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Review

Micro- and nanoplastic toxicity: A review on size, type, source, and test-organism implications

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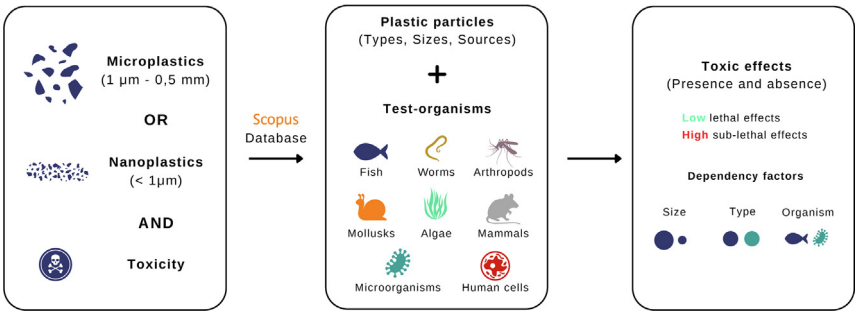
HIGHLIGHTS

- Plastic particles have mostly sub-lethal toxicity rather than significant lethality.
- Toxic effects are dependent on particle size, types and organisms exposed.
- 90.9 % of publications with exposure to nanoplastics used PS particles.
- Arthropods (19.5 %) and fish (19.4 %) are the two classes of organisms most evaluated.

GRAPHICAL ABSTRACT



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ABSTRACT

Polymeric wastes are among the current major environmental problems due to potential pollution and contamination. Within the spectrum of polymeric waste, microplastics (MPs) and nanoplastics (NPs) have gained ground in recent research since these particles can affect the local biota, inducing toxic effects on several organisms. Different outcomes have been reported depending on particle sizes, shape, types, and exposed organisms and conditions, among other variables. This review aimed to compile and discuss the current knowledge and possible literature gaps regarding the MPs and NPs generation and their toxicological effects as stressors, considering polymer type (as polyethylene, polypropylene, polyethylene terephthalate, polystyrene, polyvinyl chloride, or others), size (micro- or nano-scale), source (commercial, lab-synthesized, or environmental) and test organism group. In that sense, 615 publications were analyzed, among which 72 % discussed micro-sized plastics, while <28 % assayed the toxicity of NPs (<1 µm). For most polymers, MPs and NPs were commercially purchased and used without additional size reduction processes; except for polyethylene terephthalate studies that mostly used grinding and cutting methods to obtain MPs. Polystyrene (PS) was the main polymer studied, as both MPs and NPs. PS accounts for >90 % of NPs reports evaluated, reflecting a major literature gap if compared to its 35.3 % share on MPs studies. Among the main organisms, arthropods and fish combined accounted for nearly 40 % of toxicity testing. Overall, the different types of plastics showed a tendency to report toxic effects, except for the 'Survival/lethality' category, which might indicate that polymeric particles induce mostly sublethal toxic effects. Furthermore, despite differences in publication numbers, we observed greater toxicity reported for NPs than MPs with oxidative stress among the majorly investigated endpoints. This study allowed a hazard profile overview of micro/nanoplastics (MNPs) and the visualization of literature gaps, under a broad diversity of toxicological evidence.

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Contents

1. Introduction

Nowadays, plastics are among the main materials used in the world, being present in several everyday applications. However, their widespread use resulted in one of the main environmental pollutants worldwide (Kang et al., 2021; Kokalj et al., 2021). Due to its resistance to degradation, plastic waste accumulates in the environment, possibly for centuries (Worm et al., 2017). Post-consumer plastics reach not only terrestrial environments and organisms, but also aquatic habitats, being transported through water resources and air, finally reaching the marine environment, where they can accumulate (Li et al., 2016; Lebreton et al., 2017).

Most plastics are low-cost, easily manufactured, high-strength, hydrophobic, flexible, and considered biologically inert materials. They are used in nearly every application in everyday life, from household utilities to automotive parts, becoming essential materials in our lives (Lebreton et al., 2019; Urban-Malinga et al., 2020). According to the annual report of Plastics Europe (2021), an estimated 367 million tons of plastics were produced in 2020, and it is expected to double its production in 20 years (Lebreton et al., 2019). The largest demand comes from the packaging sector, which reflects 40.5 % of total production, followed by the civil construction (20.4 %) and automotive (8.8 %) areas (Fig. S1A). Consequently, there is a progressive increase in the generation of plastic waste. According to Geyer et al. (2017), from 1950 to 2015, the amount of polymeric waste generated reached 6300 million tons. The United States alone generates the largest amount of these wastes (37,729 tons/day), followed by China (31,665 tons/day), Japan (19,606 tons/day), and Brazil (12,272 tons/day) (Fig. S1B).

Due to improper waste disposal, management deficiencies, and polymer degradation inertia, polymeric waste can remain in the environment for a long time, potentially requiring 20 to 450 years to be degraded (Urban-Malinga et al., 2020; Zaman and Newman, 2021). Therefore, it is not surprising that contamination of the terrestrial environment occurs, especially in areas with high anthropogenic activities, such as urban and agricultural zones (Horton et al., 2017). The same authors also suggest that the presence of smaller polymeric particles in terrestrial environments is driven by polymer degradation due to UV radiation and high temperatures. Despite the wide distribution of polymeric residues in terrestrial environments, few studies have focused on this contamination, since the extraction of microplastics (MPs) in these environments is challenging (Campanale et al., 2022). Polymeric particles can be transported within the soil, depending mainly on particle size and soil texture (Scheurer and Bigalke, 2018), through agricultural practices, such as tillage, crack formation in soils, bioturbation; or preferential water flow, which may also lead to groundwater contamination. Wind and rainwater are also capable of carrying them towards water sources and the marine environment (Peng et al., 2020; Hu et al., 2022).

The aquatic environment, mainly the oceans, represents one of the final destinations of polymeric wastes. Around 18 % of these wastes have been estimated to come from fishing activities, mainly generated by improper disposal of fishing nets (Andrady, 2011). Meanwhile, the largest share of wastes in marine environments comes from land, including waste generated on beaches (Andrady, 2011), and is transported to water resources like rivers and, eventually, oceans, due to strong rains and winds. Out of the top ten rivers that contribute most to ocean polymeric pollution, eight are in Asia, and three of them are in China (Lebreton et al., 2017). A significant portion of polymeric waste can also reach the oceans through extreme events, such as storms, floods, cyclones, and tsunamis (Eriksen et al., 2016), or even through accidental shipping losses and simple runoff into water bodies during industrial polymer processing (Andrady, 2011). Lebreton et al. (2017) developed a model to estimate the amount of polymeric waste entering the oceans through rivers, projecting between 1.15 and 2.41 million tons of input per year.

According to Eriksen et al. (2014), it is estimated that a minimum of 5.25 trillion polymer particles are present on the oceans' surface, weighing a total of 268,940 tons. Van Sebille et al. (2015) estimated an even higher number, ranging from 15 to 51 trillion, with an equivalent lower mass of 93 to 236 thousand tons. Once the polymers reach the oceans, they can be routed to accumulation zones in the subtropical ocean gyres. These are caused by wind-driven surface water movements from Ekman's transport model, resulting in rotational fields that carry the floating debris to its center (Van Sebille et al., 2012). The largest polymeric residue accumulation zone in the oceans is known as “The Great Pacific Garbage Patch” (GPGP), located between California and Hawaii, which covers an estimated area of 1.6 million km^2 (thrice the size of France, approximately), with a mass of 80,000 tons of polymeric waste in its densest part (Lebreton et al., 2018).

Studies indicate that the degradation of polymeric materials can generate particles in the micro and nanoscale, which can be released into the environment (Luo et al., 2019; Malankowska et al., 2020; Song et al., 2020; Gonçalves and Bebianno, 2021), thereby expanding polymeric waste impact way beyond the obvious macro impact of its improper disposal. Due to their size, micro- and nanoplastics can be accidentally ingested or absorbed by several organisms, such as crustaceans, fish, shellfish, turtles, mammals, and seabirds, among others, and may cause some level of toxicity (Barnes et al., 2009; Ter Halle et al., 2017; Mueller et al., 2020; Sendra et al., 2021a, 2021b). This toxicity can be enhanced by the adhesion of compounds, such as heavy metals and high molecular weight organic substances, to these polymers (Teuten et al., 2009; Davranche et al., 2019;

Zhang et al., 2020) or through the leaching process of plastic additives (Bermúdez and Swarzenski, 2021). Nevertheless, the toxicity caused by micro- and nanoplastics to organisms, including humans, is still an emerging area that needs further study (Lehner et al., 2019).

