Nanoplastics in the oceans: Theory, experimental evidence and real world

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ABSTRACT

This review critically analyses > 200 papers collected by searching on Pubmed the word "nanoplastics", a group of emerging contaminants which are receiving growing attention. The present review intends to provide an overview of current knowledge on nanoplastic pollution starting with the theory of polymer degradation, passing to laboratory confirmation of nanoplastic formation and ending with the possible occurrence in sea water samples. Most of the observations proposed focus the attention on polystyrene (PS) because the majority of research knowledge is based on this polymer. Moreover, we thoroughly describe what effects have been observed on different organisms tested in controlled conditions. Nanoplastics formation, fate and toxicity seem to be a very dynamic phenomenon. In light of this, we identify some aspects retained crucial when an ecotoxicological study with nanoplastics is performed and which elements of nanoplastics toxicity could be deeper covered.

1. Introduction

Searching on PubMed the word "nanoplastics" (NPs) a list of 224 papers appears, among which, the oldest dates back to 2012 (research conducted on February 5th, 2020). Respect to microplastics, nanofraction of the marine litter, represent a new infant field of investigation as suggested by the number of papers published in 2019 which doubled the studies of the previous year and that the first 2 weeks of 2020 have recorded almost the entire 2018 scientific production on this topic (Fig. 1).

Some authors adopted the definition taken from the nanomaterial including particles smaller than 100 nm, others prefer to raise the upper size limit to 1 µm, typical border of colloids (Alimi et al., 2017; Cole and Galloway, 2015; Gigault et al., 2018; Costa et al., 2016). In addition, most researchers include in the definition, either primary (manufactured) and secondary (originated from degradation) nanoplastics. Personal care products, industrial abrasives, paints, and particles used in drug delivery can be considered primary nanoplastics (Alimi et al., 2017; Hernandez et al., 2017; Guterres et al., 2007). Also recent technologies, as 3D printing, have proved to be sources of ultrafine particles (Azimi et al., 2016). Nevertheless, although nanomaterials productions is ever-expanding (Inshakova and Inshakov, 2017), nanoplastics represents only a thin slide of the market (Vance et al., 2015).

Mostly because of technological limitations and dilution effect, nowadays, it is laborious to extract and quantify nanoplastics in marine environment. Despite the impetus in the research which took place in recent years, there are still many lights and shadows on the subject which, this review aims to highlight. The majority of the observations proposed in this review focus the attention on polystyrene (PS) because most of research knowledge (about 97%) is based on this polymer. It will be discussed what are the knowledges on polymer degradation, initially reviewing laboratories experiments and consequently reporting evidence of the occurrence in marine samples. Laboratory evidence of biological effects on aquatic organisms will be also briefly reported. Finally, some aspects retained crucial for ecotoxicological studies with nanoplastics will be pointed out and elements of research on nanoplastics which could be deeper investigated will be suggested.

2. Polymer degradation theory, lab and field evidence

2.1. The theory of polymer degradation

A full-bodied bibliography on polymer degradation is available on Pubmed. The degradation of plastic polymers is induced by either abiotic or biotic paths (Gewert et al., 2015). Generally abiotic degradation precedes biodegradation following the photo-oxidative degradation, the thermal and ozone-induced degradation, the mechanochemical degradation and the catalytic degradation (Singh and Sharma, 2008). Such mechanisms lead to changes in polymer properties resulting in bond scissions and consequent chemical transformations.

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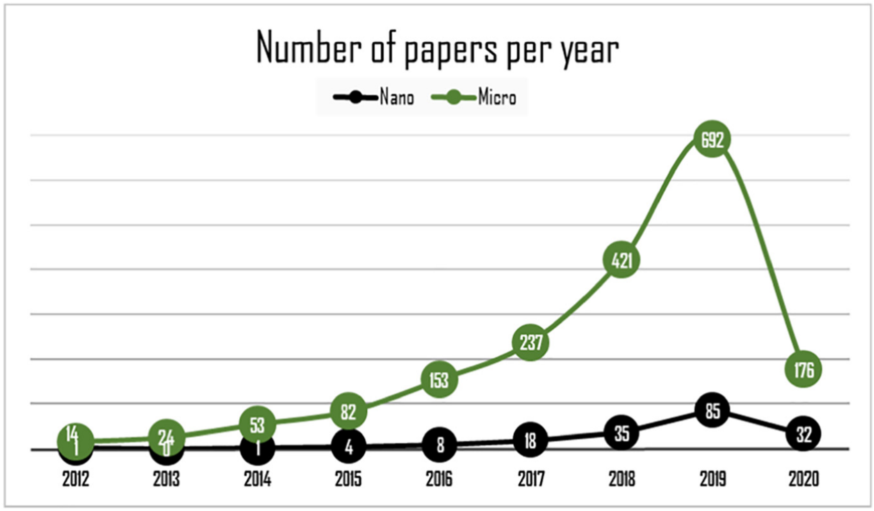

Fig. 1. Number of papers published, from 2012 to 2020, searching the term "nanoplastics" and "microplastics" on PubMed (February 5th, 2020).

First, the modification involves the surface of the item: the visual effects are micro-cracking, discoloration, erosion and crazing. Subsequently, the inside polymer, can be subjected to further degradation such as microbial attach. Two different pathways exist, depending on whether we consider carbon-carbon backbone plastic (polyethylene, PE; polypropylene, PP; polystyrene, PS; polyvinylchloride, PVC) or plastics with heteroatoms (polyethylene terephthalate, PET; polyurethane, PUR). Henceforth, the attention will be put on PS.

