

Review

# Micro and Nano Plastics Distribution in Fish as Model Organisms: Histopathology, Blood Response and Bioaccumulation in Different Organs

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**Featured Application:** Current evidence indicates that micro- and nano-plastics can be absorbed by aquatic organisms as well as mammals. The topic focuses on the important role of the biodistribution of micro- and nano-plastics (MP/NPs) and the role of blood biomarkers in the teleosts used as model organisms.



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**Abstract:** Micro- and nano-plastic (MP/NP) pollution represents a threat not only to marine organisms and ecosystems, but also a danger for humans. The effects of these small particles resulting from the fragmentation of waste of various types have been well documented in mammals, although the consequences of acute and chronic exposure are not fully known yet. In this review, we summarize the recent results related to effects of MPs/NPs in different species of fish, both saltwater and freshwater, including zebrafish, used as model organisms for the evaluation of human health risk posed by MNPs. The expectation is that discoveries made in the model will provide insight regarding the risks of plastic particle toxicity to human health, with a focus on the effect of long-term exposure at different levels of biological complexity in various tissues and organs, including the brain. The current scientific evidence shows that plastic particle toxicity depends not only on factors such as particle size, concentration, exposure time, shape, and polymer type, but also on co-factors, which make the issue extremely complex. We describe and discuss the possible entry pathways of these particles into the fish body, as well as their uptake mechanisms and bioaccumulation in different organs and the role of blood response (hematochemical and hematological parameters) as biomarkers of micro- and nano-plastic water pollution.

**Keywords:** micro-nano plastics; fish; organism model; histopathology; blood biomarkers



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## 1. Introduction

Micro-nano plastic pollution is a global problem that has generated great interest among scientists and attracted public attention because of the potential health risks associated with exposure to MP/NPs through air [1], water [2,3], and food [4,5]. In the past years, we have seen an increase in the number of scientific works regarding the topic with the declared purpose of understanding the mechanisms underlying pathological manifestations in various organs, through the study of toxicity on cells and tissues to extrapolate risks to mammals [6,7]. This is a very complex topic due to the difficulty involved in analyzing the effects of various types of plastic. In fact, plastic particles discharged into the environment are often contaminated with different chemical pollutants [8,9] and

other contaminants [10–13], including pathogens [14]. In this regard, in fact, some authors argue that microplastics can act as a vector to convey pathogens from the environment to organisms [15,16]. Therefore, adverse effects of microplastics and nano plastics may result from a combination of intrinsic toxicity of plastics and ability to absorb, concentrate, and release environmental pollutants into living organisms [17]. In addition, it has become well documented that the different particles sizes exert effects through different physiological pathways, in relation to quantity and quality in different species at different developmental stage and in different tissues [18]. Micro plastics are small synthetic solid particles mostly composed of blends of polymers and functional additives with different chemical compositions, shapes, colors, sizes, and densities. The European Food Safety Authority (EFSA) [19] defined “micro plastics” all plastic particles having a diameter < 5 mm. MPs including nano plastics (NPs), which are particles with dimensions below 0.1  $\mu\text{m}$  (1–100 nm) [20]. They are classified as primary and secondary based on their origin. Primary micro plastics are any plastic particles that are directly released into the environment. These include microfibers, fragments, microbeads, and plastic pellets. Secondary micro plastics, on the contrary, originate from the degradation of larger-sized plastic products once exposed to the marine environment with regular or irregular shape [21]. They are derived from abrasion, mechanical wear as a wave action, photo oxidation and biological degradation of small pieces of plastic [22,23]. MP/NPs, once released into the environment do not degrade and therefore accumulate in aquatic organisms entering the food chain until they are ingested by humans through food. MPs/NPs acute toxicity are correlated with plastic size [24], concentration [25], and cellular/tissue uptake and accumulation [26]. In general, small size, high dose, and the presence of toxic additives or pollutants in the micro/nano plastics appear to induce cellular toxicity [27]. In fact, small sizes of the microplastics enhance their translocation across the biological membranes with various effects on aquatic organisms via endocytosis-like mechanisms resulting in internalization into cells and tissues with potential adverse consequences on health [20]. In contrast, particles larger than 100  $\mu\text{m}$  do not have any effect [28,29]. In terrestrial vertebrates, the main entry route of microplastics into the body, is the digestive pathway through food and water with pathological changes consisting of gut barrier dysfunction, intestinal inflammation, and gut micro biota dysbiosis [30–33]. In fishes, the absorption routes are various, beyond the digestive way, absorption occurs through the gills and the skin. Therefore, scientists started to document the effects of microplastics on fish [34] and the signs of toxicity observed in these animals are, chain can be illustrated [35]. More specifically, the purpose of this review is not only to investigate the uptake routes and accumulation of MPs in fish, including zebrafish, but also to reveal the toxic effects of MPs in different organs (gut, liver, gills, and kidney), including the brain, with implications on growth and food consumption. In addition, we focused our attention on the central nervous system to study the neurotoxicity effects due to the crossing of the blood-brain barrier as well as on the blood response to MP/NPs. Microplastics cause anemia and alterations in hemato-biochemical parameters. Therefore, representing important blood biomarkers of water pollution by micro- and nano-plastics. Blood tests represent useful experimental tools to monitor the response of fish to the aquatic load of micro and nanoplastics. We have reviewed 219 publications until 2021. Below, we report the data collected for each organ, focusing on structural and molecular effects caused by the exposure to micro and nano plastics.