2. Overview of micro- and nanoplastics generation

Micro- and nanoplastics (MNPs) are small polymeric particles, mainly differentiated by the scale at which their dimensions are found. Nevertheless, both MNPs have particle size definition divergences among the scientific community. Microplastics (MPs), for example, have their dimensions defined between 1 μm and 5 mm according to some authors (Arienzo et al., 2021; Wang et al., 2021) and <5 mm by others (He et al., 2019; Rillig and Lehmann, 2020). For nanoplastics (NPs), some authors recommend that their definition should be based on the specification of nanomaterials, i.e. with dimensions of 1–100 nm (Mendoza et al., 2018), while others consider the entire nanometer range, i.e. 1–1000 nm (Da Costa et al., 2016; Gigault et al., 2018; Schwaferts et al., 2019). More recently, definition and classification criteria have been reviewed, discussed, and proposed, yet to be fixed as the field evolves, helping scientific and regulatory communities to avoid ambiguous terminology and uncertainties (Hartmann et al., 2019; Bermúdez and Swarzenski, 2021).

MNPs have mainly been studied due to the concern that they can negatively affect organisms and microorganisms that ingest, absorb, or are exposed to them. MPs have been found in the oceans and inside organisms in several studies (Fang et al., 2018; Ribeiro et al., 2019; Suaria et al., 2020; Zhang et al., 2020). They originate in two distinct ways: by a direct introduction from a leaching process, known as primary generation, or by secondary generation, as fragmentation of larger particles, such as mesoparticles (pellet-sized) and macroparticles (measuring up to 10–15 cm) of polymeric waste due to environmental degradation (Andrady, 2011).

Regarding the primary generation, the leaching process of micro- and nanoparticles typically present in cosmetic products (Fendall and Sewell, 2009) and industrial sandpaper and abrasives processing (Andrady, 2011) has been reported. MPs used in hand cleansers and facial scrubs have replaced traditionally used natural ingredients, including ground almonds, oatmeal, and pumice stone (Derraik, 2002; Fendall and Sewell, 2009). Typically commercially sold and known as “microspheres” or “microbeads”, these MPs vary in shape, size, and composition (Fendall and Sewell, 2009). According to Anagnosti et al. (2021), >90 % of microspheres in cosmetics show a polyethylene (PE) composition, although polypropylene (PP), polymethylmethacrylate (PMMA), polystyrene (PS) and polyethylene terephthalate (PET) have also been used. The same study also highlights the existence of polymeric nanoparticles in exfoliants, even though they do not fulfill the exfoliation function due to their small size. As the authors state, the presence of these nanoparticles in such products is not purposeful and may have been generated from microparticle degradation during their preparation. This may also be the case of 3D printer waste, where NPs can be generated when resin residues are dissolved in alcohol and exposed to UV radiation (Rodríguez-Hernández et al., 2020).

On the other hand, secondary generation of MNPs originates from the fragmentation of larger plastic particles, fibers, and foams (Mendoza et al., 2018), mainly because of larger fragments of polymeric debris weathering in coastal environments (Andrady, 2011), in both the sea and the land (Ryan et al., 2009; Thompson et al., 2004). Chemical, physical, and biological processes play a role in to particles degradation (Zbyszewski and Corcoran, 2011) reducing the structural integrity of polymeric waste debris' over time and resulting in its fragmentation (Browne et al., 2013). Within the marine environment, they can degrade from exposure to UV radiation and mechanical abrasion (Song et al., 2017). After long-term periods, exposure to UV radiation can result in the photodegradation of polymers, as solar radiation combined with oxygen can lead to oxidation in the polymer matrix, resulting in the polymer chains splitting (Moore, 2008; Barnes et al., 2009; Andrady, 2011; Browne et al., 2013). This oxidative degradation can generate a fragile and brittle layer on their surface, which can develop fissures/cracks. Therefore, polymer microparticles (MPs) can be derived from these degraded brittle surfaces, as they are susceptible to fractures due to moisture-induced stress, temperature variations, and abrasion by grains of sand on beaches (Andrady, 2011). The generation of these surface micro-cracks has been observed in mesoplastic debris collected on beaches (Cooper and Corcoran, 2010) and is mainly present in high-density polyethylene (HDPE) (Chaochanchaikul et al., 2012; Umar et al., 2012), low-density polyethylene (LDPE) (Küpper et al., 2004; Ranjan and Goel, 2019); and polypropylene (PP) materials (Badji et al., 2017; Nakatani et al., 2020) which are the polymers with the highest demand worldwide (Plastics Europe, 2021).

According to Andrady (2011), a sample of polypropylene rope that had been on a pier for several years, when extracted with distilled water, generated large amounts of MPs. However, the weathering, and hence, the degradation suffered by polymeric residues, occurs differently in beach sand, on the surface, or in deep waters. Due to the low specific heat value of sand (664 J/kg°C), polymeric residues present in the sand can reach higher temperatures (over 40 °C) than in water, depending on the polymer pigment color. This phenomenon, combined with great oxygen availability and direct exposure to UV radiation, accelerates the photo-oxidative degradation on beaches and, consequently, the generation of microfragments from larger fragments; leading to embrittlement, cracking, and yellowing of the polymeric samples (Andrady, 2011; Barnes et al., 2009; Moore, 2008). Meanwhile, the lower temperature and oxygen availability of the marine environment results in a milder photooxidation process (Andrady, 2022). Anyway, upon loss of structural integrity, polymers become further susceptible to fragmentation by abrasion, wave action, and turbulence (Barnes et al., 2009; Browne et al., 2013). This process is ongoing, causing the polymeric fragments to become smaller and smaller, reaching the submicron scale (Fendall and Sewell, 2009; Rios et al., 2007; Ryan et al., 2009). MPs are also considered to degrade further, reaching the nanometer scale. The studies by Gigault et al. (2016) and by Lambert and Wagner (2016) point to the possibility that even nanoplastics can be generated from the weathering of larger polymeric waste, and according to Hernandez et al. (2017), they can be generated from the fragmentation of microspheres used in shampoos and scrubs. The authors also highlight agricultural activities as a generator of polymeric nanoparticles, with the application of sewage sludge as fertilizer representing a significant source. Indeed, it has been estimated that agriculture soils receive around 14 % of total plastic waste in the environment, releasing 4–23 times more microplastic particles to land than to oceans (Horton et al., 2017); although partially migrating to waterbodies (as recently reviewed by Moeck et al., 2023).

The detection and quantification of NPs in environments are particularly complex due to their size and chemical composition since it is very similar to organic matter in general (Lehner et al., 2019). Due to their large surface area relative to their volume, NPs are susceptible to contamination by various aqueous pollutants, including metals (Ashton et al., 2010; Davranche et al., 2019; Baudrimont et al., 2020); endocrine-disrupting chemicals (Ng and Obbard, 2006), and persistent organic pollutants (POPs) (Andrady, 2011; Rios et al., 2007). These pollutants are typically found in higher concentrations in the uppermost layers of the oceans, where MPs of lower densities are also more abundant (Ng and Obbard, 2006; Rios et al., 2007; Teuten et al., 2009). These compounds are lipophilic-stable chemicals that adhere and concentrate on the hydrophobic surface of plastics, with environmental concentrations recorded in the range of ng/g to μg/g (Barnes et al., 2009; Teuten et al., 2009). Besides the MP fragments themselves, additives intentionally used in polymer synthesis and processing have been reported as potential hazards to biota as they can leach out from the polymers (Cole et al., 2011).

In this context, the current study aimed to review and compile studies that evaluated the toxicity of MNPs exposure in several different test organisms. For that, a comprehensive review evaluated and discussed polymer characteristics, such as size distribution, type and source, and their assayed potential toxic effects, highlighting literature gaps that need to be covered in the future, involving toxicological studies using polymeric particles.

3. Methodology

This study was conducted based on Scopus platform searches using the keywords "microplastics" OR "nanoplastics" AND "toxicity" published until December 31st, 2021, retrieving a total of 1033 scientific articles. This selection was then filtered for data refinement and standardization under the following exclusion criteria: 1) review articles, as they did not perform exposure tests; 2) publications with no toxicity assays; 3) publications with incomplete information, missing either the type of polymer used, particle size, particle obtaining source or test organism studied (Fig. 1A).

