranes such as endosomes, mitochondrial membranes, and chloroplast membranes, with subnm resolution. TEM provides the most compelling direct evidence for Nanoplastic cell penetration and toxic interactions at the organelle level

(Bouwmeester et al., 2015). Nanoparticle tracking analysis (NTA) is a technique that uses laser light to observe the Brownian motion of individual particles in a liquid suspension and measures their hydrodynamic diameter and concentration distribution. Although this rapid measurement method can measure particle sizes from 30 to 1000 nm at concentrations of 10^8–10^{12} particles/mL, it cannot distinguish between many organic and inorganic particles occurring in water Nanoplastics, because they are yielding less information (Gigault et al., 2017). Asymmetric flow field-flow fractionation (AF4), affecting multi-angle light scattering (MALS), dynamic light scattering (DLS), inductively coupled plasma mass spectrometry (ICP-MS) or Raman spectroscopy together, are currently the most powerful integrated analytical platform for characterization of environmental plastics. This method allows simultaneous phase separation and online composition determination with particle size sensitivity from 1nm– 1µm (Gigault et al., 2017). However, routine applications and comparisons between facilities are severely limited by process complexity, high equipment cost, low sample size (typically only 2-4 samples analyzed per day) and lack of standardized methods (Primpke et al., 2020).

4.3 Key Analytical Limitations and Standardization Imperatives

All of the research challenges go beyond the limitations of individual methods and reveal methodological shortcomings that need to be addressed through international standardization efforts to achieve the analytical precision needed for regulatory applications in freshwater plastic nanoparticle science (Cowger et al., 2020; Primpke et al., 2020). Size below 100 nm is a major challenge for all current research assemblies. In this approach, spectroscopic techniques exhibit lower signal intensities as the particle size decreases due to reduced scattering and absorption cross sections. Interference during sample preparation for electron microscopy analysis can change the size distribution of the original particles, and the separation efficiency of AF4 decreases as it gradually undergoes Brownian diffusion (Hartmann et al., 2019). The lack of reference materials for Nanoplastics in the environment is a major obstacle. Without standard reference materials with known polymer composition, particle size distribution and concentration under normal freshwater conditions, interlaboratory validation of results is not possible. The Joint Laboratory of the Council of Europe has initiated a project to develop standards, but there are still no standards for detecting microplastic particles in freshwater environments, and so far research has focused on the particle size of microplastics, rather than Nanoplastics.

In environmental Nanoplastics research, contamination of samples by airborne fibers and particles is a widespread and often underestimated problem in sample collection, processing, and analysis. Cowger et al., (2020) found in a systematic quality assurance review that only 24% of published studies fully reported all quality assurance measures, including methodological deficiencies, and fewer than 10% applied corrections to small counts. These design flaws mean that serious analytical errors, which are difficult to distinguish from actual environmental contaminants, occur in a significant number of studies reporting environmental Nanoplastic levels. It is therefore important to develop and implement standardized minimum quality requirements for research on environmental Nanoplastics (Cowger et al., 2020).

5. Environmental Fate and Transport

The environmental fate of Nanoplastics entering freshwater systems is determined by complex physical, chemical, and biological interactions. These processes determine the distribution of particulate matter in water, sediments, and ecosystems, the temporal and spatial distribution of pollutants, and the chemical composition of pollutants entering receiving ecosystems (Alimi et al., 2018; Koelmans et al., 2019). A thorough understanding of the technical details of these processes is essential for predictive models that can reliably predict hazards and environmental risk assessments. However, quantitative measurements of the mechanisms governing the fate of various

Nanoplastics under real-world environmental conditions are still incomplete (Besseling et al., 2019).

5.1 Colloidal Stability and Aggregation Dynamics

The stability of Nanoplastics in water depends on electrostatic repulsion and van der Waals force interactions, whether dispersed as single colloidal particles or aggregated into large aggregates. These interactions are theoretically described by the classical DLVO model (Dürgen-Landau-Förj-Overbeek) (Oriekhova & Stoll, 2018). The model of Orekhova and Stoll (2018) can also be used. Most of the synthetic polymeric Nanoplastics carry a negative charge in the environmental range of pH 6-8. This negative charge results from the addition of carboxyl, sulfonate or hydroxyl functional groups during polymer synthesis or gradually acquired by surface oxidation upon exposure to ambient atmospheres (Jiang et al., 2019). However, dissolved electrolytes are present in most biological freshwaters at concentrations of 0.5–5mM, mainly divalent cations (Ca^{2+}, Mg^{2+}), which gradually shield the surface charge and reduce the electrostatic repulsion barrier. It promotes aggregation by providing a mechanism for cross-linking between negatively charged surface groups of particles, especially divalent cations (Nasser & Lynch, 2016; Oriekhova & Stoll, 2018). Oriekhova & Stoll (2018) showed that the critical aggregation of 100 nm polystyrene Nanoplastics in calcium chloride solution is about 2 mM, which is much lower than the concentration of calcium in natural freshwater. The corresponding critical accumulation concentration in sodium chloride solution is about 200 mM, which is much higher than the ionic strength of ordinary fresh water. This means that most Nanoplastics are added to natural freshwater within hours or days, rather than remaining suspended in colloidal solution for weeks as predicted by stability models based on monovalent salts, and aggregation depends on the density and size of individual clusters.

In ecosystems where the concentration of natural colloids is higher than Nanoplastics, heterogeneous aggregates (aggregates made of various natural colloids, including Nanoplastics and dissolved organic aggregates, layered clay minerals (kaolinite, montmorillonite and hydroxides), hydromass such as geotite); and biopolymers of phytoplankton and emerging bacteria) tend to be more pervasive than homogeneous aggregates (aggregates composed solely of Nanoplastics) (Oriekhova & Stoll, 2018). The density of these heterogeneous aggregates depends on the density of mineral colloids (typically 2.0–5.0 g/cm³) compared to the density of Nanoplastics in the aggregate (typically 0.9–1.4 g/cm³). The settlement can be estimated using a modified Stokes law, where the fractal dimension of the aggregate (the fractal dimension of surrounding aggregates, typically df = 1.8–2.3) and the effective density of the aggregate are important factors. Because the formation of heterogeneous aggregates depends strongly on clay mineral type, surface charge, and formation conditions, there is large spatial and climatic variation in Nanoplastics settlement rates, and this variation is not explained by current sedimentation models (Alimi et al., 2018).