## **2. Micro- and Nano-Plastics in Aquatic Ecosystems Can Be Taken-Up by Fish and Reside in Their Brain**

Since microplastics (MPs) and nanoplastics (NPs) ingestion by aquatic organisms is reported by laboratory and field studies with a regard to commercial species too [36–38], an unavoidable concern is whether MPs and NPs, one ingested by fish, can be absorbed by the gastrointestinal (GI) tract and can pass into other body districts. However, this field of knowledge just started to be investigated recently. A proof of the MPs and NPs movement from intestine to other body compartments is represented by the evidence that, once these plastics are ingested, they can be found in different organs, outside the GI tract [39]. Once

the gastrointestinal barrier is bypassed, also the brain may be at risk of exposure when micro and nanoplastics are able to pass the blood brain barrier (BBB). According to Ding's study on *Oreochromis niloticus* [40], MPs reach the fish brain along with blood circulation. This agrees with Sökmen [41] and his team, hypothesizing that since nanoplastics can pass into red blood cells, as shown by Geiser's in vitro study [42], they can reach the brain through the blood. Indeed, nanoplastic particles have been identified in the brain of fish after waterborne [43] or food-mediated exposure [35], indicating that they are capable of crossing a highly selective permeability barrier as the blood brain one [44]. Mattsson et al. [35] demonstrated that, once ingested, 53 and 180 nm sized polystyrene plastics can arrive into the brain of Crucian carp (*Carassius carassius*). Ding et al. [45] demonstrated that 0.3, 5, and even 70–90 µm polystyrene MPs could be accumulated in the brain, indicating their tissue translocations within red tilapia (*O. niloticus*). In their reviews, Barboza [46] and Campanale [47] agree that about 10 µm or smaller microplastics might penetrate organs and cross the blood-brain barrier reaching the brain (Table 1). Furthermore, micro and nanoplastics could reach the brain before the BBB is established and this could result in an uptake of NPs by the brain at early stages of fish life. Indeed, Sökmen et al. [41] demonstrated that 20 nm diameter polystyrene nanoplastics (PS-NPs) injected to yolk sac can reach the brain and bioaccumulate there. Even when zebrafish (*Danio rerio*) embryos are just exposed to NPs, these ones can accumulate in the yolk sac and migrate to brain [48]. However, Sökmen [41] and his team also hypothesized that the presence of PNPs in the brain may be due to the alteration of the hemodynamic physiology induced by these plastics. These and other pathways traveled by MPs and NPs to the brain add up: NPs can reach the head of young zebrafish that have just undergone an in-water exposure [49] and MPs can cross the gills, reach the fish head, and accumulate there [50]. Therefore, it is necessary to investigate the effects micro and nanoplastics have on the brain.