After inclusion and exclusion criteria, the dataset resulted in 59.5 % (615) eligible publications for evaluation; while 27.1 % (280) were review papers; 7.5 % (77) did not evaluate toxicological effects and 5.9 % (61) lacked information (Fig. 1B). The eligible articles were analyzed in terms of type, size, and source of the polymeric particles, as well as the test organisms and the toxic effects that were evaluated. Some of these studies employed more than one particle type/size and/or organism; therefore, they accounted for each exposure condition independently.

Dataset categorization was performed by distributing polymers by type: polyethylene (PE), polypropylene (PS), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), or 'others', which included the remaining polymers, such as PLA, PA, PHB. For polymer size, the nanoscale was considered to range between 0 and 1000 nm (Gigault et al., 2018; Schwaferts et al., 2019) and the microscale between 1 μm and 5 mm (Al Hamra and Patria, 2019; Wang et al., 2021), while polymer source was differentiated into 'commercially available', 'lab-generated', and 'environmentally sampled'. Regarding the toxicity evaluation, the test organisms were categorized among the following groups: arthropods, 'fish', 'algae', 'mollusks', 'microorganisms', 'worms', 'human cells', 'mammals', and 'others'. The category 'human cells' was separated from the category 'mammals' mainly because it reflected only in vitro protocols.

To evaluate a broad range of toxic effects reported for different organisms after exposure to various types of MNPs, we chose to concentrate them among 20 arbitrary categories, as described in Table 1.

4. Polymer types, sizes, and sources

As some of the publications reported exposure of more than one particle type/size, the total of 615 publications resulted in 965 independent exposure condition records. Around 69 % of them discussed micro-sized plastics, and ≈31 % assayed NP toxicity (<1 μm). Regarding the studies using polymer particles in the microscale, PS was the most studied polymer, presented in 35 % of the studies, followed by PE (30 %), 'Other' (16.1 %), PVC (8.5 %), PET (5.4 %), and PP (5 %) (Fig. 2A). Regarding the abundance of the polymeric particle types at the nanoscale, PS was again the main polymer used in the studies, being present in 90.9 % of the evaluated studies, followed by 'Others' (6.1 %), PE (12 %), PET (0.7 %) and PVC (0.3 %). This significant difference in the types of polymers used at the micro- and nanoscale might be mainly related to the difficulty in obtaining/producing nanoparticles of certain polymers versus other polymers, e.g., PS, which are easier to produce in the laboratory (Loos et al., 2014).

Furthermore, by providing an overview of the annual production of polymers by type, it is possible to compare the MNP toxicity publication as a share of their annual production (Fig. 2B). Among the main polymers produced annually, PS has a smaller share of its peers; however, it is by far the most studied polymer regarding toxicity in organisms. This discrepancy may create an information gap on polymer type-specific effects since both the type of the polymer and its size can interfere with toxicity in organisms (Chae et al., 2019; Renzi et al., 2019; Sendra et al., 2021b). This difference is also evident when comparing the types of polymers studied, both at micro and nanometer scales over time. Regarding studies with microparticles, PE was the most studied type of polymer until 2019, when polystyrene surpassed it (Fig. 2C) due to a large increase of concurrent studies employing PS nanoparticles, which often also use micrometric particles for comparative purposes. Overall, the studies that used PS employed smaller particles than the other polymers (Fig. 3A). This might be because PS particles can be easily synthesized in a wide range of dimensions, including the nanometer scale, and even with different surface functionalization (Loos et al., 2014). Although PET and PE have shown some dispersion in the size scale throughout the studies, their average sizes are close to the other two polymers studied (PP and PVC), reaching values between 100 and 300 μm. It is also evident that, excluding PS, all polymers show a deficiency in studies using particles below 1 μm, and specifically for PP, below 10 μm. In addition, PE, PS, and PVC have fewer publications on larger particles, such as above 1 mm (Fig. 3A).

Most polymers employed were acquired from commercial sources, and therefore, they represent polymers that can be commercially purchased as MNPs (Fig. 3B), without further processing for size reduction, for example. PS has the larger share of commercial particle reports, a predominance that could be reinforced by their easy production. PET and other publications extensively employed lab-synthesized particles, in which polymeric particles were acquired from laboratory synthesis or obtained commercially and then subjected to laboratory processes, aiming to reduce their dimensions, such as milling and or UV exposure. The articles included in the 'commercial' category usually employed spherical shapes with low dispersion of sizes, whereas articles in the 'lab' category typically used fragments obtained from mechanical milling showing a wider range of particle sizes. Most PET publications obtained their particles from the grinding of PET bottle pieces, as a proxy to recapitulate environmental effects and impact on these materials, which is the most likely reason the average size of PET particles is higher than its peers (Fig. 3A). On the other hand, 'environmental source' refers to polymer particles recovered from environmental samples, mainly water, but also soil or even within organisms. Despite being more realistic, studies with particles collected in the environment have more comparative limitations, due to the wide variation in particle sizes, types of plastics, and contaminants, among other factors.

5. Toxic effects of micro- and nanoplastics

Assessing plastic particle toxicity in organisms can be very complex. This is because it is influenced by several factors, such as shape, size, and type of plastics employed, the exposed organism, the concentration and exposure conditions (Xia et al., 2022; Jiang et al., 2023; Rodrigues et al., 2023).

For instance, secondary MPs (e.g., those formed mainly from degradative processes in the environment) have mostly irregular surfaces (Xia et al., 2022), and polymeric particles produced by processes such as ball milling, grinding using a homogenizer or cryogenic milling methods also present random shapes (Xu et al., 2022). However, commercially available MNPs usually consist of spherical, regularly sized particles (Xu et al., 2022). Considering that it has been reported that non-spherical MPs, like fragments and fibers, have higher toxicity on organisms (Jung et al., 2021), we expected to observe more studies reporting the presence (over absence) of toxicity on lab-synthesized versus commercial particles. However, at least under a relative number of publications, there were no apparent differences in reporting polymeric particle toxicity of 'commercial' and 'lab'-sourced particles (Fig. 4A).

Regarding the organisms studied (Fig. 4B), arthropods (19.5 %) are the main test organisms employed to evaluate MNPs toxicity, followed by fish (19.4 %), algae (11.6 %), mollusks (10.1 %), worms (10 %), microorganisms (9.4 %), human cells (5.5 %), and mammals (4.5 %). The remaining publications including reptiles, amphibians, and plants, among others, constitute the last category ('others'), which did not reach >3 % individually, and combined, represent 10 % of the total dataset analyzed. Among arthropods, *Daphnia* is the most studied organism in toxicity tests and is indeed considered a model organism for this type of research (Trotter et al., 2021; Cunningham et al., 2022). *Daphnia magna* is present in 73 studies, representing ≈39 % of the arthropod reports and 8 % of the total publications. Fish are the second most studied group, and zebrafish (*Danio rerio*) are the main species of the entire dataset. This organism is present in 45 % of the publications that used a fish species and represents 9 % of the total studies, corroborating zebrafish as a model organism for