5.2 Eco-Corona Formation and Natural Organic Matter Interactions

Natural organic matter (NOM) is a complex chemical mixture of humic acids, folate, polysaccharides, proteins, lipids, and organic matter, with dissolved carbon carbon typically in the range of 1 to 20 mg/L. When these materials enter biological water, they rapidly adhere to the surface of Nanoplastics and form organic layers or bioprotein/organic layers in minutes to hours. This concept is similar to the formation of bioprotein layers on the surface of synthetic nanoparticles in body fluids (Nasser & Lynch, 2016; Monopoli et al., 2012; Cedervall et al., 2007). The formation of these organic compounds has distinct, sometimes overlapping, effects on the environmental effects of Nanoplastics. From the biohazard perspective, the adsorption of organic compounds (NOCs) is positive, because NOCs tend to increase the water solubility of plastic nanoparticle surfaces (water contact angle in NOC-rich water decreases from about 90° to about 20-40°). In addition, addition of long-chain

polymer to achieve stoichiometric ratio improves templating and reduces adhesion efficiency between Nanoplastics, mineral surfaces, and organic aggregates, thus reducing sedimentation rate, increasing water retention time, and greatly reducing bioavailability at the entrances of filtering. On the other hand, the environment significantly modifies the analytical properties of Nanoplastics by altering Fourier transform infrared (FTIR) and Raman spectra, masking surface functional groups, and increasing the hydrodynamic diameter, enhancing adsorption in environmental matrices. This makes it difficult to identify and characterize Nanoplastics (Adeleye et al., 2018).

Environmental envelopes are not dynamic, but evolve, with constantly changing properties. As Nanoplastics move in the environment, they are affected by factors such as organic composition, pH value, temperature, ionic strength and biological activity (Monopoli et al., 2012). This dynamic change in coatings indicates that the surface properties and bioactivity of Nanoplastics are constantly changing along their transport pathways in the environment. These variables cannot be evaluated by considering only the primary effects of particles (Nasser & Lynch, 2016). The adsorption of proteins on the surface of Nanoplastics, which results from the dissolution of biological fluids and organic compounds, can modify cells according to particle shape. Significant differences in environmental response are often observed between newly synthesized Nanoplastics and Nanoplastics isolated from the environment or previously synthesized from natural biological sources (Cedervall et al., 2007; Monopoli et al., 2012). This highlights the importance of biodynamics in toxicology management and prevents direct generalization of laboratory results to determine field environmental risks associated with changes (Nasser & Lynch, 2016).

5.3 Trophic Transfer and Bioaccumulation

In the freshwater food chain, the transfer of Nanoplastics from primary producers to herbivores and higher predators is a key mechanism leading to increased food contamination. This mechanism is of particular concern due to the accumulation of Nanoplastics and related contaminants in fish tissues consumed by humans (Cedervall et al., 2012; Rochman et al., 2015). Cedervall et al. (2012) conducted a major study that demonstrated the efficiency of Nanoplastics transferred at three trophic levels (from algae to Daphnia magna, carp (*Carassius carassius*)) using 57nm polystyrene particles labeled with carbon nanotubes as tracers. Energy transfer from algae to Daphnia magna is estimated to be between 30% and 80%, depending on particle size, surface chemistry of Nanoplastics, and feeding conditions (Cedervall et al., 2012). Mattsson et al. (2017) extended this analysis. Initial experimental studies showed that 53 nm polystyrene nanoparticles accumulate in the brain and liver of cadaver flies, leading to neurobehavioral changes. This is the first case of nanoparticle neurotoxicity in the food chain, and behavioral disturbance in a predator carries wide ecological consequences, as it can affect ecosystem function through prey frequency and food community variables (Mattsson et al., 2017).

Whether Nanoplastics actually bioaccumulate, that is, as nitrous oxide levels gradually increase at subsequent trophic levels, as an effect of lipophilic organic pollutants (PCBs and DDT), is a matter of scientific debate and a major environmental threat (Koelmans et al., 2016). As bioaccumulative organic matter regulated by lipid and water diffusion and metabolic resistance, Nanoplastics are removed not only by physical processes such as secretion, gill washing, ciliary cell washing, and cell extrusion, but by accumulation dynamics and classical diffusion models of biological growth (Farrell & Nelson, 2013; Koelmans et al., 2016). According to a 2016 gel modeling study, the expected outcome of polymer fragments in a typical freshwater food web scenario is biodegradation, rather than biological growth, where the number of polymers at all trophic levels is less than below unity it is in biological tissues (Bakir et al., 2012; Koelmans et al., 2016). In environmental risk assessment, it is important to distinguish between macromolecular development (absence) and development (potential) of relevant contaminant transport. This highlights the need

for a comprehensive research protocol that considers plastic nanogels as a single effector, rather than considering each chemical agent separately.

6. Ecotoxicological Impacts on Aquatic Organisms

Ecotoxicity studies of Nanoplastics on freshwater microbes contain a diverse and growing body of evidence spanning a variety of tissues, from molecular and cellular damage to physiological disturbances in ecosystems and population effects on populations. Toxicity pathways include direct and indirect effects, including co-infection and contamination (Bhatt et al., 2021; Besseling et al., 2019; Rochman et al., 2017). The mechanisms responsible for the toxicity of Nanoplastics involve at least five mechanisms, often operating simultaneously. The particles interact with cell membranes and accumulate intracellularly, causing direct physical damage to biofilms and cellular structures. Oxidative stress from particle-induced reactive oxygen species (ROS) generation in mitochondria and via Fenton-type surface chemistry. The plasticizers and chemicals (phthalates, bisphenol a, alkylphenols, brominated flame retardants) in the polymer matrix penetrate biological membranes, causing endocrine disruption. The microbial toxicity occurs when pollutants accumulate on the surface of Nanoplastics and reach high levels that can be achieved in biological tissues. Indirect consequences include food chain disruption, habitat modification, and conversion of pollutants to the plastisphere biofilm (Lu et al., 2016; Chen et al., 2017; Chen et al., 2018).