The European demand of polystyrene in 2018 was 6.4%, mainly destined for the production of food packaging (e.g. egg tray), coffee cup, lid, eyeglasses frames and building insulation (Plastic Europe, 2019; Ho et al., 2018). Once released into the environment, the polystyrene is subjected to degradation. Specifically, when irradiated with UV-light, the phenyl ring get excited and the excitation energy is transferred to the nearest C—H bond (Yousif and Haddad, 2013). The PS is thus deeply modified: the cleavage of the hydrogen, the formation of a polymer radical cross-linking and chain scission lead to the formation of ketones, olefins and styrene monomers as the main volatile product (Yousif and Haddad, 2013; Singh and Sharma, 2008). Although PS is susceptible to outdoor weathering, it is considered a polymer very resistant to biodegradation (Mor and Sivan, 2008). Furthermore, additives and UV-stabilizers decrease its degradability (Ho et al., 2018).

An interesting suggestion on additional polymer degradation pathway is given by the physical size alteration of virgin polyethylene microplastics (31.5 μm) occurred after ingestion by Antarctic krill (Dawson et al., 2018). Following a 4-day static assay, the mean particle size isolated from the krill was 78% smaller than the original plastic particles (7.1 μm ± 6.2 SD). The paper suggests a new pathway of degradation that it would be interesting to deeper investigate even with other polymers.

The theory of abiotic polymer degradation is well known, but less is the knowledge about laboratory confirmation of such phenomenon in the nanoscale. Few studies reproduce realistic conditions for the marine environment.

2.2. Laboratory confirmation of nanoplastics formation

Laboratory evidence shows that the formation of nanoscale particles from bigger plastic items is very plausible. Shim et al. (2014) firstly reported fragmentation of expanded polystyrene (EPS) beads to micro- and nano-sized particles in laboratory. The study mimed conditions at beached or riverbanks, by submitting infield expanded polystyrene (EPS) beads to a monthly accelerated mechanical abrasion with glass beads and sand. Subsequently, they combined effects of UV exposure and mechanical abrasion on different polymer types (polyethylene PE, polypropylene PP and expanded polystyrene EPS) demonstrating that, in laboratory conditions, a large proportion of the particles had fragmented into undetectable submicron particles, within 12 months (Song et al., 2017). In particular, after 2 months the surface of the EPS pellets became yellow, brittle and showed cracks. In addition, powder-like white fine particles were produced on the surface of EPS. After 12 months, micrometer-sized fine particles were detected inside the cracks. Finally, aggregations of small particles (lower than 1 μm) were confirmed on the inner surface of cracks under high magnification. The study by Lambert and Wagner (2016) is a further laboratory example reporting how the degradation of plastic materials can lead to the release of nano-sized plastic particles to the environment. They used a Nanoparticle tracking analysis to characterize the formation of nanoplastics during the degradation of a polystyrene (PS) disposable coffee cup lid. In detail, the PS sample was cut into 1 cm squares, placed in glass vials, immersed in 20 mL demineralized water, and placed in a weathering chamber. After 56 days' exposure the concentration of nanoplastics was 1.26×10^8 particles/mL (average particles size 224 nm) compared to 0.41×10^8 particles/mL in the control. Nevertheless, the exposure conditions were kept static with the temperature set to 30 °C, 24 h exposure to light in both the visible and ultra-violet (UV; 320 and 400 nm) range; conditions quite distant from those found in the environment.

In addition to photo- and mechanical degradation, the biodegradation pathway of PS particles has been also investigated. PS is considered a polymer very resistant to biodegradation. Tian et al. (2017) calculated the mineralization of ^{14}C-PS polymers in liquid medium (pH 7.5) with the fungi *Penicillium variabile* for 16 weeks after ozonation pre-treatment. The study reports only a very slow mineralization ability expressed by the terrestrial fungi. Most of other studies have been conducted burring in soil the PS (Atiq et al., 2010; Oliveira et al., 2010). Marine-like experiments are still missing.

Whilst there is evidence on PS degradation into nano-size particles, less is known about other polymers. Recently Enfrin et al. (2020) demonstrated how polyethylene microplastics extracted from a commercial facial scrub exposed to a wide range of shear forces may fragmented into NPs. Specifically, the study reports that a daily use of 4 g of scrub could release up to 1011 particles of 400 nm in size per litre of wastewater. The mechanism at the base of the fragmentation into nanoplastics smaller than 10 nm is supposed to be a combination of turbulences created by mixing or pumping followed by a crack propagation and failure mechanism. Finally, only strong, and far-removed from reality methods have been applied in order to produce PET nanoplastics

(Magrì et al., 2018). The details will be provided in the Section 4.2.

Some studies report the fragmentation and degradation of plastic to nanoplastics under laboratory conditions but evidence of their occurrence in environmental samples is still poor. In addition, the relative importance of this process still has to be validated in the field.

2.3. Nanoplastics occurrence in marine samples

Ter Halle et al. (2017) first reported the occurrence of the nanofraction (1–999 nm) of the marine litter in environmental samples. Sea water samples collected in the North Atlantic Subtropical Gyre, were ultra-filtrated and analyzed under a dynamic light scattering (DLS). After a concentration of 200 times, the DLS showed a relaxation of the light intensity over time, indicating the presence of colloidal materials. They didn't obtain an accurate size distribution, due to sample dilution, but the analysis reports the presence of particles between 1 and 1000 nm. In order to obtain a chemical fingerprint of colloidal samples and confirm their plastic nature, they performed a pyrolysis coupled to gas chromatography–mass spectrometry. PE, PS, PVC and PET have been detected in the sample. There are no other studies demonstrating the occurrence of nanoplastics in marine samples, by so far.