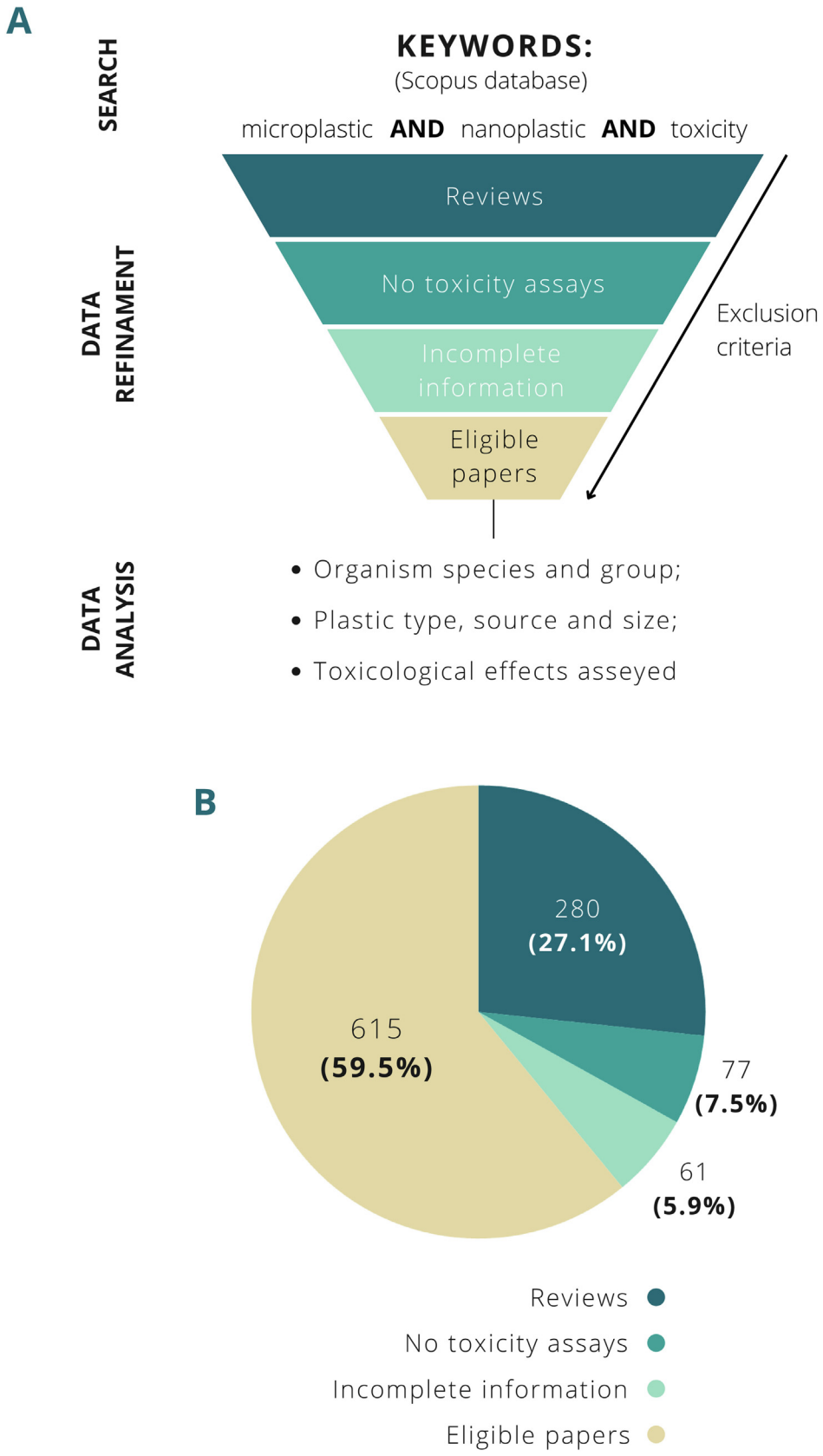

Fig. 1. Methodology applied for data search, refinement, and analysis. (A) "Microplastic", "nanoplastic", and "toxicity" were employed as keywords in the Scopus database. Review articles and publications without toxicity assays or incomplete data were excluded from further evaluation. Eligible publications were analyzed for plastics size, source and type, test organism, and toxic effects assayed. (B) The absolute and relative amounts of publications retrieved after data refinement are presented.

Table 1
Categorization of toxic effects reported with respective content description considered within each group.

Categories	Description
Abiotic	Alteration of physical parameters of the medium such as ph and dissolved carbon, effluent treatment, dewaterability, production of antibiotics
Absorption/bioaccumulation	Uptake, internalization, ingestion, accumulation and distribution in tissues and absorption of particles
Behavior	Behavioral changes, locomotion, spontaneous movements,
Biomass	Body mass, weight, growth rate, growth curve
Cytotoxicity/cell death	Apoptosis, autophagy, cell necrosis, cytotoxicity
Development	Alteration of organism development factors, such as hatching, heartbeat, molting
Feeding	Change in consumption of water, food, and nutrition
Genotoxicity/epigenetics	Epigenetic mechanisms, DNA damage
Hematology	Injuries and alterations in the hematologic system, biochemical blood counts
Histopathology	Structural changes in tissues/organelles, regeneration, calcification/whitening
Inflammation/immunity	Tissue inflammation, immune/immune response or effect, alteration in immune defenses
Metabolism	Metabolic responses/routes; Metabolomics, mitochondrial changes, protein/carbohydrate content, lipids, calcium content
Microbiota	Bacterial diversity, composition and structure in the intestinal flora or community (specific for the microorganism category)
Morphology	Morphological changes, morphometric measurements, biometric measurements, malformations
Neurotoxicity	Neurodegeneration, changes in AChE[a]
Oxidative stress	Antioxidant defenses, antioxidants, alteration in oxidative balance, redox imbalance, ROS[b]
Reproduction/transgenerational	Fertility, fecundity, offspring, sperm count, sperm quality, Transgenerational effects and multiple generations
Survival/lethality	Death, EC50[c], LC50[d]
Transcriptome/proteome	Protein profiling, Gene ontology, RNA-seq
Xenobiotic	Chemical/pesticide biotransformation, chemical stress, metal bioavailability

[a] Acetylcholinesterase.
[b] Reactive oxygen species.
[c] Half maximal effective concentration.
[d] Lethal concentration for 50 % of the test-organism.

environmental toxicity evaluation, as it presents ease maintenance, high fecundity, and short life cycle (Bhagat et al., 2020).

Aquatic organisms account for 67 % of the dataset, as detailed in Table S1. For these species, water physical-chemical characteristics play an important role in toxicity since it influences particle aggregation, bioavailability, chemical adsorption/desorption, and weathering (Atugoda et al., 2020). This is evident when comparing fresh and marine environments, with enormous differences in ionic strength, pH and natural organic matter, among several characteristics (Arini et al., 2022). Very few publications retrieved reported specific assays for abiotic parameters (18/22), mainly studies with microorganisms, because most studies employ the optimal conditions for the species being assayed. Among the aquatic organism cohort (Fish, Mollusks, Worms, Arthropods, Algae and Others), we identified that 52.1 % of the studies employed freshwater media over

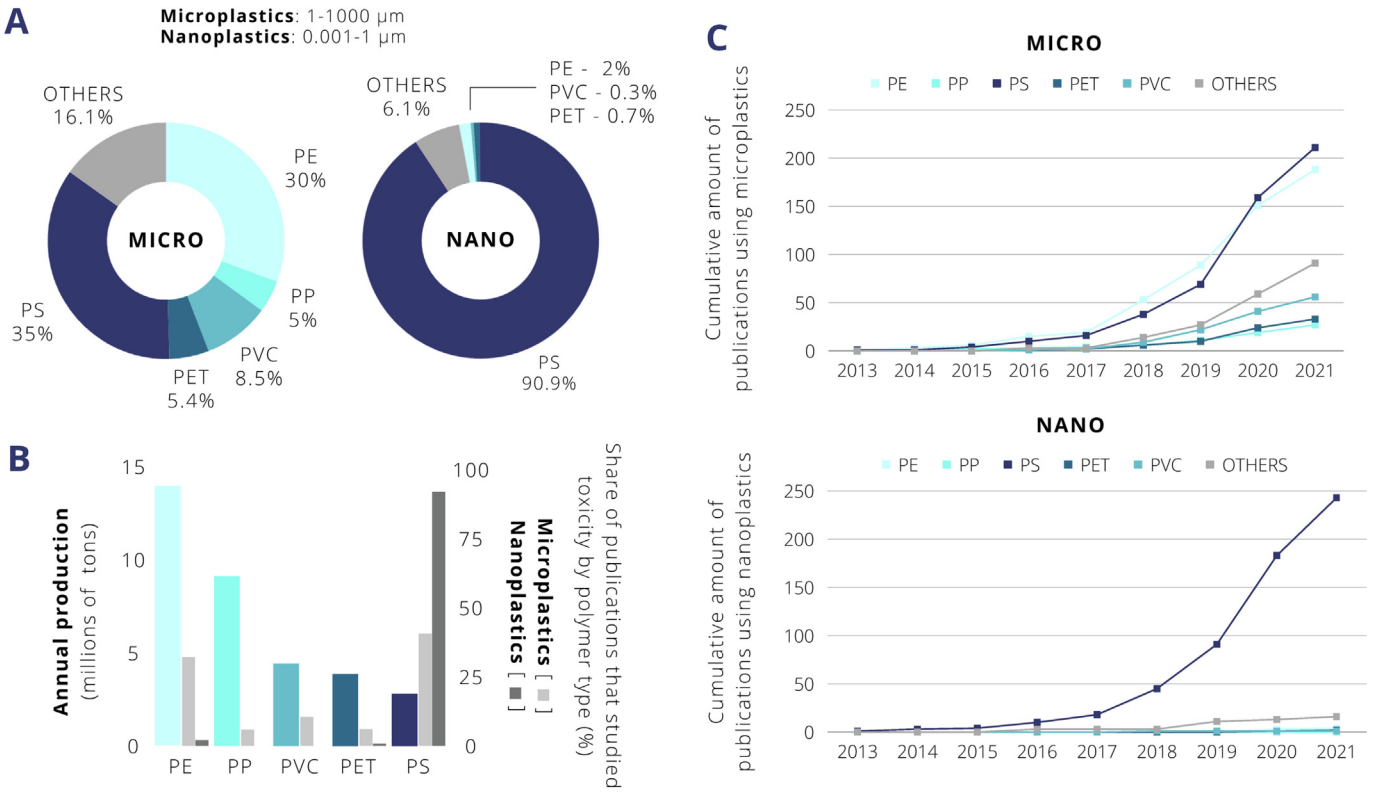

Fig. 2. Overview of types, scales, annual production, and number of publications that evaluated the toxicity of different plastics within the dataset. (A) Polymer type distribution within each size-scale; (B) relative comparison between the share of MNPs toxicity publications versus their respective annual productions in absolute numbers; (C) cumulative amount of studies targeting plastic particles of different types at both micro and nanometer scale. (PE: polyethylene, PP: polypropylene, PET: polyethylene terephthalate, PS: polystyrene, PVC: polyvinyl chloride).