6.1 Ecotoxicological Effects on Microalgae and Phytoplankton

Algae mainly form the energetic and structural basis of freshwater food webs, and because of their important ecological roles and sensitivity to photosynthetic signals, there is active research on their response to Nanoplastics (Long et al., 2017; Mao et al., 2018). Growth inhibition, measured by photosynthetic biomass indicators such as cell division rate or chlorophyll content reduction, is the most commonly defined parameter in studies of the ecotoxicology of Nanoplastics in algae. Canniff & Hoang (2018) reported 96-hour EC50 values of 12.5 mg/L for *Raphidocelis subcapitata* exposed to 200-nm polystyrene, with reductions in maximum photosystem II quantum yield (Fv/Fm, measured by pulse amplitude modulated fluorimetry) of 15– 40% at exposure concentrations of 10–100 mg/L, confirming photosynthetic pathway perturbation at concentrations substantially below acute lethality thresholds. The EC50 value after 72 hours was about 30 mg/L. Long et al. (2017) reported that the EC50 value of *Rhaphidocelis subcapitata* after 96 hours of incubation with 200 nm polystyrene particles was 12.5 mg/L. At concentrations of 10-100mg/L, the peak value of photosynthetic system II (Fv/ Fm measured by pulse-width modulated fluorescence) was reduced by 15- 40%, confirming the inhibition of the photosynthetic pathway even at concentrations much lower than the concentration magnitude. Long et al. (2017) described several physiological mechanisms that distinguish algal toxicity from chemical or oxidative stress. Specifically, the aggregation of heterogeneous Nanoplastics in algal cells and their adhesion to the cell surface results in physiological photo-blocking effects. This is called a "contrast accumulation light-blocking" device. Although concentrations of Nanoplastic can reach 1mg/liter, the biological significance of this mechanism at concentrations of Nanoplastics commonly found in freshwater (< 1 µg/L) is unclear.

At the molecular level, increased levels of intracellular reactive oxygen species (ROS) are the most common biochemical changes in algae exposed to Nanoplastics, and are associated with growth inhibition and reduced photosynthesis. *Chlamydomonas reinhardtii* showed a significant increase in ROS levels at concentrations of 0.1–1 mg/L Nanoplastics, as measured by the membrane-permeable fluorescein probe 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA). These levels may be within normal limits in highly polluted urban freshwaters (Bhatt et al., 2021). At exposure to 5 to 50 mg/L, lipid peroxidation (measured by malondialdehyde (MDA)), protein carbonylation, and single-

stranded DNA damage were observed in many algae, consistent with the pattern of gradual decline of cellular antioxidant defense systems damage chloroplast microstructure (TEM) during polystyrene nanoparticle concentration increased from 10 to 50 mg/L. This impairment is demonstrated by a reduction in carbon fixation function, including disruption of the thylakoid membrane, irregular arrangement of granules, and aggregation of starch granules (Bhatt et al., 2021). Taken together, these results suggest that exposure to ecologically significant low concentrations of Nanoplastics may disrupt photosynthesis in polluted freshwater ecosystems over time, but not lethally, and may slightly reduce key crops without high mortality rates undetected by traditional methods so the main crop.

6.2 Ecotoxicological Effects on Zooplankton

6.2.1 Acute and Chronic Toxicity in *Daphnia magna*

Daphnia magna has emerged as an important model organism for studying the biology of Nanoplastics in freshwater, due to its filter biology (facilitating direct uptake of suspended particles), well-characterized genomic and physiological functions, and established roles in OECD standardised ecotoxicological test guidelines (TG 211 for long-term reproduction tests), and its important role as a major consumer of zooplankton, linking phytoplankton production to higher trophic levels (Besseling et al., 2014; Rosenkranz et al., 2009). Besseling et al. (2014) compared the risks of polystyrene nanoparticles (60 nm) with carbon dioxide in *D. magna*. Specifically, this study shows that in a 21-day long-term reproductive efficacy test, the non-detected effective concentration (NOEC) was 0.05 mg/L and the minimum effective concentration (LOEC) was 0.5mg/L, which is several orders of magnitude below the threshold value of impaired reproductive efficacy. In environmental risk assessment, there is a significant difference of about 1000 times between the threshold values for acute and chronic disease. If risk assessment is based solely on acute toxicity data, the risk may seem relatively low relative to ambient concentrations of nanoparticles, but long-term amplifying effects can occur even at certain levels measured in the environment.

Nasser & Lynch (2016) further complicated environmental risk assessments using environmental risk data from untreated laboratory particles. They found that when the surface of Nanoplastics was ecologically attracted by organic compounds from the surrounding water, feeding inhibition and reproductive complications in *D. magna* were greatly reduced compared to untreated particles at the same concentration. In their experiments, polystyrene Nanoplastics pretreated with 10 mg/L dissolved NOM showed about 50% reduction in toxicity at different concentrations compared to untreated particles at the same concentration. This is because the organic compounds reduced the hydrophobicity and surface stability of the particles. These results also suggest that while current environmental data on particulate matter systematically overestimate the aging and toxicity of NOM-coated Nanoplastics, environmental data from freshwater environments with low-NOM concentrations (freshwater, brackish, brackish water) may it is more promising. Rosenkranz et al. (2009) were the first researchers to successfully compare the uptake of nanoparticles (polystyrene with sizes of 20, 100 and 1000 nm) and microparticles (1–10 µm) by *D. magna*. Confocal microscopy was used to show that 20 nm particles move through blood cells and the stomach via non-phagocytic pathways, whereas particles in the 1–10 µm range are captured in the intestinal lumen. This indicates that particle size is an important factor for efficient penetration of biological barriers and supports the specific concerns regarding environmental toxicity that arise regarding Nanoplastics of different sizes.

Besseling et al. (2014), demonstrated the potential for the transmission of Nanoplastics to infants through breast milk. Researchers found these particles in the intestinal mucosa of infants and reported that 15–25% of the total amount of nanoparticles in the maternal body is transferred during each stage of lactation. This means that reproductive function can be affected if the mother is continuously exposed to these trace elements throughout her reproductive year. This finding was also confirmed in a multigenerational

cross-sectional study by Bhat et al. (2021) and the author. This study showed that second-generation *Daphnia* (F2) exposed to polystyrene Nanoplastics (100 nm) for multiple generations, starting immediately after birth, showed a more gradual decline in fertility than first-generation *Daphnia* (F1). Gene expression of oxidative stress response genes (very high levels of catalase, glutathione peroxidase, and superoxide dismutase mRNA) was altered, and these changes were maintained across generations. This suggests that epigenetic changes may be less frequent in long-term polluted freshwaters (Bhatt et al., 2021).