3. Biological effects in aquatic organisms

Given the lack of protocols of extraction and detection of nanoplastics in wild organisms, the only possible effects of nanoplastic interaction with biological systems are confined to laboratory contexts. Due to their small size < 1 μm, lower than the average dimension of vegetal and animal's cellular mean diameter (10–30 μm), they are potentially able to cross the contact surfaces (gills, gastrointestinal tract, cellular wall) translocate to inner organs and directly interact at a cellular level (Rossi et al., 2014; Forte et al., 2016). Similarly to microplastics, PS nanoparticles can exert an intrinsic toxicity or act as vectors for other pollutants (Chen et al., 2017a, 2017b; Chen et al., 2017a; Ma et al., 2016; Cui et al., 2017; Shen et al., 2019a; Barría et al., 2019; Shen et al., 2019b). Further, they can interact with organisms at the base of trophic chain and be transferred to top consumers (Cedervall et al., 2012; Chae et al., 2018). More than half of the experiments (54.8%) deployed invertebrates followed by fish (16.9%), algae (12.7%), microbes (9.4%), cell lines (5.2%) and rat (only one paper) (Fig. 2). Within of the huge, and biologically speaking extremely different category of invertebrate, crustaceans have been tested in almost half of the cases (43.1%) followed by worms (23.5%), molluscs (21.6%), rotifers (7.8%), and sea urchin (3.9%).

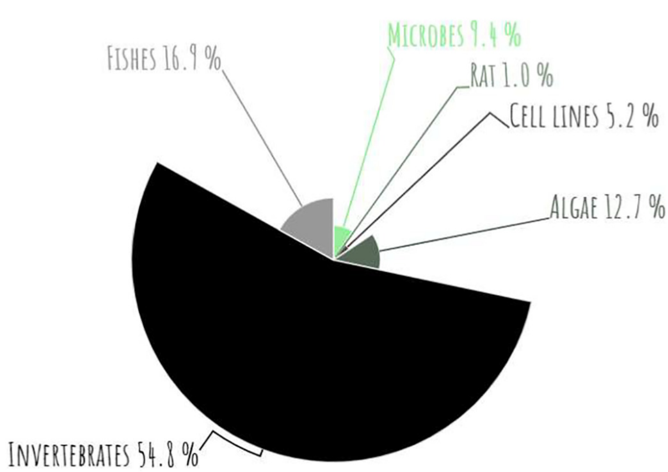

Fig. 2. Percentage of biological categories used in ecotoxicological studies with nanoplastics. Invertebrates (divided in crustaceans, molluscs, worms, rotifers and sea urchins) are deployed in more than half of the cases.

3.1. Toxicity to microbes

Some studies highlighted negative effects (especially on gut microbiome biodiversity) but others did not exhibit any observable toxicity. Among the first, particles from 50 to 100 nm induced significant impacts on gut microbiome of soil oligochaete *Enchytraeus crypticus* after a 7-day exposure of PS nanoplastics leading to a reduction in the relative abundance of *Rhizobiaceae*, *Xanthobacteraceae* and *Isosphaeraceae* families (Zhu et al., 2018). However, the majority of the effects were found in the highest exposure rate of 10% nanopolystyrene, which is roughly 250–10,000 times bigger than the estimated environmental concentration (as suggested by the authors themselves). The disrupted microbiome in *E. crypticus* is also reported by Ma et al. (2020) who, in addition, investigated the combined effect of NPs and tetracycline on antibiotics resistance genes (ARGs). ARGs increased after 14 days of exposure and their diversity and abundance was not completely restored after a period of recovery. Changes in the gut microbiota and contents of amino acids and fatty acids in the shrimp *Litopenaeus vannamei* were reported by Chae et al. (2019) suggesting possible consequences on nutritional values of the shrimp. The single and combined effects of amino polystyrene NPs (70 nm; 0.2 mg/L) and perfluorooctane sulfonate (PFOS: 0.1, 1.0, and 5.0 mg/L) on metabolism of thermophilic bacteria has been investigated by Chen et al. (2020). NPs alone resulted in a 53.9% reduction in hydrogen production, however, the combined exposure revealed an antagonistic effect (31.6%). One of a kind, PS-NH_2 at 5.0 mg/L had no effect on the growth of *Microcystis aeruginosa* but combined with glyphosate showed antagonistic effects significantly alleviating the inhibitory effect of the herbicide. Sun et al. (2018) studied the toxic effects of 50-nm PS-NPs on the marine bacterium *Halomonas alkaliphila*. The results showed growth inhibition and differences on chemical composition and ammonia conversion efficiencies at concentration of 80 mg/L. Moreover, oxidative stress and increased extracellular polymeric substances were detected. Finally, *Vibrio fischeri* did not exhibit any observable toxicity to 26 or 100 nm dialysed-PS (< 100 mg/L) or poly-methyl-methacrylate (PMMA) nanoparticles (86–125 nm; ≤1000 mg/L) (Booth et al., 2016; Heinlaan et al., 2020).