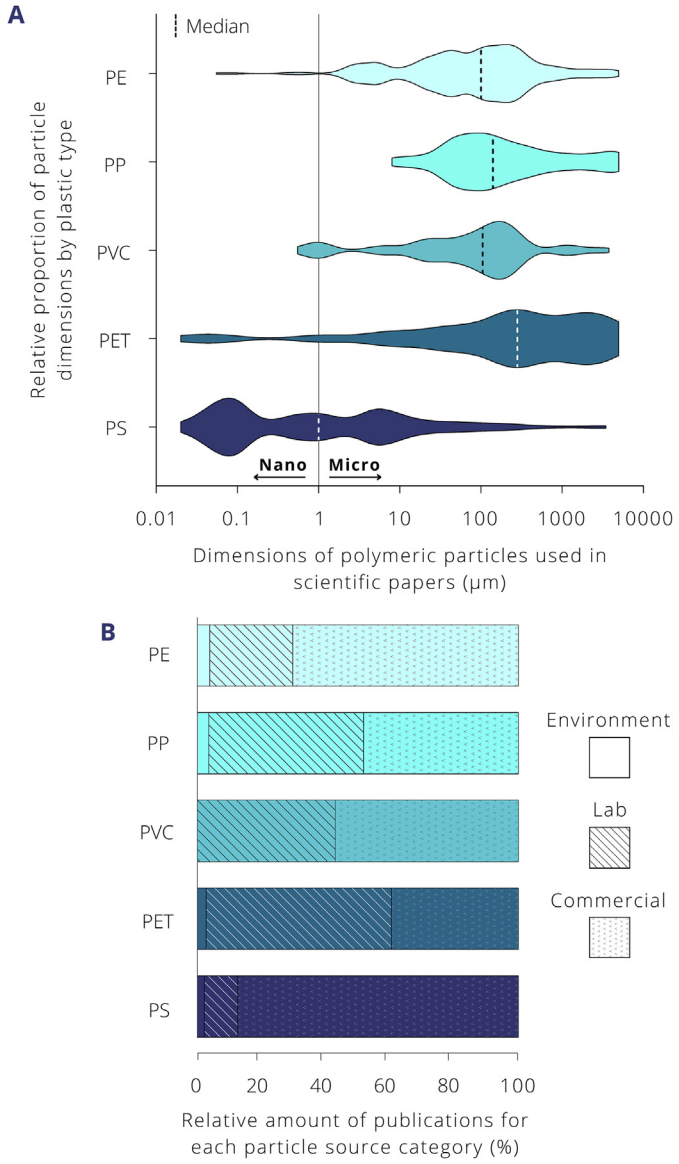

Fig. 3. Size and source spectrum of polymer particles reported among the publications evaluated. (A) Dimensions distribution of the main plastic particle types; (B) relative number of publications for each particle source category – Environment, Laboratory (Lab), and Commercial. (PE: polyethylene, PP: polypropylene, PET: polyethylene terephthalate, PS: polystyrene, PVC: polyvinyl chloride).

47.9 % that used saltwater; and this equilibrium is not maintained within each test organism group (e.g. fish: 79 % with freshwater species). The exception was observed for Algaes, with greater balance between freshwater (56.3 %, $n = 63$) and saltwater (43.7 %, $n = 49$) studies. This similar distribuition is maintained even when splitting studies between microplastics (56 % for freshwater and 44 % for seawater) and nanoplastics (56.8 % for freshwater and 43.2 % for seawater). Since the majority of aquatic organisms evaluated have a specific habitat (freshwater or saltwater), no comparative effect on particle outcomes is possible within the same species. Some organisms, however, are found in wave transition waters and, consequently, are exposed to variable gradients of salinity, which impacts particles' colloidal behavior. More recently, microfluid devices have been proposed to better mimic such environments during ecotoxicity assays using swamp or estuarine species and showing an underestimation of toxicity when overlooked (Venel et al., 2021; Arini et al., 2022).

Plastic type and size also play a role in toxicity (Jeong et al., 2016; Verla et al., 2020; Bobori et al., 2022). Aiming to investigate the main toxic effects reported for MNPs exposures, a heatmap was constructed based on the number of publications reporting either the presence or absence of each toxic effect category, for each plastic type at both micro- and nanometer scales (Fig. 5). The heatmap shows the discrepancy in the number of studies employing the different materials. Despite that, an overabundance of toxic effects for both micrometric and nanometric particles seems predominant in most categories. The limited (and in some cases) nonexistent information combining plastic types with some categories of toxicity is quite evident, especially at the nanometer scale.

Some categories, such as 'oxidative stress', 'metabolism', and 'histopathology', presented, in general, the presence of toxicological effects combined with more publications. Studies evaluating exposed organism survival/lethality of polymeric particles on a micrometric scale reported mostly low toxicity, which was not observed for nano-sized plastics, although a more robust comparison is limited due to the significant difference in the number of publications with nanoplastics. When analyzing only PS MNP, a polymer having a similar number of reports for micro- ($n = 90$) and nanometric ($n = 68$) studies in this category, this overall tendency is maintained, with higher toxicity of PS NPs compared to PS MPs under the 'survival/lethality' category. These data corroborate the suggestion that particles on the nanometer scale are more toxic than particles on the micrometer scale (Jeong et al., 2016; Bobori et al., 2022). In addition, the data also corroborate that the different types of polymers may differ in their toxicity predominance (Zhu et al., 2019; Zimmermann et al., 2020), as illustrated within categories such as 'behavior', 'biomass', and 'xenobiotic effects' in Fig. 5. Based on the relative number of publications, a graphical representation of micro- versus nanoplastic toxicity reports for each category is also available in Fig. S2 (Supplementary Material).

5.1. Polyethylene (PE)

PE has multiple applications, such as manufacturing containers, dispensing bottles, wash bottles, tubing, plastic bags for computer components, and laboratory equipment. PE is one of the world's most-produced plastic polymers, the type most frequently identified in marine litter (Beiras et al., 2018; Muñiz-González et al., 2021), and the most detected MPs in surface waters in general (Barboza et al., 2020). These particles are also relevant for their hydrophobicity (due to the presence of -CH_2 groups) and high molecular weight, which allows environmental pollutant adsorption (Castro et al., 2020). However, PE is susceptible to photooxidation, and, therefore, additives such as UV stabilizers are often added to its formulation (Beiras et al., 2018).

Despite being the most produced polymer, PE is the second most studied plastic in terms of toxicity to organisms, totaling 207 publications. Micro-sized particles were used in 97 % of the studied papers, while only 3 % used nano-sized particles, and the most used particle sizes (73 %) in the exposure tests were between 10 and 1000 μm. Fish were the main test organisms used to test PE toxicity (22 %), followed by arthropods (20 %). The least used organism categories were mammals (only 2 %) and human cells (4 %). Most PE studies employed commercially available particles (70.1 %), while lab-produced ones accounted for 25.8 %, and environment-recovered particles accounted for only 4.1 %.

The exposure and toxicity caused by PE particles are generally widespread in the literature, covering all the toxicity categories of this study. The most studied toxicity category for both PE MPs was 'survival/lethality' ($n = 71$), which resulted in the presence of effects in only 41 % of publications, followed by 'oxidative stress' ($n = 62$) and 'biomass' ($n = 59$), which presented 80 % and 46 % presence of effects, respectively. Other than 'biomass' and 'survival/lethality', 'xenobiotic effects' is the only category that showed less presence than the absence of effects (43 %, $n = 14$). All other toxicity categories had associated toxicological effects.