6.3 Ecotoxicological Effects on Fish

6.3.1 Tissue Distribution and Histopathological Responses

Fish dominate the freshwater food chain and come in direct contact with humans through their food. Therefore, it is important for environmental risk assessment and food safety to understand the impact of Nanoplastics on this population in the environment (Rochman et al., 2015; Lu et al., 2016). Lu et al. (2016) conducted a randomized comparative study on the distribution of Nanoplastics in Zebrafish (*Danio rerio*) tissue. After providing equal amounts of water and food for 7 days, significant differences were observed in the accumulation of 70 µm and 5 µm polystyrene particles in Zebrafish. The 5 µm particles accumulated mainly in the digestive tract and were mainly cleared within 24 hours of release. Nanoparticles of 70 nm are widely distributed in liver, gill and intestinal epithelium, but persist after injection and cause severe pathological reactions on organ surfaces, such as lipid accumulation (lipolysis), glycogen, helicogen, sputum, inflammasome, inflammation and inflammatory infiltrates (Lu et al., 2016). Because adult Zebrafish can breathe about 10 liters per hour, the most important site of exposure to Nanoplastics in the aquatic environment is the gill tissue. Increased permeability, epithelial cell proliferation and associated nasal fiber attachment have been observed in the cervical mucosa of several exposed specie. A 2017 study showed that Japanese medaka (*Oryzia latipes*) showed an increased incidence of liver disease after 100 days of exposure. Glycogen depletion, lipid vacuole formation, cell proliferation, and inflammatory infiltration were performed on hematoxylin and eosin-stained tissue sections using a standard histopathological scoring system, providing rare evidence that the inflammatory response is similar to that measured in contaminated freshwater.

6.3.2 Oxidative Stress and Molecular Biomarker Responses

Oxidative stress is an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense capacity of cells and is a prominent molecular mechanism of plastic nanoparticle toxicity in cichlids and fish, including tiger (*Danio rerio*), carp (*Cyprinus opis*), European a bass (*Cyprinus*) (*Discent labrax*) (Bhatt et al., 2021; Lu et al., 2016; Chen et al., 2017). Bhatt et al. (2021) carried out detailed mechanical studies on carp (*Cyprinus carpio*) exposed to 100 nm polystyrene Nanoplastics at a concentration of 10 mg/L for 21 days. In this study, we confirmed that malondialdehyde (MDA), a stable endpoint of lipid peroxidation and reliable biomarker of oxidative stress, was associated with a 45% decrease in hepatic glutathione (GSH) levels. This suggests that the decrease in glutathione is due to an increase in reactive oxygen species (ROS). A compensatory effect of antioxidant enzymes was also observed, with a 68% increase in superoxide dismutase (SOD) activity and a 52% increase in catalase (CAT) activity compared to the control group. This specific pattern of increased lipid peroxidation, decreased glutathione (GSH), and activation of antioxidant enzymes reflects the typical biochemical patterns of oxidative stress found in fish muscles (Bhatt et al., 2021). Chen et al. (2017) extended these results by applying transcriptomics to Zebrafish exposed to 100 nm pure polystyrene and found that cytoprotective heat shock proteins (hsp70, hsp90), inflammatory cytokines (il-1β, tnf-α) and xenobiotic-metabolising cytochrome P450 enzymes (cyp1a1, cyp1b1) was increased in liver tissue, while regulators of lipid metabolism (pparα, fabp3) were decreased, suggesting a transcriptomic profile with a combination of oxidative, inflammatory and metabolic changes in liver tissue.

6.3.3 Neurotoxicological and Behavioural Impairment

Mattsson et al. (2017) demonstrated through a three-step transfer assay that contamination of *Daphnia daphnia* with 53 nm polystyrene nanoparticles in the food chain induces behavioral problems in carp. These results are one of the most important and frequently published studies in the field of biological toxicity of Nanoplastics. Fish exposed to food chains contaminated with Nanoplastics showed significant decreases in physiology (−42% decrease in feeding activity in field trials), decreased feeding activity (−38% decrease in successful predation) and changed social behavior (increased individual distance and decreased group cohesion). These behavioral deficits negatively impact the ability of fish to adapt and survive in natural freshwater ecosystems. Neurochemical analysis of brain tissue revealed a −31% decrease in acetylcholinesterase (AChE) activity, a biomarker representing cholinergic neurotoxicity. This suggests that disruption of the enzyme results in an accumulation of the neurotransmitter acetylcholine at synapses. In addition, this study revealed changes in dopamine and dopamine receptor expression, as well as dopamine signaling pathways gene expression, suggesting that disruption of cholinergic and dopamine signaling pathways may be the mechanistic basis for the observed behavioral changes (Mattsson et al., 2017). Chen et al. (2017) suggested that exposure to Nanoplastics during aging may lead to impaired neurodevelopment that persists into adulthood. This problem is similar to problems with neurotoxicity caused by methyl mercury and organophosphorus pesticides in fish and other vertebrates.

6.3.4 Endocrine Disruption and Reproductive Toxicity

Several synthetic metabolites, including bisphenol A (BPA), di-(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), nonylphenol, and polybrominated diphenyl ethers (PBDE), as additives (plasticizers, UV stabilizers, antioxidants). These materials can migrate from Nanoplastics into aqueous environments and biological tissues, and thermal and microchemical environments can accelerate the diffusion of these inclusions (Chen et al., 2018; Rochman et al., 2013). Since the surface area to volume ratio of Nanoplastics is higher than that of microplastics, the chemical release per unit mass of Nanoplastics is much higher than that of microplastics. Low intestinal pH increases the activity of emulsified lipids and bile acids, thereby increasing the solubility and absorption rate of detergent additives (Bakir et al., 2012). Chen et al. (2018) reported that vitellogenin (VTG) gene expression was induced in male Zebrafish exposed to BPA-releasing polycarbonate nanoparticles. Vitellogenin is an estrogen indicator in male fish, and the pathway for vitellogenin synthesis is known to be more active in males than in females. At a concentration of 100 μg/L, vitellogenin gene levels increased fourfold compared to the untreated control group. Zebrafish exposed to polyvinyl chloride nanoparticles during a 60-day reproductive cycle showed concentration-dependent changes in genital index (ratio of genital weight to body weight) at 100 μg/L diethylhexyl phthalate (DEHP) (Chen et al., 2018). These concentrations correspond to threshold concentrations of DEHP and BPA measured in highly contaminated freshwater systems supplying industrial and municipal wastewater. This suggests that endocrine disruption due to Nanoplastics in polluted systems is a potential environmental hazard and not just a laboratory phenomenon.