3.2. Toxicity to algae

How nanoplastics can interact and affect algae has been the object of some studies. Generally, toxicity seems to be influenced by a variety of factors such as the surface charge of the particles, the medium hardness and the particle concentration (Nolte et al., 2017; Bergami et al., 2017), the presence of natural organic polymer (Liu et al., 2019a), the size of the particles (Sjollema et al., 2016) and the algal life stages (González-Fernández et al., 2019). Chae et al. (2018) studied the trophic transfer and individual impact of 51 nm PS nanoplastics in a four-species freshwater food chain included the alga *Chlamydomonas reinhardtii*. After an exposure to a maximum 100 mg/L, they reported little or no mortality although the confocal laser microscopy showed the attachment to the surface of zoospores and the penetration of the outer layer during cell division. Other experiments reported the adsorption of NPs on microalgae (Bergami et al., 2017; Nolte et al., 2017). In some cases algal growth was reduced (Bergami et al., 2017; González-Fernández et al., 2019; Sendra et al., 2019b; Sjollema et al., 2016; Besseling et al., 2014), in others it was not affected (Venâncio et al., 2019; Baudrimont et al., 2020; Heinlaan et al., 2020). The decrease of photosynthesis activity of *Chlorella* and *Scenedesmus*, probably caused by the light shading effect of the adsorbed PS particles (20 nm) and/or by obstructed CO_2 gas flow and nutrient uptake pathways has been reported by Bhattacharya et al. (2010). ROS production, morphological alterations, decreases in chlorophyll content and esterase activity, DNA damage and depolarization of mitochondrial and cell membrane are some of the impacts reported by freshwater and marine algae (Bellingeri et al., 2019; González-Fernández et al., 2019; Sendra et al., 2019a, 2019b).

3.3. Toxicity to invertebrates

Rotifers are major components of zooplankton in freshwater and coastal marine ecosystems throughout the world and could be useful indicator species. A handful of studies have been conducted on rotifers. For example, Jeong et al. (2016) evaluated the accumulation and adverse effects of PS micro- and nanoplastic (6 µm, 500 nm, 50 nm) in the rotifer *Brachionus koreanus*. Using different concentration (0.1-1-10-20 mg/L), all sizes led to significant size- dependent effects, including reduced growth rate, reduced fecundity, decreased lifespan and longer reproduction time. They reported also significant differences in antioxidant enzymes such as SOD, GR, GPX and GST. Manfra et al. (2017) reported an increase of mortality rate of *Brachionus plicatilis* after 24 h and 48 h of exposure to cationic ($-NH_2$) PS. Significant differences were detected at concentrations upper than 5 mg/L. Also PMMA-NPs were able to induce mortality in rotifers at concentrations higher than 4.69 mg/L with an estimated 48 h median lethal concentration of 13.27 mg/L (Venâncio et al., 2019).

Della Torre et al. (2014) investigated the disposition and toxicity of two surface modified polystyrene nanoparticles (-COOH and -NH_2, 40 and 50 nm respectively) in early development of sea urchin embryos (*Paracentrotus lividus*). PS-COOH accumulated inside embryo's digestive tract but no embryotoxicity was observed up to 50 mg/L. PS-NH_2 were more dispersed and caused severe developmental defects in addition to induce cas8 gene at 24 h post fertilization (at the concentration of 3 mg/L), suggesting an apoptotic pathway. A significant concentration and time-dependent decrease in lysosomal membrane stability and apoptotic-like nuclear alterations were observed in phagocytes of sea urchin upon exposure to 50 nm PS-NH_2 at concentration of 10 and 25 mg/L, although the multixenobiotic resistance phenotypes was not altered (Marques-Santos et al., 2018).

Copepods are widely used as model species in ecotoxicity and nanotoxicity test (Baun et al., 2008; Jarvis et al., 2013; Bergami et al., 2016; Luo et al., 2018) too. A size-dependent effect of micro- and nanopolystyrene particles in the marine copepod *Tigriopus japonicas* has been investigated by Lee et al. (2013). The study reported some effects on survival and development of first (F0) and second (F1) generation, after administration of 50 nm PS particles at concentration higher than 1 mg/L. Further, the 500 nm PS treated individuals, reported a decrease in fecundity, which was not recorded for the smaller 50 nm particles. Several studies demonstrated the accumulation of nanoplastics in different organs and developmental stages of *Daphnia* spp. (Brun et al., 2017, Rist et al., 2017; Cui et al., 2017; Liu et al., 2019b; Liu et al., 2018; Liu et al., 2020; Saavedra et al., 2019). A wide range of effects have been recorded: the decrease in survival, reproduction and body size (Besseling et al., 2014; Cui et al., 2017), abnormal development, low hatching rate and differences in levels of genes encoding key stress defence enzymes (Liu et al., 2018; Lin et al., 2019a; Liu et al., 2020) as well as induction of the heat shock proteins HSP70 and HSP90 (75 nm; ≤ 1 mg/L) (Liu et al., 2019a, 2019b, 2019c). Accumulation but no mortality and toxicity effects on *Daphnia magna* have been reported in a trophic transfer experiment (60 nm) (Chae et al., 2018) as well as in experiment performed with PMMA nanoparticles (86–125 nm; ≤ 1000 mg/L) (Booth et al., 2016) or with dialyzed PS-NPs (26 and 100 nm; ≤ 100 mg/L) (Heinlaan et al., 2020). Finally, during a 14-days experiment, the presence of 50 nm NPs significantly enhanced the bioaccumulation of phenanthrene-derived residues in daphnid body (NPs: 5 mg/L; Phe: 0.1 mg/L) (Ma et al., 2016). *Artemia* spp. has been also deployed in ecotoxicological studies highlighting the ability to bioaccumulate nanoplastics and several sub-lethal effects (Bergami et al., 2016; Bergami et al., 2017; Mishra et al. 2019; Sendra et al., 2020).