**Table 1.** Micro and nanoplastics crossing the blood brain barrier in fish.

Specimens	Micro and Nano-Plastics Type	Micro and Nanoplastics Size	References
Data not referred to a particular species	Data not referred to a particular micro and nano-plastics type	<10 µm	[46,47]
Red tilapia ( <i>Oreochromis niloticus</i> )	Polystyrene	0.1 µm	[40]
Red tilapia ( <i>Oreochromis niloticus</i> )	Polystyrene	0.3; 5 µm 70–90 µm	[45]
See-through medaka ( <i>Oryzias latipes</i> )	Polystyrene	39.4 nm	[43]
Crucian carp ( <i>Carassius carassius</i> )	Polystyrene	53 and 180 nm	[35]

### 3. Neurotoxicity of Micro and Nano-Plastics in Fish

Nanoplastics are small hydrophobic particles that can reach the brain by crossing the blood-brain barrier [35,43,51]. Nanoplastic particles have been identified in the brain of fish after waterborne or food-mediated exposure, indicating that they are capable of crossing the blood-brain barrier [44]. Then, in aquatic models, the brain is essential for evaluating the toxic effect of nanoparticles (NPs) [52,53]. In fish at different stages of development, NPs accumulate in the tissues, resulting in multiple negative effects including the nervous system [54]. Ding et al. [45] suggest that the effects of NPs and MPs on the nervous system depend not only on the size and time of exposure but also on indirect mechanisms such as oxidative stress. In this regard, Barboza et al. [51] observed higher levels of LPO and greater AChE activity in the brains of *Dicentrarchus labrax*, *Trachurus*, and *Scomber colias* exposed to plastics than in unexposed specimens of the same species, probably because

higher concentrations of LPO induce the break of acetylcholine-containing vesicles resulting in increased neurotransmitter released in synaptic clefts and increased production of acetylcholine as a compensation mechanism [51]. Wan et al. [55] showed that neurotoxicity following polystyrene microplastics (PS-MPs) exposure in zebrafish larvae could be associated with some metabolic changes. Instead, Ding et al. [45] show, in adult specimens of tilapia, a downregulation of the amino acids phenylalanine and tyrosine, associated with neurological functions and consequently the polystyrene nanoplastics (PS-NPs) PS-MPs exposure. According to Ding et al. [45], amino acids associated with neurological functions could interact with nanoplastics and microplastics resulting in alteration of the metabolic pathways of these amino acids and alterations in the formation of various neurotransmitters. Furthermore, it has been observed in tilapia that PS between one and 100 micrometers in size can induce more severe stress than 0.3 micrometers particles and this stress seems to be mainly due to oxidative stress and damage caused by LPO, more severe in MPs than NPs exposure. This could be explained by the fact that medium-sized MPs can move freely between cells and induce mechanical injury and alterations in biochemical pathways such as oxidative damage and inflammatory response [45]. Furthermore, NPPs can favor the accumulation of reactive oxygen species (ROS) and cause negative effects at the cellular level [43,56,57]. In adult specimens of medaka fish (*Oryzias latipes*) polystyrene nanoplastics in both organs and blood have been found. Furthermore, after exposure for seven days at 10 mg/L, polystyrene nanoplastics crossed the blood brain barrier in this species and get to the brain [43]. The central nervous system is particularly vulnerable to oxidative stress, in fact it determines developmental and motility alterations and neurotoxicity [58,59]. The results of Chen et al. [60,61], who evaluated specific biomarkers of oxidative stress, the activity of catalase (CAT), glutathione, peroxidase activity (GPx), and the reduced form of glutathione (GSH), confirm the role of oxidative stress in neurotoxicity [60,61]. Behavioral anomalies in fish exposed to pollution by micro and nano plastics have been observed [62]. It has been observed that zebrafish exposed to plastic particles show uncontrolled movements probably due to an improper activation of neurons, due to an altered expression of important neurotransmitters such as acetylcholine, glutamate, and  $\gamma$ -aminobutyric acid [63–67]. Such alterations can be considered indicators of neurotoxicity [68,69]. In fish, exposure to microplastics triggers the activation of cellular oxidative stress processes with consequent peroxidation of cell membranes [46,70]. The damage from lipid peroxidation in the brain can cause the break of the vesicles membranes containing neurotransmitters with consequent increase of the neurotransmitter concentration in the synaptic junctions [71,72]. The neurotoxic effects of microplastics were confirmed by measuring the increase in activity of the enzyme acetylcholinesterase [46,73]. These results are severe because the activity of cholinesterase (ChE) enzymes is essential for cholinergic neurotransmission in neuromuscular junctions and in cholinergic brain synapses [51,74]. Acetylcholinesterase (AChE) activity regulates brain function and is considered an important biomarker for neurotoxicity, also in zebrafish [75,76]. A decrease in AchE activity [61,77], as well as the inhibition of neurotransmitters dopamine, melatonin, aminobutyric acid, serotonin, vasopressin, kisspeptin, and oxytocin [77], in zebrafish exposed to NPs was found [78]. Inhibition of acetylcholinesterase (AChE) enzymatic activities also in Medaka, *O. latipes*, *Pomatoschistus microps* exposed to micro and nano plastics has been reported [10,54,79]. It has been also observed that, in zebrafish larvae, the inhibition of AChE activity interferes with the functioning of the nervous system, causing growth retardation, paralysis, and death [75,76]. Since EE2 (17 $\alpha$ -ethinylestradiol) modulates the development of the neuroendocrine system, it was considered a positive control in the evaluation of neurotoxicity [60,80,81]. In groups of larvae exposed to MPP or coexposed with MPP and EE2, the activity of AChE decreased [60]. Moreover, polyethylene microplastics (1–5  $\mu$ m) inhibited AChE activity in *P. microps* [60,73]. The small particles can inhibit AChE activity causing neurotoxicity with adverse effects in the cholinergic system. Therefore, the suppression of locomotor capacity in zebrafish exposed to nanoparticles and nanoplastics can be explained by the inhibition of acetylcholinesterase activity [60]. Downregulation of genes associated