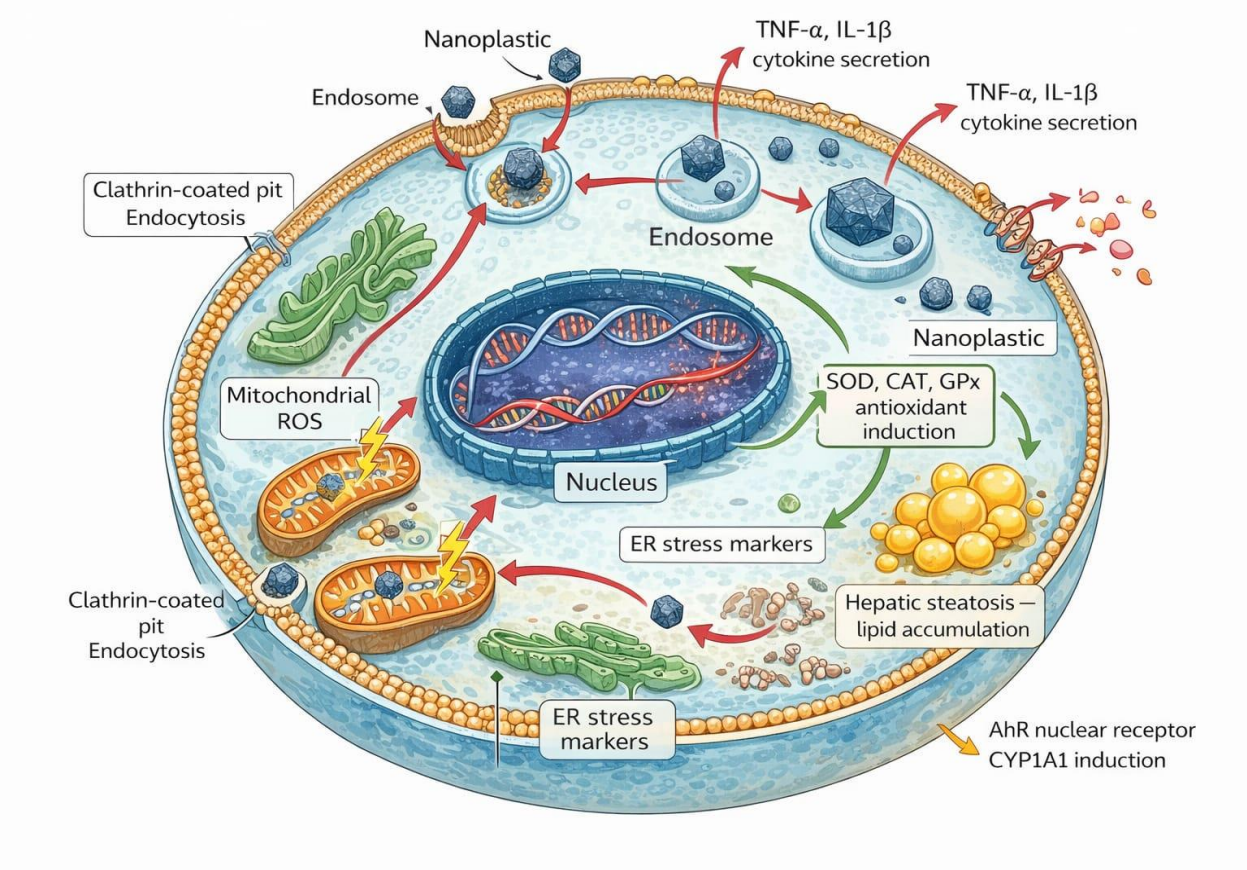

Figure 2: Molecular mechanisms of toxicity induced by Nanoplastics penetrating freshwater fish liver cells

7. Interaction of Nanoplastics with Co-Contaminants: The Trojan Horse Effect

Nanoplastics act as potential environmental contaminants of the aquatic environment on surfaces or polymeric systems and then deliver them to biological tissues for uptake under chemical conditions. It is one of the most important and environmentally threatened components of this species (Bakir et al., 2012; Koelmans et al., 2016). This phenomenon, sometimes called the "Trojan horse effect", "phytotoxicity" or "free pollution transport", may expand the environmental risks of plastic nanoparticle pollution beyond the effects of simple physical particles, as Nanoplastics evolve from inert physical to active systems of transportation of the pollutant (Bakir et al., 2012; Hartmann et al., 2017). The thermodynamic basis for this concern is firmly established by principles of physical chemistry: the extreme specific surface area of Nanoplastics (10^3–10^6 m^2/kg for 10–1000 nm particles), combined with the hydrophobic character of non-polar polymer surfaces, the π-electron systems available for π-π stacking interactions on aromatic polymers such as polystyrene, and the surface functional groups (carboxylate, hydroxyl, amine) available for electrostatic and coordination interactions with ionic contaminants, collectively generate high-capacity and rapid-equilibration sorption systems for a structurally diverse range of priority environmental contaminants (Hüffer & Hofmann, 2016).

7.1 Sorption of Hydrophobic Organic Contaminants

Aqueous pollutants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl

ethers (PCBs), organochlorine pesticides (OCP: chlorpyrifos, isodrin, aldrin, DDT), and polybrominated diphenyl ethers (PBDEs), are well localized on the surface of hydrophobic polymers due to hydrophobicity repulsion with water, positive van der Waals potentials, and π-electron interactions with the polymer matrix (Bakir et al., 2012; Wang et al., 2018). The adsorption coefficient (Kd, L/kg) of hydrophobic materials on Nanoplastics ranges from about 104 to 107 L/kg, depending on the degree of hydrophobicity (log Q) of the solution, polymer type, and size. Poorly soluble compounds, such as pyrene or PCB-52, have gelatinization rates similar to or higher than those of organic carbon in water (Bakir et al., 2012; Koelmans et al., 2016). Size-dependent adsorption mechanisms of Nanoplastics. The specific surface area of a 100 nm particle is larger than that of a 1 mm plastic microparticle made of the same polymer, and the adsorption equilibrium time for a single organic hydrocarbon compound ranges from days to minutes for microplastics or hours for Nanoplastics. This indicates that Nanoplastics in the environment rapidly reach adsorption equilibrium with different concentrations of organic hydrocarbons dissolved in the ambient water (Hüffer & Hofmann, 2016). Acidic pH values in this region (5-6 in posterior fish intestine), high temperatures and the presence of lipids and bile acids promote HOC release from Nanoplastic surfaces, allowing precise delivery of high HOC concentrations in more sensitive biological environments (Bakir et al., 2012).