Bivalve molluscs are abundant from freshwater to marine ecosystems, where they are extensively used in biomonitoring programs but also in studies of nanoparticles toxicity (Canesi et al., 2012; Rocha et al., 2015). Among the first studies focused on possible effects of nanoplastics on feeding behaviour, Wegner et al. (2012) recorded an increase in the total weight of the faeces and pseudofaeces in specimens of *Mytilus edulis* exposed to 30 nm PS NPs and a reduction in their filtering activity quantified as valve opening. However, it is necessary to note that the exposure concentrations were very high: 0.1–0.2-0.3 g/L. More recently, the increase in faecal production after exposure to polyethylene nanoparticles on specimens of *Corbicula fluminea* has been demonstrated by Baudrimont et al., 2020 using concentrations more environmentally relevant, namely 1000 µg/L. A handful of studies highlight the variability of ingestion and egestion rates of micro and nanoplastic particles describing a mechanism that is dependent on multiple variables such as developmental stage, particle size and surface properties of the particles (Cole and Galloway, 2015; Al-Sid-Cheikh et al., 2018; Rist et al., 2019). Decrease in fertilization success and in embryo-larval development (50, 500 nm, 2 µm; 0.1, 1, 10 and 25 mg/mL) (Tallec et al., 2018), significant alterations in the expression of genes associated with biotransformation, DNA repair, cell stress-response and innate immunity (110 nm; ≤ 50 mg/L) (Brandts et al., 2018a), consequences on haemocytes in terms of motility, apoptosis, ROS and phagocytic capacity (Canesi et al., 2015; Canesi et al., 2016; Sendra et al., 2019a) are some of the impacts induced by NPs with different surface functionalization (NH_2, COOH). The presence of amino- or carboxylic functional groups on the surface of the particles greatly affects their behaviour, stability and potentially their toxicity. In fact, different responses were recorded by González-Fernández et al. (2018) who tested the toxicity of 100 nm PS-COOH and PS-NH_2 on oyster *Crassostrea gigas* gametes. A significant increase of ROS production was observed in sperm cells after 1 h of exposure at concentration of 1, 10 and 100 mg/L of PS-COOH. On the contrary, spermatozoa exposed to PS-NH_2 were not significantly affected and no significant differences were observed in the percentage of motile spermatozoa or movement linearity after exposure.

In the last two years, several research groups put the attention on the possible interaction and toxicity of nanoplastics on soil worms (Zhu et al., 2018; Qu et al., 2018; Dong et al., 2018; Kim et al., 2019; Qu et al., 2019; Shao et al., 2019; Qu et al., 2019; Kim et al., 2020; Qiu et al., 2020; Yang et al., 2020), however, how marine worms could be affected by this new form of contamination remain poorly clarified. Bioaccumulation study of nanoplastics and polycyclic aromatic hydrocarbons (PAHs) in the clamworm *Perinereis aibuhitensis* has been conducted by Jiang et al. (2019), who indicated a NP-adsorbed pyrene of < 1% of the total pyrene accumulation in the clamworm body when the concentration of NPs in seawater was as low as 0.4 mg/L. Cholinesterase activity and burrowing are endpoints considerable impacted on Polychaeta *Hediste diversicolor* which, on the contrary, did not exhibit differences in most of the parameters associated with oxidative stress (100 nm; 0, 0.005, 0.05, 0.5, 5, 50 mg/L) (Silva et al., 2020).

3.4. Toxicity to fish

Laboratory experiments demonstrate the trophic transfer of polystyrene nanoplastics from primary producers to fishes which, in turn, highlighted effects. In particular, Cedervall et al. (2012) reported weight loss, differences in triglycerides/cholesterol ratio in blood serum and differences in the distribution of cholesterol between muscle and liver of *Carassius carassius*. Moreover, the time it took the fish to consume 95% of food was more than doubled, indicating behavioural disorders in fishes exposed to 24 nm polystyrene nanoparticles. *Oryzias sinensis* and *Zacco temminckii* after a 7-days trophic transfer exposure to 51 nm PS, reported several abnormalities with differences in liver histopathology, slight increase in the total amount of cholesterol in the blood serum and locomotor deficits (Chae et al., 2018). Kashiwada (2006) reported the accumulation of 39 nm PS particles in gills, brain, testis, liver, blood, and intestine of *Oryzias latipes* when exposed at a concentration up to 10 mg/L. In vitro experiment on fathead minnow (*Pimephales promelas*), demonstrate significant degranulation increase and induction of neutrophil extracellular trap release at a dose of 0.1 g/L PS (Greven et al., 2016). Evidence of immune system of fish compromised by exposure to 45 nm PMMA NPs ($\leq$20 mg/L) are reported by Brandts et al., 2018b who also evidenced the increase of m-RNA transcripts related to lipid metabolism, pparα and pparɣ. Behavioural changes have been detected in *Sebastes schlegelii* specimens exposed to 0.5 and 15 μm PS particles (190 μg/L), which reported cluster, reduction of swimming speed, but also increasing in oxygen consumption and ammonia excretion and lower protein and lipid contents (Yin et al., 2019).