5.2. Polypropylene (PP)

PP is one of the most produced synthetic polymers in the world, mainly due to its properties and variety of applications. It can be used in a rigid

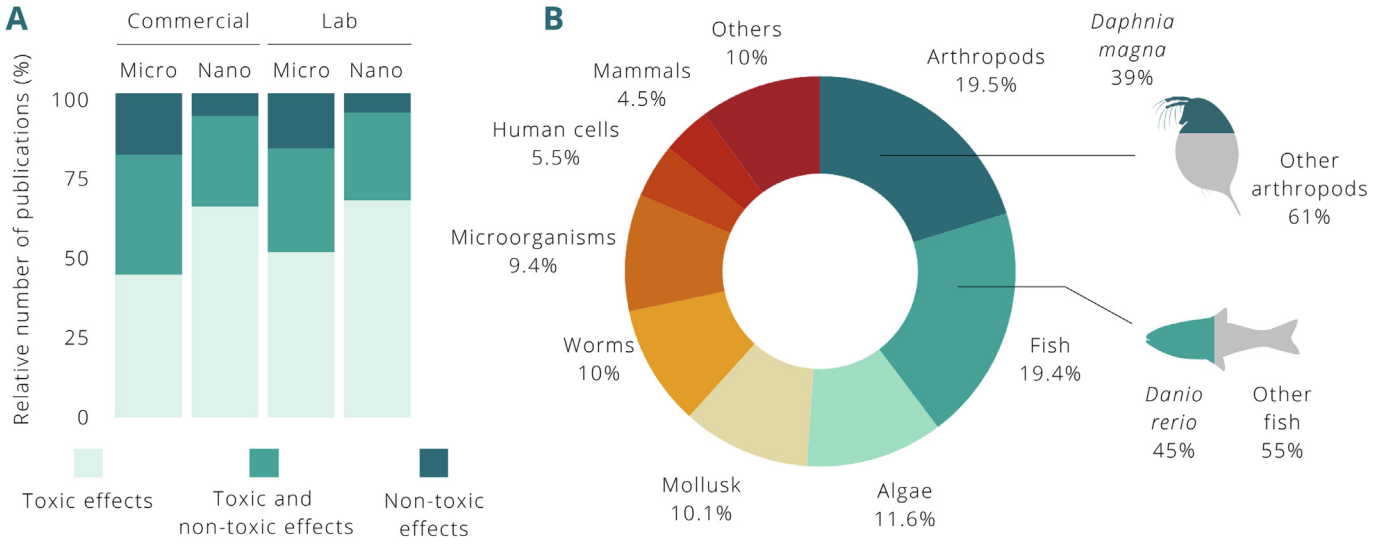

Fig. 4. (A) Presence or absence of toxicity after exposure to plastic particles from 'commercial' and 'lab' sources; (B) distribution of test-organism classes used for toxicity assays among the publications evaluated.

format, by injection and blow-molding, or even in the form of fibers, films, fabrics, and sheets, among others (Maddah, 2016). Despite being considered a durable polymer and persistent material, PP is very susceptible to degradation when exposed to UV radiation (Lestari et al., 2022). This degradation may be responsible for altering its physicochemical properties, and the weathering process can lead to chemical modification of the polymer surface (Zhu et al., 2020; Jeon et al., 2021; Li et al., 2021). This process can generate carboxylic acid groups that may have more affinity for protein binding, which can affect the biological behavior of organisms exposed to these materials (Pfleging et al., 2009).

Even though polypropylene is the second most produced polymer and one of the most prevalent micropollutants in the aquatic ecosystem, toxicity studies in both aquatic and terrestrial organisms are still scarce. Under our methodological approach, we identified 33 publications that evaluated the toxicity of PP in organisms. Like PE, most of the PP particles used for exposure ranged between 10 and 1000 μm (80 %). No toxicity studies were found with nanometric PP among the eligible articles of this study. Regarding test organisms, arthropods (30 %), worms (15 %), and algae (15 %) were the main groups assayed, while only one report using mammals and two reports using mollusks and microorganisms were identified. Interestingly, the categories 'commercial' and 'lab' as sources of PP particles showed an equivalent number of publications (48.1 % each) and only 3.8 % were from the 'environment' category.

'Survival/lethality', 'oxidative stress', and 'biomass' were the most studied toxicity categories in the exposure tests with PP, and toxicological effects were present in 58, 100, and 75 % of the cases, respectively. However, other than 'survival/lethality' ($n = 12$), no categories presented at least a dozen studies evaluating PP toxicity. This indicates a limitation in comparative terms with other types of polymers in terms of the toxicological effects caused.

5.3. Polyethylene terephthalate (PET)

PET is a thermoplastic polymer of the polyester family and is used in liquid containers, food trays, clothing, and thermoforming applications, among others (Sinha et al., 2010; Palacios-Mateo et al., 2021). It is considered an inert material with excellent mechanical and thermo-mechanical properties (Damayanti et al., 2021), including being an effective barrier for gases such as oxygen and carbonic gas, and it is widely used to manufacture soft drink bottles (Sinha et al., 2010). However, despite being a weathering-resistant material, PET is subject to fragmentation caused by degradation, photooxidation, and hydrolysis (Gewert et al., 2015), all very common in aquatic environments, which results in polymer chain splitting, a phenomenon that leads to the generation of MPs (Arhant et al., 2019).

In aquatic environments, PET is prone to sink due to its density of 1.37–1.45 g/cm^3 (Parolini et al., 2020), and since these polymers are denser than seawater, they are more commonly found in benthonic regions (Woodall et al., 2014), although some studies also show the presence of PET microparticles on the water surface (Suaria et al., 2016; Hendrickson et al., 2018; Wang et al., 2020). Particle surface area and shape, water turbulence, and temperature are among several factors that may influence the behavior of PET in water (Enders et al., 2015; Schwarz et al., 2019).

As with PE, studies with PET are scarce compared to other types of plastics. Here we identified 38 publications using PET particles, from which 95 % used microparticles and only 5 % used nanoparticles. An interesting point is that approximately one out of three publications used particles between 1 and 5 mm, which are considered large MPs, and they were mostly lab-sourced. This category accounts for 57.6 % of PET particles assayed and is usually obtained by grinding or cutting plastic bottles, therefore corroborating the larger MP dimensions reported. The main test organisms exposed to PET were arthropods (24 %) and fish (18 %), and no studies with mammals were found.

Regarding toxicity categories evaluated in PET studies, 'biomass' had the highest number of publications ($n = 16$) and presented the lowest presence of toxic effects (25 %). Compared to other types of plastics, this is the lowest amount reported among all materials. However, since there is still a limitation in the number of publications, exposed organisms, and particle concentrations and sizes in PET studies compared to other plastics, only comparative studies will allow to robustly indicative if PET presents less risk to biomass modification than other polymeric MNPs.

5.4. Polyvinyl chloride (PVC)

PVC is currently the third highest-demand synthetic polymer. Being thermoplastic, PVC can be melted and reshaped many times. Its polar characteristics allow the incorporation of various additives, expanding its application range, which includes the manufacture of pipes, cables, food packaging, textiles, and medical and industrial applications (Patrick, 2004; Fernández-González et al., 2022). Other common uses for PVC include building materials and applications requiring flame-retardant properties (Levchik and Weil, 2005; Engler, 2012).