7.2 Heavy Metal Sorption and Delivery

Trace metals such as cadmium (Cd^{2+}), lead (Pb^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), and arsenic (As^{3+}/As^{5}) bind to the surface of Nanoplastics in different ways. These binding mechanisms include negatively charged surface functional groups (carboxyl, hydroxyl and sulfonate groups), interactions with metal-binding functional groups generated by surface oxidation during UV damage, and physical attachment to microporous surface structures induced by weathering (Gao et al., 2019). Gao et al. (2019) systematically characterized cadmium sorption onto polystyrene Nanoplastics, demonstrating capacities of 0.8–12.5 mg Cd per gram of Nanoplastic depending on surface functionalisation and solution pH, with carboxylated surfaces about five times higher due to the higher concentration of metal-coordinating functional groups. The main result of the risk assessment was that environmentally degraded Nanoplastics, with oxygen functional groups removed by UV radiation, had a higher metal binding capacity than the original nanoparticles. This scenario shows that mature Nanoplastics found in natural environment have higher metal binding capacity than unprocessed nanoparticles used in laboratories and most hazardous substances from environment. Metal transport efficiency is higher than toxicology efficiency (Jiang et al., 2019). This discrepancy suggests that studies using nanoparticles themselves systematically underestimate the risk of metal toxicity from these particles. PH- dependent adsorption of metals on the surface of plastics. Nanoparticles (adsorption rate is faster at acidic pH value (5-6) of fish stomach than at neutral pH value (7-8) in ambient freshwater) is a pH-stimulated release mechanism that can deliver metal levels to bacteria (Bakir et al., 2012; Gao et al., 2019).

7.3 Antibiotics, Pharmaceuticals, and Antimicrobial Resistance

Binding of antibiotics and micropollutants on the surface of Nanoplastics in freshwater systems is a process that combines two major environmental hazards: plastic pollutants and antioxidants (Guo & Wang, 2020; Zettler et al., 2013). Tetracyclines, fluoroquinolones (ciprofloxacin, enrofloxacin), and sulfonamides are the most commonly used antibiotics in human, veterinary, and aquaculture, exhibiting moderate to high affinity for surface plastics. Adsorption mechanisms include cation exchange interactions of ionized amino groups, π-π stacking interactions between aromatic rings of antibiotics and polymer chains, and hydrophobic dispersion of nonpolar antibiotic structures (Guo & Wang, 2020). The adsorption constants (log Kd) of tetracyclines and ciprofloxacin for polypropylene and polyethylene terephthalate nanoparticles ranged from 3.5 to 4.6. This indicates that even at low nanoparticle

concentrations (μg/L), the concentration of bound antibiotics can be much higher than soluble antibiotics (Guo & Wang, 2020). Plastic rings, a distinct group of microorganisms that inhabit plastic surfaces in aquatic environments, were first identified in marine systems by Zetler et al. (2013) and subsequently reported in freshwater environments. Several studies have shown that these rings exhibit higher levels of anti-inflammatory gene (ARG) expression than environmental microorganisms in water or water. Estrogenic compounds, including 17α-ethinylestradiol (EE2), the active ingredient in contraceptives and one of the most potent endocrine disruptors in freshwater ecosystems, exhibit log-Kd absorbance values in the range of 3.5 to 4.8, with typical doses ranging from 1.80 to 1.8.8. This condition increases the amount of EE2 from Nanoplastics, which can be released into the reproductive tissues of fish even at concentrations lower than those appearing in soluble compounds, causing endocrine disruption (Chen et al., 2018).

8. Environmental Risk Assessment

Environmental risk assessment of Nanoplastics in freshwater ecosystems typically uses a traditional four-step regulatory approach (hazard identification, risk assessment, impact assessment, and risk interpretation) to evaluate these complex pollutants. Nanoplastics cannot be reduced to a single entity with predictable physical, chemical, and toxicokinetic properties (Besseling et al., 2019; Koelmans et al., 2022). Because polymorphism of Nanoplastics shows a continuous spectrum including different polymer types, particle sizes, shapes, surface chemistry, additives, and environmental conditions, single toxicity profiles, exposure models, or exposure thresholds cannot describe the risks posed by plastics at all will system (Hartmann et al., 2019). A 2019 World Health Organization (WHO) review of microplastics in drinking water found that Nanoplastics are an important regulatory target, requiring improved systems for environmental risk assessment, and current scientific knowledge is insufficient to establish regulatory levels and health data.

8.1 Exposure Concentration Modelling and Measured Environmental Data

Environmental risk modeling for Nanoplastics requires a large number of inputs, such as emissions, environmental transport, runoff and water concentration, to obtain water quality standards. However, for almost all parameters, experimental data are insufficient to adequately constrain the input data (Koelmans et al., 2017). The SimpleBox4Plastics bulk model developed by Koelmans et al. (2017), estimates the concentrations of Nanoplastics in European freshwater using a simple accessible steady-state modeling framework and predicts concentrations in the range of 10^{-6}–10^{-3} mg/L for polyethylene nanoparticles under current emission conditions. In heavily polluted river systems (especially in industrialized Asia), the measured concentration of Nanoplastics reaches 10—1mg/L (Wang et al., 2018), which is 3–4 times higher than the lower limit predicted by the model for European conditions. This increase mirrors an increase in plastic production in developing countries and a decline in wastewater treatment (Koelmans et al., 2019).

8.2 Species Sensitivity Distributions and PNEC Derivation

Species sensitivity distributions (SSDs) model links cumulative loss of threatened species to environmental concentrations and provides the basis for projected number-neutral-impact (PNEC) assessments for aquatic ecosystem protection and environmental regulatory risk assessments (ERAs) (Besseling et al., 2019). Besseling et al. (2019) developed a solid suspension (SSD) of polystyrene Nanoplastics in freshwater ecosystems, including algae, cladocerans, water worms, annelids and fish, using 47 ecotoxicity data points. Thus, the effective concentration (HC5) for 5% degraded species ranged from 0.02 to 0.09 mg/L, depending on the selected assay protocol and exposure time during hardening. Applying a 10-point analytical factor to HC5, applied to products with sufficient biotoxicity data according to the European Chemicals Agency (ECHA) PNEC-based SSD derivation method, yields PNEC values between 0.002 and 0.009 mg/L. This is 2-3 times higher

than the lower limit for Nanoplastics in Europe (Koelmans et al., 2017; Besseling et al., 2019). However, the upper limit of measured environmental concentrations in urban rivers (especially in Asia) reaches or exceeds the PNEC value, and in the worst case, the risk ratio (RQ = measured environmental concentration / PNEC) has been shown to exceed unity for worst-case exposure scenarios (Wang et al., 2018; Besseling et al., 2019). Considering the Trojan horse effect, meaning if the actual concentration of a poison exceeds the concentration of compounds in solution, the risk level increases 10 to 1000 times, and antibiotics also contribute to this situation (Bakir et al., 2012; Koelmans et al., 2016).