Danio rerio, better known as zebrafish, proved to be a good model organism in nanoparticle toxicity studies (Chakraborty et al., 2016). Nano PS distribution and accumulation has been studied at different developmental stages (Pitt et al., 2018a, 2018b; Van Pomeren et al., 2017; Chen et al., 2017a, 2017b) highlighting the ability to localize in different tissues (e.g. head, yolk sac, gastrointestinal tract, gall bladder, liver, pericardium, pancreas, etc.). Some effects reported are altered larval behaviour, significant inhibition of acetylcholinesterase (AChE) and genotoxic effects (50 nm; 1 mg/L) (Chen et al., 2017a, 2017b), but also decreased glucose level (25 nm; 0.2, 2, 20 mg/L) (Brun et al., 2019). Chen et al. (2017a, 2017b) reported an upregulation of gfap and α1-tubulin, nervous system related genes, after 5-days exposure at 1 mg/L 50 nm PS. Pitt et al. (2018a, 2018b) demonstrated the maternal transfer of 42 nm PS particles to offspring in zebrafish through dietary exposure (at a final NPs concentration of 10% of the food by mass). The co-parental exposure did not significantly affect the reproductive success but reduced glutathione reductase activity. They concluded that PS NPs could bioaccumulate and passed on to the offspring but does not cause major physiological disturbances. Finally, the combined effects of gold ions, PAH and PS NPs was investigated by Lee et al. (2018) and Trevisan et al. (2019). In the first case, PS NPs alone (50, 200, and 500 nm; 0.1 mg/ml) induced only marginal effects on survival, hatching rate, developmental abnormalities and cell death of zebrafish embryos, but exerted a synergistic effect on gold toxicity. The second experiment reports opposite results: nanoplastics adsorbed contaminants but potentially decrease their uptake due to particle agglomeration (PS:44 nm; 0.1, 1, 10 ppm; PAHs: 5.07 to 253.65 ng/ml).

4. What's been done and future studies

Fig. 3 summarizes the main field of interest on nanoplastic issue, suggesting which ones have been more done and what remain to be investigated. Of 224 papers, 40.4% of research efforts regarded ecotoxicological studies as reviewed above, followed by reviews often including considerations on microplastics (15.3%) and papers on methods of detection (8.9%). The possible role of NPs as vector of contaminants has been examined by 9.4% of cases. Studies on nanoplastics behaviour, degradation, fate and transport represents only a small slice (5.1%, 2.6% and 2.6%, respectively). Human risk related publications represent 6.4% of the total (Table 3, Suppl. Materials). Undoubtedly, the possible implications of nanoplastic contamination in terrestrial and freshwater ecosystems are understudied (5.5%), however, this percentage does not take into account the toxicological studies performed on zebrafish, a freshwater species used in 11 out of 18 cases when fishes are tested (Table 1, Suppl. materials), Daphnia spp. (17 papers), soil worms (10 papers) and freshwater microalgae/cyanobacterium (7 papers). Terrestrial/freshwater related studies investigated, for example, the transport of polystyrene nanoplastics in natural soils, their formation in agricultural ecosystems, the inhibition of activated sludge by PS NPs, but also review and toxicological effects in freshwater dipteran or plants (Table 4, Suppl., materials). Maybe due to practical difficulties, trophic transfer of nanoplastics has been examined only by Chae et al. (2018) in a four-species freshwater food chain and by Cedervall et al. (2012) reproducing a food chain transport *Scenedesmus* sp.-*Daphnia magna*- *Danio rerio*.

Taking into account the results obtained in laboratory experiments on different organisms, the framework on nanoplastics toxicity seems to be alarming. However, it is proper to highlight how the laboratory context represents only an oversimplification of a phenomenon, in many ways, unknown and complex. In the following paragraphs we aim to pointing out some aspects which are retained crucial when an ecotoxicological study with nanoplastics is performed and which elements of nanoplastics toxicity could be deeper covered.

4.1. Concentration

Since the first record of Ter Halle et al. in Ter Halle et al., 2017 which reported the occurrence of nanoplastics in marine samples from the North Atlantic Sea, the detection and quantification of the nanofraction remains a challenge. To date, twenty papers proposed different methods for the analysis of submicrometric particles in the environment (Table 2, Suppl. materials). However, its concentration and distribution are still unknown and, accordingly, it is difficult to size the phenomenon spotting the time and space scales. For these reasons, the concentrations used in laboratory studies are based on evidence on microplastics concentrations. It remains to clarify whether they are overlapping and if the relative polymers abundance is the same. Further, as reported above, many studies used rather high concentrations, sometimes up to g/L. However, the use of such high concentrations in dose-response experiments could be justified because it provides important insights on toxicity thresholds (Paul-Pont et al., 2018). Always in the context of laboratories studies, some possibilities have been recently proposed for the tracking of very low concentrations (< 15 μg/L) of nanoparticles in order to assess their bioaccumulation in aquatic organisms and/or to investigate their fate and behaviour in complex matrices. In the first case Al-Sid-Cheikh et al. (2018) used ^{14}C-radiolabelled nanopolystyrene for studying the uptake, distribution and depuration of 24 nm and 250 nm NPs in *Pecten maximus*. In the second case, a metal tracer made of palladium was added to a polyacrylonitrile (PAN) core ad spiked into sludge (Mitrano et al., 2019). Further studies are needed to better understand these aspects.

4.2. Reference materials

Most studies used polystyrene as reference materials, mainly because it is quite cheap and the only available in the market. Only 7 papers (out of 224) are based on polymers different by polystyrene. In detail: regarding polyethylene nanoplastic Paço et al. (2017) focused the attention on the ability of marine fungus *Zalerion maritimum* to biodegrade PE microplastics; Baudrimont et al., 2020 explored the ecotoxicity of PE nanoplastic on different marine organisms; Panizon et al. (2015) developed coarse-integrated models of polyethylene and polypropylene aimed at the study of the interaction with lipid membrane. Regarding on PMMA nanoparticles, Booth et al. (2016); Brandts et al. (2018a, 2018b) and Venâncio et al. (2019) tested the toxicity on different organism (algae, rotifer and fish). Finally, Magrì et al. (2018) explored the possible toxic effects of 100 nm PET NPs by in vitro studies on human Caco-2 intestinal epithelial cells.