with neuronal functioning (glutamate receptors, potassium channels, synapsin), genes implicated in neuron differentiation and axon genesis (*neurod4*, neuronal differentiation 4, Gene ID: 266958; *lrnm2*, leucine rich repeat neuronal 2, Gene ID: 558051) support the neurotoxic effects of plastics in zebrafish [82] and plastics seem to influence the visual system by upregulating the gene expression of *zfrho* (rhodopsin, Gene ID: 30295) [60,78]. In zebrafish, *gfap* (glial fibrillary acidic protein, Gene ID: 30646) and  $\alpha 1$ -*tubulin* (alpha-1 tubulin, Gene ID: 842782) genes are expressed in the central nervous system during the early stages of development and are considered important biomarkers of neurotoxicity [83].  $\alpha 1$ -*tubulin* is essential in the formation of microtubules, while *gfap* intervenes in the development of astrocytes [84,85]. In zebrafish, the *gfap* gene and the corresponding protein are highly conserved and exhibit functions similar to those of mammals [86]. Moreover, the up-regulation of *gfap* is an indicator of neurotoxicity, also observed in mammals [86,87]. The use of *gfap*'s upregulation in neurotoxicity screenings is now accepted, and its use by the US Environmental Protection Agency (EPA) has been recommended [88]. Instead, the up-regulation of *gfap* and  $\alpha 1$ -*tubulin* in the central nervous system promotes an increase in the expression of *zfrho* and *zblue* and this alteration may be a side effect of neurotoxicity [87]. Zebrafish larvae exposed to nanoplastics showed more severe hypoactivity phenomena when co-exposed to NPPs E EE2 due to oxidative damage [60]. In treatments with MPP and MPP + EE2, the up-regulation of sight-related genes such as opsin which is expelled by photoreceptor cells of the retina [89] has been observed [60]. Upregulation of the opsin gene cannot indicate alterations in movement [90].

In *Clarias gariepinus* the effects of PVC microparticles, considering a battery of biomarkers of oxidative stress (superoxide dismutase, catalase, glutathione peroxidase), neurotoxicity (acetylcholinesterase) and lipid peroxidation, have been evaluated [53]. It has been shown that in the presence of PVC the activity of GPx, Sod, Cat, and AchE is inhibited while the levels of LPO have increased, all progressively with time [53].