8.3 Limitations of Current ERA Approaches for Nanoplastics

Several design limitations of current environmental risk assessment frameworks are important factors in identifying risks associated with Nanoplastics, and guidelines for risk assessment of these pollutants should explicitly acknowledge these limitations (Hartmann et al., 2019; Koelmans et al., 2022). Perhaps the central question is the relationship between exposure and outcome. Currently, almost all environmental toxicity data from laboratories are based on monodisperse fluorescently labeled polystyrene nanoparticles or simple polystyrene fragments in their unsaturated state. The properties of these materials are very different from heterogeneous mixtures of polymer compounds, surface damage, and organic coated Nanoplastics (Nasser & Lynch, 2016; Hartmann et al., 2019). Risk assessment based on biotoxicity data for polystyrene can reduce the risk of degradation of key polymer chemicals at various levels, including polyethylene, polypropylene, polyethylene terephthalate, polyvinyl chloride and polyamides. Complex murders present additional systemic challenges. Nanoplastics are often associated with dissolved chemical pollutants in ecosystems, and their role as mediators of the bioavailability of these pollutants is not evaluated in conventional environmental risk assessment programs. Based on the general concept of combined toxicity of dissolved compounds, the goal of this program was to evaluate the toxicity of each compound individually (Koelmans et al., 2016). Long-term chronic side effects are the third major difference. Although most available ecotoxicological data measure acute (24-96 hours) or short-term subchronic (21-28 days) effects, freshwater bacteria are present in wastewater systems throughout their lives, and only a few models currently require multigenerational data, which is important for population estimates (Besseling et al., 2019; Bhatt et al., 2021).

8.4 Regulatory Frameworks and Governance Developments

The current regulatory framework for Nanoplastics is heterogeneous and varies considerably between jurisdictions. To date, no jurisdiction has defined environmental quality standards for priority products under the EU Water Policy Directive (Rochman et al., 2019; ECHA, 2022). In 2022, the European Chemicals Agency (ECHA) proposed a ban under REACH for microplastics, which are solid polymer fragments between 1 µm and 5 µm in diameter, from products placed on the EU market (ECHA, 2022). While this prohibition explicitly mentions the intentional addition of Nanoplastics, secondary Nanoplastics resulting from the environmental degradation of plastic products are completely excluded from the regulation. Although the EU Environmental Protection Agency (EPA) has not yet issued specific regulatory guidance on Nanoplastics, it addresses them in the Safe Drinking Water and Clean Water Act as part of its pollution control efforts, although without specific regulatory obligations (Rochman et al., 2019). In 2022, the United Nations Environment Program (UNEP) began negotiations on a legally binding international agreement on plastics under UNEP Resolution 5/14. This represents a very important regulatory development in the global management of Nanoplastics, and objectives related to the protection of freshwater ecosystems and the monitoring of these particles are currently under consideration (UNEP, 2023). The introduction of environmental quality standards (EQS) for Nanoplastics in freshwater, based on saturation-based predicted no-impact confidence

intervals (PNECs) and consideration of "Trojan horse" effects and long-term risks, represents a major regulatory gap in recent global applications (Besseling et al., 2019; Koelmans et al., 2022).

9. Future Directions

Research on Nanoplastics has grown significantly since 2015 but fewer than 50 peer-reviewed articles published before 2015, increasing to more than 500 by 2022 (Hartmann et al., 2019) However, significant knowledge gaps still exist in environmental risk identification, mitigation strategies and regulatory decisions, which are significantly limiting in many ways (Koelmans et al., 2022; Rochman et al., 2019; Besseling et al., 2019).

9.1 Analytical Method Harmonisation and Reference Material Development

The lack of consistent and reliable analytical methods is a major obstacle in advancing scientific investigations on Nanoplastics in freshwater, making it impossible to reliably compare quantitative environmental data between experiments and laboratories (Primpke et al., 2020; Cowger et al., 2020). Primpke et al. (2020) reported that when Nanoplastics were measured by different analytical platforms in both hard water samples, the relative standard deviation between laboratories exceeded 100%. This assessment variability undermines the reliability of data collection between assessments and greatly weakens the judgment needed to derive the codes below. Immediate priorities are plastic nanoparticle standards for particle sizes from 10 to 1000 nm for common polymers (PS, PE, PP, PET, PVC) by working groups at National Institute of Standards and Technology (NIST) and Joint Research Center (JRC), and the need for ISO 4 and ISO 1 in other approaches to development and implementation. Locations, particle recovery experiments, detection limit specifications and quality control results should be described and reflected in peer-reviewed articles (Cowger et al., 2020). In addition, investments should be made in next-generation sensor platforms, such as Raman spectroscopy based on machine learning, SERS based on plastic Nanoplastic-selective sensors, single-particle mass spectrometry and nano-flow cytometry with polymer identification capability (Zhu et al., 2020; Hartmann et al., 2019).

9.2 Environmentally Realistic Test Materials and Exposure Scenarios

In this study, we investigated key differences between pure, monodisperse, and well-defined polystyrene nanoparticles, used in virtually all published ecotoxicity studies, and composite mixtures of filtered plastic polymer nanoparticles found in natural freshwater systems, and characterized by fractured, surface structures, multi-2 (Hartmann et al., 2019; Koelmans et al., 2022; Nasser & Lynch, 2016). For future ecotoxicity studies, it is important to use customized and technology mature Nanoplastics, with customized ripening mechanisms and UV-drying systems, including surface oxidation states, polydispersity, and actual stratification ability. In addition, exposure tests should be performed on polymer mixtures sensitive to realistic environmental conditions containing real contaminants, including organochlorines, metals, and associated chemicals, measured at room temperature. Long-term multigenerational exposure models have been used to capture long-term pollution population effects (including epigenetic effects in a single generation) at different trophic levels in freshwater models (Bhatt et al., 2021; Besseling et al., 2019).