Phthalates, lead compounds, organotin compounds, and adipates are among the main additives in commercial PVC composition (Patrick, 2004; Fernández-González et al., 2022), making it a leader material in compatible additives (Rosato et al., 2020). Consequently, their leaching into the

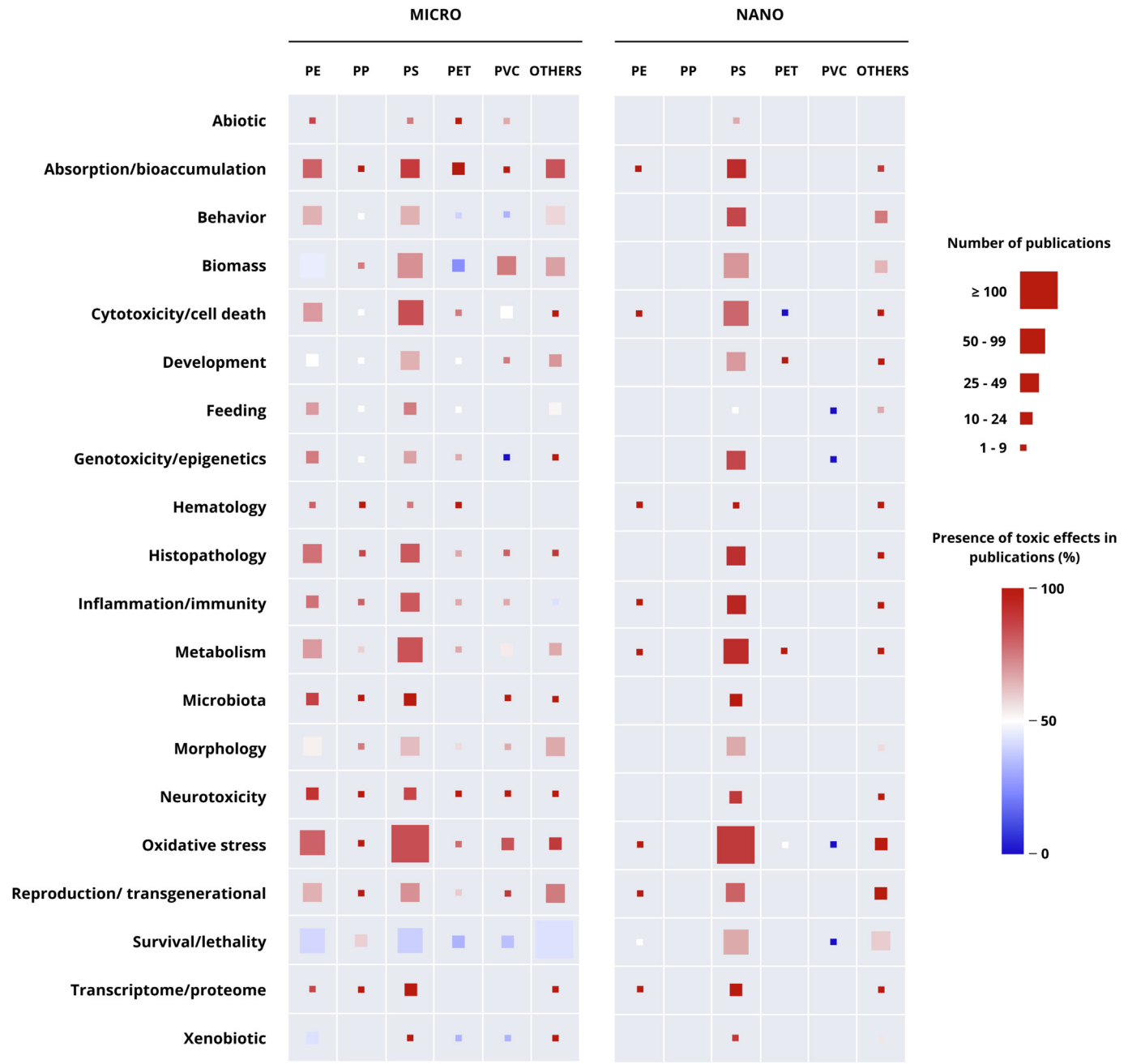

Fig. 5. Effect of micro- and nano-scale plastic particle exposure among the different predetermined toxicity categories (Table 1) for each plastic type. Square size is directly proportional to the number of publications assaying a toxic effect, while square color reflects the predominance of the presence/absence of such effects. The absence of squares indicates the absence of publications retrieved for that condition. (PE: polyethylene, PP: polypropylene, PET: polyethylene terephthalate, PS: polystyrene, PVC: polyvinyl chloride).

environment has been reported through both chemical and natural processes (Dopico-García et al., 2007; Chen et al., 2019; Meng et al., 2021), eventually reaching organisms and resulting in toxic effects that have been studied over the past few years (Luo et al., 2022; Sridharan et al., 2022).

Xia et al. (2022) point out that PVC is easily fragmented compared to other thermoplastics. PVC MPs have been found in many environments such as soils from agricultural activities, as well as marine and fresh waters (Savoca et al., 2019; Ding et al., 2020; Huang et al., 2021). This is because, despite the stabilizing additives that can slow down its degradation, PVC is extremely susceptible to photodegradation and its MPs can remain for prolonged periods in the environment (Fernández-González et al., 2022).

PVC is the third most produced type of plastic and the third most studied relative to the toxicity caused by its particles, totaling 58 papers. Among these, only one reported the effect of nanoparticles. As for PE and PP, most studies employed PVC particles between 10 and 1000 μm (72 %). Unlike other plastics, algae (26 %) were the main test organisms, followed by arthropods (17 %) and fish (17 %). The least studied organisms were mollusks (3 %) and human cells (3 %), and no mammal studies were identified with PVC. Regarding particle sources, 56.9 % of the PVC studies used commercially available particles, 43.1 % used particles generated from laboratory processes, and, interestingly, no study used particles collected from the environment. As with other polymers, the most studied category was 'biomass' ($n = 26$), which showed one of the highest prevalences of toxic

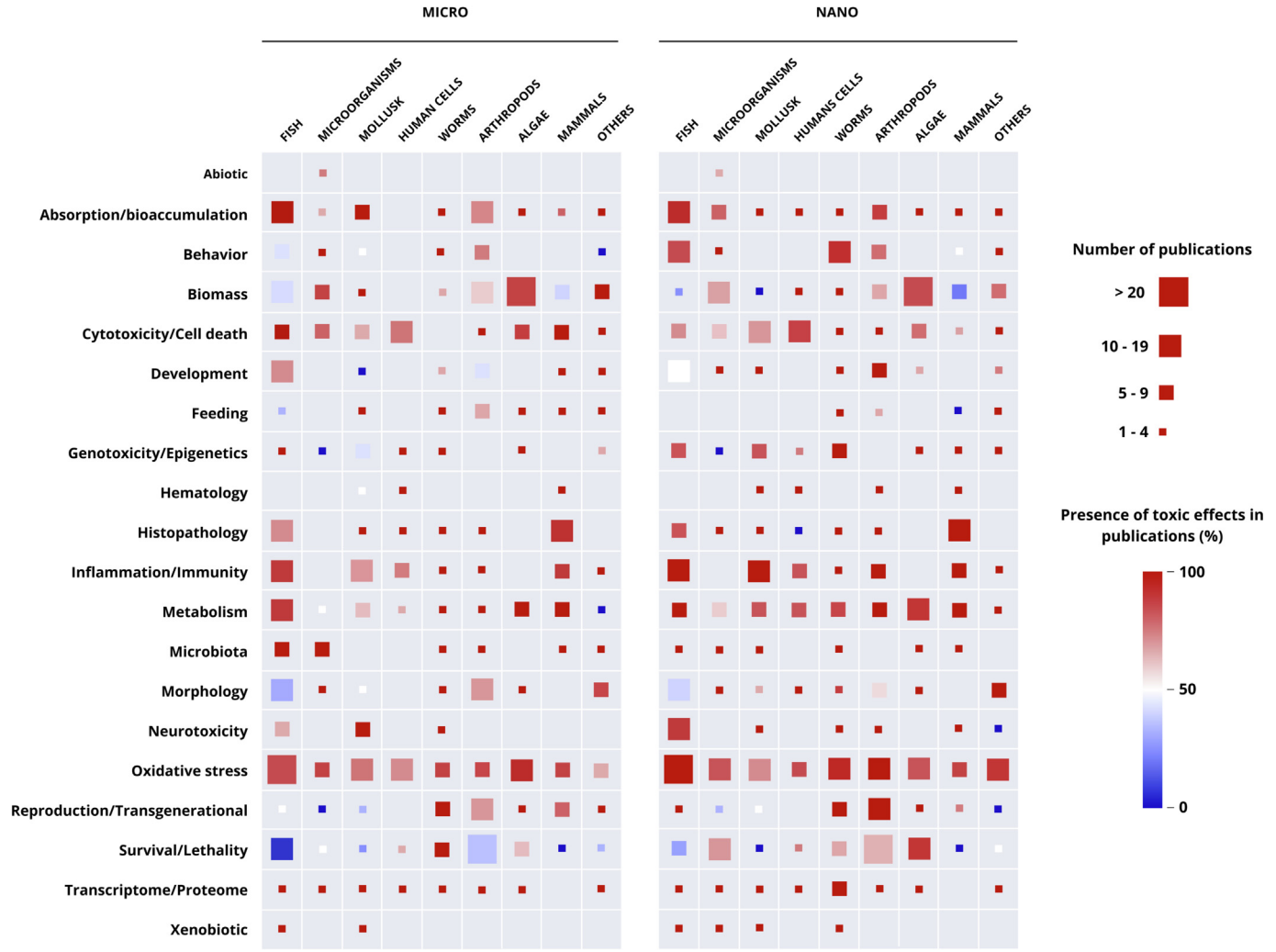

Fig. 6. Toxic effects of polystyrene (PS) micro and nanometer particles exposure in different organisms. Toxicity categories were predetermined in Table 1. Square size is directly proportional to the number of publications assaying a toxic effect, while square color reflects the predominance of the presence/absence of such effects. The absence of squares indicates the absence of publications retrieved for that condition.

effects in the dataset (73 %), followed by 'survival/lethality' (36 %) like most other plastics.