If polystyrene can be considered the dominant polymer, the same applies to the bead shape. Gigault et al. (2018) state that secondary nanoplastics completely differ from the manufactured polystyrene nanomaterials used as reference material in eco-toxicological researches. Nanomaterials are characterized by a homogeneity of size (< 100 nm), type of polymer, physical/chemical surface properties and often are sold in dispersion spiked with surfactants (e.g. Sodium dodecyl sulfate, SDS) and antimicrobials that affect their water behaviour. On the opposite, secondary nanoplastics are supposed to be very motley as the result of the degradation process (Potthoff et al., 2017). Nanoplastics, similar to microplastics, in natural soils and water are supposed to undergo various transformation as result of interaction with inorganic

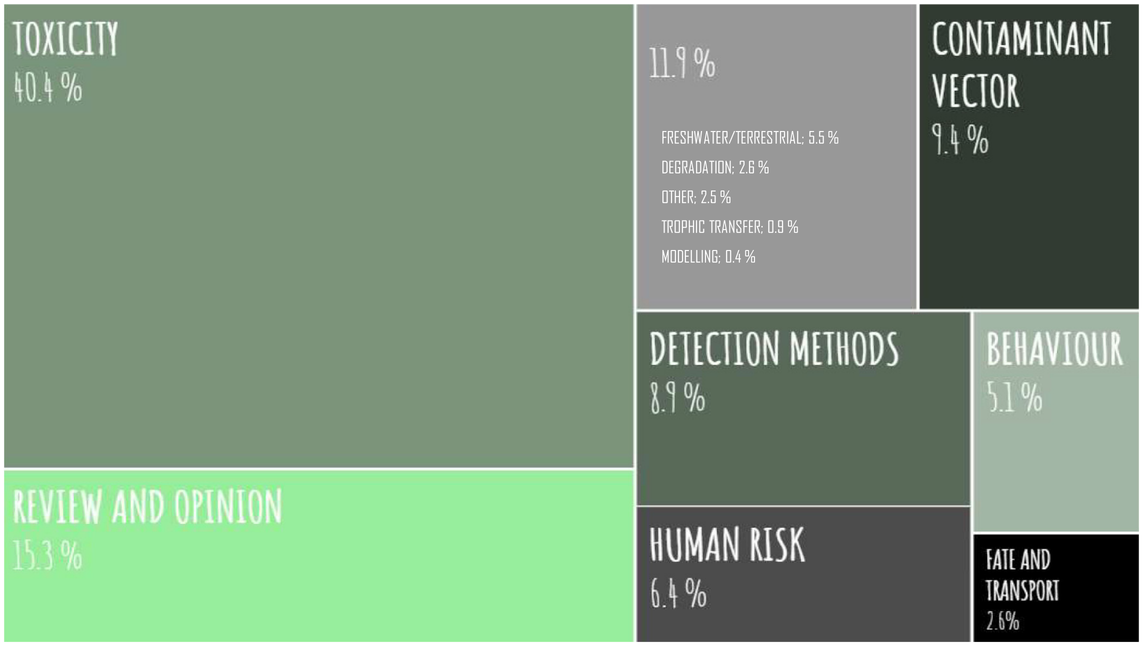

Fig. 3. Paper published on "nanoplastics" divided for main categories. "Other" includes studies on nanomaterials, protein interaction and leaching of fluorescent dye.

and organic factors present in natural water (e.g. differences in density, surface properties, charge, particles size distribution, particle shape distribution) (Potthoff et al., 2017). This heterogeneity plays a crucial role within nanoparticles behaviour and can affect the fate once in the environment.

Attempts on PS- alternative nanoparticles production only recently begin to appear. Magrì et al. (2018) applied a top-down approach which allows obtaining PET nano-sized particles starting from bulk scale materials. In detail, laser ablation of commercial PET film was performed and allowed to mimic real environmental nanoplastics (e.g. size and shape heterogeneity, weak acid group). Nanoparticles of polymethylmethacrylate (PMMA) prepared by microemulsion polymerization of styrene with sodium dodecyl sulfonate have been also tested on *Mytilus galloprovincialis* and *Dicentrarchus labrax* (Brandts et al., 2018b; Brandts et al., 2018a). Baudrimont et al., 2020 produced PE nanoparticles through the ultra-sonication of a solution consisting of PE reference powder and Milli-Q water for 1 h with pulses of 5 µs in an ice bath. After sonication, the solution was filtered at 0.45 µM and then evaluated with total organic carbon measurements and trace amounts of titanium by ICP-MS.

The development of new techniques for the nanoplastic production is needed to expand the knowledge on other polymers (e.g. PE, PP) and shapes (e.g. fragments) in order to make experiments more realistic. Meanwhile, the use of PS beads can help to investigate some aspects believed to be possible drivers in nanoparticle behaviour in water matrices. Anyway, a nanoplastics primary (focused on the particle itself) and secondary (on its interactions once in the medium) characterization before the ecotoxicological experiment is strongly required (Lambert et al., 2017; Fabrega et al., 2011; Bergami et al., 2016). Electron microscopy, Dynamic Light Scattering (DLS) or Nanoparticle Tracking Analysis (NTA) are essential tools for achieving these objectives (Ter Halle et al., 2017).