9.3 Adverse Outcome Pathway Framework Development

The system of adverse effects associated with Nanoplastics, as per the OECD toxicity code product map, has not been defined or validated. This framework is a formal mechanistic picture of the progression of negative effects from molecular initiation events (MIEs) to larger intermediates in particles. This implies more important regulatory mechanisms with direct regulatory effects (Hartmann et al., 2019; Koelmans et al., 2022). Potential mechanical toxicity from Nanoplastics includes damage to mitochondrial membranes and generation of reactive oxygen species (ROS), as well as damage to estrogen, androgen, and plasminogen receptors by protein on the surface of

Nanoplastics. Although these mechanisms have been demonstrated in individual model organisms, population-level effects have not yet been formally captured by AOP interactions (Chen et al., 2017; Mattsson et al., 2017; Lu et al., 2016). The application of different genomic technologies (transcriptomics, proteomics, metabolomics and epigenetics) to different freshwater samples exposed to real Nanoplastics facilitates the systematic construction of AOP interactions. Submitting the developed AOPs to the OECD AOP Knowledge Base (AOP-Wiki) allows them to be used for cross-validation through peer review (Hartmann et al., 2019).

9.4 Ecosystem-Level Research and Global Monitoring

Previous studies of Nanoplastics in freshwater ecosystems have failed to bridge the gap between laboratory ecological studies of individual species in the mesosphere or natural ecosystems and local studies. Such community assessments should include trophic interactions, competitive dynamics, habitat diversity, and biogeochemical cycles of indirect effects (Besseling et al., 2019). According to a systematic review by Besseling et al. (2019), at least five mesosphere-level studies have been conducted worldwide investigating the impact of Nanoplastics in freshwater environments. This is clearly insufficient, considering the environmental complexity and legal implications of local risk profiles. Thus, long-term field observations (macroinvertebrate indices, fish community surveys, and crop monitoring in particular) that combine measurements of environmental Nanoplastics concentrations with assessments of environmental condition should be used. Such projects should include water quality programs that include risk assessment models that incorporate various plastic nanoparticle pollutants and validate regulatory concentration limits from laboratory conditions and real-world environments (Koelmans et al., 2022). An important geographic finding of this study is the lack of freshwater systems in areas with increased plastic production and more vulnerable wastewater treatment, such as South Asia, Southeast Asia, sub-Saharan Africa, and small island developing countries. This suggests that risk assessments based on monitoring data from Europe and North America significantly underestimate the magnitude and magnitude of the global risk posed by Nanoplastics in freshwater (Misra et al., 2021; Koelmans et al., 2019).

9.5 Advanced Removal and Mitigation Technologies

To effectively reduce freshwater pollution by nanoparticles, concurrent actions are needed in the following areas: to contain nanoparticle sources, improve nanoparticle removal from wastewater treatment plants, and develop in-situ treatment technologies for already polluted systems (Talvitie et al., 2017; Oriekhova & Stoll, 2018). Electrocoagulation technology is a method of solidifying iron or aluminum hydroxide in situ by electrolysis of an expanded metal electrode. In laboratory tests, this technology achieved an efficiency of about 99% for Nanoplastics larger than 100nm, making it a promising technology for the modernization of wastewater treatment (Oriekhova & Stoll, 2018). Membrane bioreactors (MBRs) achieve theoretical removal rates in excess of 99.9% for particles larger than the membrane cross-section by combining biological treatment and ultrafiltration membranes with nominal pore sizes of 0.01–0.1 µm. However, membrane fouling by natural organic matter and high energy consumption of pressure membrane filtration present significant operational challenges for MBRs that are widely used at the municipal level (Talvitie et al., 2017). Advanced oxidation pathways (AOPs), including UV/hydrogen peroxide and ozone oxidation, convert Nanoplastics into smaller molecules, ultimately producing carbon dioxide and water (Oriekhova & Stoll, 2018). Nature-based solutions, such as constructed water, river barrages and flood restoration, offer an environmentally friendly approach with synergistic effects on biodiversity, water quality and flood protection. While removal rates of microplastic particles in experimental plants have been reported to be between 40% and 80% (Hurley et al., 2018), there is considerable uncertainty about the effective removal of nanoparticles in natural systems. This suggests a

substantial knowledge gap that needs to be addressed at the exam level through targeted exam research.

10. Conclusions

Nanoplastic contamination of freshwater ecosystems is becoming a serious problem due to scientific challenges and environmental understanding, and the aquatic science community is beginning to explore the potential consequences of this contamination. This review summarize data from scientific publications (most published between 2009 and 2024), highlight the current scientific consensus, and present key issues that represent important frontiers for future research and policymaking. The sources and formation pathways of freshwater Nanoplastics are diverse, complex, and still not fully understood. Large plastic fragments are exposed to UV radiation, mechanical corrosion, and the main cause of their associated damage is generation. At the same time, major sources, including personal care products, rubber particles, and industrial wastes from manufacturing, also continue to release Nanoplastics into freshwater via, urban storms, agricultural precipitation and atmospheric precipitation. Nanoplastics are found all over the world, from remote mountain lakes to aquatic ecosystems and urban river systems in densely populated areas. Nanoparticle concentrations in sediments, which form long-term storage, are significantly higher than in sediments. Several studies suggest that colloidal behavior mechanisms, including heterogeneous aggregation of natural colloids and eco-corona formation, play an important role in the deformation of Nanoplastics. Regulated environmental toxicants are diverse, have different mechanistic properties, and have different effects. Algae have been shown to negatively affect photosynthetic efficiency at concentrations of 1–30 mg/L, while in crustaceans reproductive function is impaired even at concentrations below 5 mg/L. Maternal heritability has also been demonstrated oxidative tissue damage, liver toxicity, neurodegeneration due to disruption in the food chain: Plastic a nanoparticles releasing pollutants, heavy metals, antioxidants, and endocrine disruptors at high target doses at 10^7 by the "Trojan Horse" tool due to chemical a release. Total environmental risks that cannot be measured by standard environmental assessment systems.

Currently, a number of major experimental, methodological, and data gaps hinder the translation of these research findings into credible environmental policies. These gaps include lack of analytical capacity to detect particles smaller than 100 nm, reliance on unrefined polystyrene particles in biological tests, lack of correlation between acute and chronic toxicity, local to individual species, and lack of global impact monitoring systems to matter. This gap represents a structural inequality in science and governance that calls for urgent international collaboration. Freshwater ecosystems, which provide drinking water for more than two billion people, are the energy and water base, link terrestrial and marine ecosystems, are home to about 10% of known species, and although less than 1% of the Earth's surface. The new findings reviewed in this article show that it is not the biological risks of Nanoplastics in freshwater ecosystems, but Nanoplastics concentrations, environmental factors, molecular mechanisms, and their long-term effects on ecosystems.

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