5.5. Polystyrene (PS)

PS is employed in several applications: (1) food and non-food storage and transport components, including packing foam, food containers, disposable cups, plates, and cutlery (Ho et al., 2018); (2) office and household items, such as toothbrushes, toys, clips, cassettes and compact disks (Kik et al., 2020); (3) products of aquaculture and marine sectors, including fish boxes, net floaters, floating docks and life jackets (Turner, 2020), as well as (4) insulation in building construction, and (5) electric, electronic, and automobile industries (De-la-Torre et al., 2020).

PS is chemically characterized by a carbon backbone with aromatic benzene rings (Schröter and Ventura, 2022) because of free radical vinyl polymerization of styrene monomers (Turner, 2020), which is originally produced from ethylene and benzene (Kik et al., 2020). This synthetic polymer is an amorphous and colorless thermoplastic (De-la-Torre et al., 2020), expanded or melt-formed (Kik et al., 2020), usually rigid or foamed, that displays low elasticity, high durability, and resistance to biodegradation (Schröter and Ventura, 2022). The latter occurs at extremely low rates; therefore, it is considered nonbiodegradable (Ho et al., 2018). Although recyclable, transportation of its lightweight and bulky size is not cost-effective (Ho et al., 2018), resulting in persistent solid waste and potential PS-derived contaminants as previously reviewed (Turner, 2020).

Under this scenario, PS was the main material employed to assay MNP toxicity, totaling 502 reports. Of the total, 35 % of MP and 90.9 % of NP studies evaluated were performed using PS (Fig. 2A). Among only PS studies, nano-sized particles accounted for 53 % of the reports, while 47 % of the studies were assayed with micro-sized particles, among which, only ≈16 % used particles >100 μm. In both micro and nano scales, fish were the main test organisms employed to address PS toxicity (20 % each), where *Danio rerio* was the leading species. Regarding particle origin, 88 % of PS studies employed commercially available particles, while lab-synthesized and environment-recovered PS accounted for only 10 and 2 %, respectively.

Due to the higher number of studies, PS is the only material with available data for all toxicity categories analyzed in both MNP sizes (Fig. 5), allowing a more detailed analysis, considering the distinct test organism categories individually (Fig. 6). By far, the most studied toxicity category was 'Oxidative stress' (n = 237), in which toxic effects were reported in 83 % of MP studies and 89 % in studies with NPs. 'Metabolism' (n = 121) and 'Inflammation/immunity' (n = 90) alterations were also reported in >80 % of the studies evaluating related endpoints for both MNPs. It is also worth mentioning that 'Survival/lethality' is the only category with more reports for its absence (rather than presence) of toxicity, at least for

micro-sized PS (≈61 % reports). Despite the lower number of studies, it might indicate that exposure to PS MPs is mostly non-lethal, particularly for fish and arthropods (14/15 and 24/36 reports, respectively); although the different outcomes highlight that some classes of organisms are more susceptible to polymeric particle exposures than others. That might also be true for size since most PS nanoparticle studies (45/68) do report lethality and represent the greatest share of nano-sized reports of the full dataset, although in a much smaller number compared to larger PS particles (Fig. 3A). Thus, unlike the remaining polymers, the PS toxicity gap is among micrometric particles (1–5 mm).

5.6. Other plastics

Plastics different than previous categories had a much lower frequency of studies individually, such as PLA, PHB, PA, PU, tire rubber, and polymer mixtures (e.g., PE + PP). Out of 126 publications, only 14 % used nanometric particles, while 86 % of the studies employed micrometric particles, and the vast majority (67 %) were within the 10–1000 μm range. Particles were mainly obtained from laboratory transformation processes (57 %), such as milling and cutting, followed by commercial particles (27 %), and finally, particles collected in the environment (16 %). Most publications employed arthropods (21 %) and fish (17 %) as test organisms, while the least studied were human cells (3 %) and mammals (no studies). Within this category, mixtures of different types of plastic had the highest number of publications (n = 23), followed by polyamide (PA, n = 15) and polylactic acid (n = 13), the latter being a biodegradable polymer. This category includes plastics from both fossil and renewable sources; some are non-biodegradable materials (~72 %), and others are biodegradable materials (~28 %). The latter was one of the proposed solutions to contain polymeric waste pollution impact (Filiciotto and Rothenberg, 2021; Rahman and Bhoi, 2021). Biodegradable polymers are part of a larger group, known as bioplastics, that can be generated from raw material derived from biomass, such as sugar cane and corn, but they can also be produced from fossil sources (European Bioplastics, 2016). These materials can be degraded almost completely in 180 days in compost bins (Anderson and Shenkar, 2021; European Bioplastics, 2016). In other environments, however, bioplastics can play a similar role to petroleum-derived polymers (Anderson and Shenkar, 2021).

In terms of toxicity, 'survival/lethality' was the main endpoint assayed (n = 134), reporting toxicity in 43 % of publications with microparticles (n = 104) and 60 % with nanoparticles (n = 30). Other extensively studied categories ($n \geq 35$) were 'absorption/bioaccumulation', 'biomass', 'behavior', 'morphology', 'oxidative stress', and 'reproduction/transgenerational', and all of these showed a greater presence than the absence of effects. Another point to be highlighted is that, despite the presence of biopolymers, this group shows no apparent differences against conventional polymer categories in the heatmap (Fig. 5); although the restricted number of publications is also a limitation to more robust conclusions at this point.

6. Conclusions

With the growing demand for synthetic polymers, waste accumulation and contamination tend to continuously increase. Furthermore, current (and new) residues generate polymeric micro and nanoparticles from degradative processes, making it imperative to better define and estimate the presence of such polymeric residues in both aquatic and terrestrial areas. Realistic quantification will allow more realistic conditions for studies on their impact on the environment and the biota.

Here we aimed to compile an overview of the major toxic effects reported for micro- and nanoplastics of different plastic types on test organisms. Although some limitation is expected when choosing to predefine restrictive yet representative keywords, specific databases or publication periods, the systematic search strategy allowed an unbiased raw dataset for further analysis. Polymer type, size, and source were considered to identify the main organisms' groups and toxic effects assayed, highlighting the main gaps in the literature; to the best of our knowledge, for the first time. As expected, toxic effects reported are species-, size- and polymer-dependent. We observed greater toxicity reported for nanoparticles than for microparticles, although the smaller number of publications and the common publication bias towards the presence (rather than absence) of effects might not be overlooked. For materials with a larger number of studies (such as PS), some toxic effects were consistently reported in several organisms and for both micro and nano-sized particles (e.g., 'oxidative stress'). Also, it seems that exposure to polymeric particles has mostly sub-lethal toxicity than significant lethality for most organisms. Considering that there are several reasons to choose to report a specific parameter (clearly its relevance but also access and resources), some critical thought is also imperative regarding endpoints evaluated since the most reported toxic effect does not necessarily reflect the major source of toxicity.

It is worth noting that shape, size, type of plastic, and exposure conditions are known to play key roles in toxicity and are quite heterogeneous among publications. If on one hand such a diverse set of evidence might hinder generalizations on MNPs toxicity, on the other, it allows identification of distinct and promising processes to be more deeply assayed for underlying mechanisms. Furthermore, within the environment, these broad spectra of polymers coexist as complex mixtures, which most likely modify the behavior and toxicity of such components. That is especially true when considering the polymeric potential for chemical adsorption and desorption interactions, and only the accumulating evidence will contribute to a better understanding of their impact.

CRediT authorship contribution statement

Kauê Pelegrini: Conceptualization, data curation, editing, data presentation.
Talita Carneiro Brandão Pereira: Methodology, data curation, review and editing.
Thuany Garcia Maraschin: Data curation.
Lilian de Souza Teodoro: Data curation.
Nara Regina de Souza Basso: Review and editing.
Griselda Ligia Barrera de Galland: Review and editing.
Rosane Angélica Ligabue: Review and editing.
Mauricio Reis Bogo: Review, editing and supervising.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162954.

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