4.3. Interactions with surrounding medium, ageing effect and vector of contaminants

Due to their small size, nanoparticles have a larger surface area relative to mass, and this confers them an enhanced reactivity (Baun et al., 2008). In aquatic environment nanoplastics can form hetero-aggregates (e.g. with suspended particulate matter) or homo-aggregates with particles of the same type (Alimi et al., 2017). Aggregation, for example driven by surface charge, can convert the nanosized particles to micro-sized (Manfra et al., 2017). Nanoplastics may also interact with dissolved organic and inorganic colloids to form stable and unstable aggregates (Gigault et al., 2018; Cai et al., 2018). The marine environment has a wide variety of colloids and natural organic matter (Klaine and Alvarez, 2008). Summers et al. (2018), after an incubation study, revealed the importance of marine bacterial glycoprotein (EPS) on the formation and abundance of plastic agglomerates. Aggregation determines the fate, persistence, mobility and bioavailability of NPs (Alimi et al., 2017). How the bioavailability can change has been investigated by Ward and Kach (2009) who studied the ingestion and egestion dynamics in two bivalve molluscs (namely, *Mytilus edulis* and *Crassostrea virginica*) after an exposition to 100-nm PS nanoplastics. The plastics were delivered either dispersed or embedded within aggregates generated in the laboratory. Results show that aggregates significantly enhanced the uptake of nanoparticles. In this case, incorporation of NPs into aggregated material increased the bioavailability to suspended-feeding molluscs.

Little interest has been placed on the ageing effect to the binding of nanoplastics with contaminants. For example, Liu et al., 2019c demonstrated how the surface oxidation of PS NPs induced by UV/ozone ageing drastically affected the mobility and contaminant-mobilizing ability. Further, nanoplastics demonstrated the ability to bind different types of contaminants from naphthalene, fullerene, PCBs, PAHs, metals, POPs, glyphosate, perfluorooctane sulfonate to silver (Hu et al., 2020; Velzeboer et al., 2014; Liu et al., 2016; Town et al., 2018; Zhang et al., 2018; Jeong et al., 2018; Wan et al., 2019; Lin et al., 2019b; Ma et al., 2020; Chen et al., 2020; Lin et al., 2011). Finally, one of a kind, the possible superimposed effects of NPs and an antibiotic (i.e. tetracycline) on the microbiome of *Enchytraeus crypticus.*

have been investigated by Ma et al. (2020). In particular, the exposure significantly perturbed the abundance of some bacterial families and the microbiome was reversibly impacted.

In the light of above, it is recommended to recreate a more realistic exposure medium in order to not oversimplify the possible interactions and conduct always a secondary characterization (e.g. by DLS and/or electronic microscope).

5. Conclusions

The greater attention on nanoplastic pollution is a physiological convergence between the growing awareness on microplastic pollution and the open debate on nanomaterials toxicity. To anyone considering entering this field to embrace a blended approach between chemistry, engineering, ecotoxicology, and physics is required. This review aimed to critically analyse the body of literature in order to point out lights and shades of the phenomenon. The first reason of reflection regards the definition of nanoplastics. Gigault et al. (2018) prefer to consider nanoplastics as: "particles within a size ranging from 1 to 1000 nm resulting from the degradation of industrial plastic objects and can exhibit a colloidal behaviour". Others are more stringent to the definition taken from the nanomaterials. The latter definition leaves a link with the nanomaterials and allows the use of knowledges of decades and decades of studies in these field, but the former is maybe more consistent with the natural phenomenon. The second huge gap is represented by the quantification of the phenomenon. The occurrence of particles lower than 1 μm in marine samples suggests how 60–70 years from the boom in plastic production were sufficient to transform bigger items in colloidal (< 1 μm) particles. It still remains unclear how many nanoplastics are in the ocean and what are the rates of degradation. Koelmans et al. (2015) calculate the time scales required to reach the 100 nm scale as a function of initial plastic size. They estimated ca. 320 years needed to bring 1 mm microplastics to the 100 nm nanoscale, by joint photo-oxidation and biodegradation. In the ocean this estimation could be greater. The dilution effect, the technological limitations and the complexity of biological samples make the extraction from marine samples difficult. First attempts have been done and many others are needed.

Taking into account the results obtained in laboratory experiments on different organisms, the framework on nanoplastics toxicity seems to be alarming. Reduction in the relative abundance of gut microbiome, reduced growth and fecundity, longer reproduction time and differences in antioxidant enzymes in rotifers, inducing of *cas8* gene in *Paracentrotus lividus*, effects on survival and development in copepods, algal growth inhibition; behavioural disorders, abnormalities in liver histopathology, degranulation increase, inhibition of acetylcholinesterase and genotoxic effects in fishes are all aspects already highlighted. However, how benthic or *endo*-benthic organisms (such as marine worms) could be affected by this new form of contamination remain poorly investigated. Results obtained are all related to PS or PMMA nanoplastics an aspect which pose a debate regarding how they are a good reference material and how many important are the system conditions on bioavailability of nanoplastics and their consequent toxicity.

Nanoplastics formation, fate and toxicity seem to be a very dynamic phenomenon. It is not possible to reproduce and control all the variables in a laboratory context but even an oversimplification is counterproductive. It is therefore advisable to recreate a more realistic exposure medium in order to not oversimplify the possible interactions and conduct always a secondary characterization.

Our paper proposes a recent update in a rapidly evolving area of scientific knowledge. Linking laboratory results to real effects, estimating natural levels is essential. To date the problem of the impact of nanoplastics in the marine environment has been assessed on the basis of laboratory exposure studies. The question relating levels of nanoplastics in the natural aquatic environment must be explored with techniques that allow reliable and reproducible real levels to avoid to under- or overestimate the problem. The currently available methodological approaches need to be improved to allow for accurate and reliable determinations of nanoparticles in abiotic and biotic matrices. Nanoplastic pollution hides lights and shadows and it is difficult to take a position at the moment about the risk they pose to the biodiversity and functioning of natural ecosystems. A precautionary principle is appropriate, but this must be accompanied by solid and clear scientific evidence. At this moment they are missing, and it is not even clear how they can be obtained in a short time.

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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