Specimens of red tilapia were exposed to polystyrene particles of three different sizes (0.3, 5 and 70–90  $\mu\text{m}$ ) to evaluate their accumulation, oxidative stress, p450 enzyme activity, metabolic changes, and neurotoxicity. It was shown that only the 5  $\mu\text{m}$  polystyrene microplastics significantly inhibited the activity of acetyl cholinesterase in the brain. In this species, in treatments with PS-N and MPs (0.3, 5 and 70–90  $\mu\text{m}$ ) a decrease in the activity of the enzyme acetylcholinesterase in the brain has been observed [45], confirming previous studies in the same species [40] and in *P. microps* [91], as well as in zebrafish larva [60]. It has been shown that plastic materials are capable of conveying pollutants to the body's tissues [60,92]. Although some authors give little importance to this vector function [93–96], it is known that the toxicity of plastics and their interaction with others pollutants can affect the bioavailability and toxicity of these pollutants for the biota [92]. Plastics have shown the ability to absorb various pollutants (IPA, PCB, HCH) [97]. Furthermore, all additives added to plastics must be taken into consideration: plasticizers, flame retardants and antimicrobials whose toxicity can be altered by co-exposure with the transported toxicants [98,99]. In epoxy resins and polycarbonate plastics, the most used additive is bisphenol A (BPA). This additive can be released from plastic materials into the aquatic environment and can reach a concentration of 4  $\mu\text{g/L}$  [98,100,101]. While for years it has been considered a cause of obesity and disorders of the immune and endocrine systems [102,103], Sali et al. [104] highlighted the negative effects of BPA on the brain. The accumulation of BPA and NPPs in various zebrafish organs including the brain has been demonstrated [43,105]. Chen et al. [106] evaluated the different expression of genes and proteins as biomarkers of neurotoxicity during the development of the zebrafish central nervous system. Chen et al. [61] demonstrated that the co-exposure of NPPs and BPA not only leads to increased accumulation of BPA in zebrafish organs, but is responsible for neurotoxic effects on the central nervous and dopaminergic systems. Indeed, during co-exposure to NPPs and BPA, not only the upregulation of myelin and tubulin basic protein genes in the central nervous system and increased dopamine, but also upregulation of astrocyte-derived neurotrophic factor (MANF) expression in the midbrain and significant inhibition of acetyl

cholinesterase enzyme activity [61] were evident. The NPs have shown the ability to absorb organic pollutants (IPA, PCB, DDT, PBDE, PFOA) and heavy metals (Ni, Cu, Zn, Pb) and act as a vector for them, thus increasing their bioavailability [6,16,107,108]. They may also contain additives such as bisphenol A (BPA) or phthalates [109,110], whose negative effects on the biota are known [39,108,111]. In the work of Chen et al. [61], the effects of exposure of NNPs and co-exposure of NNPs and EE2 were evaluated. It has been observed that exposure to PS-NPs as well as the co-exposure of NNPs with EE2 inhibits the activity of the acetylcholinesterase enzyme (AChE) with consequent reduction of locomotor activity, and induces an increase in expression of the *gfap* and *alpha tubulin* genes related to the central nervous system [60]. In the work of Lee et al. [112], zebrafish embryos were exposed only to fluorescent NNPs and combined with Au ions, and an increase in deformity and mortality rate was observed as well as the activation of the inflammatory response (increase in the expression of IL6 and IL 1 $\beta$ ). Furthermore, ROS levels have increased resulting in mitochondrial damage [6,112]. Equally important are the negative effects of micro and nanoplastics on fish development. In particular, the negative effects of micro and nanoplastics during embryonic development and the protective function of the chorion in the early stages of development have been evaluated [113,114]. The protective effect of chorion against pollutants, in both de-chorionate embryos [60,115–117] and elsewhere [118], has been observed [119]. Furthermore, absorption, bioaccumulation and toxicity in zebrafish embryos and larvae have been described using 44 nm model nanoplastics [120].

#### 4. Occurrence of Micro and Nano-Plastics in Fish Gills and Consequent Effects

The occurrence of microplastics in fish gills is reported in different studies on different fish species. Studies reporting NPs and MPs detection in gills refer to animals naturally exposed to these plastics [121–124] and exposed to microplastics in laboratory condition [43,69,125,126]. Like fish collected in fish farm and mariculture areas, those exposed to MPs in laboratory conditions show a higher or, at most, a comparable accumulation of MPs in gills than in gut [127,128]. Therefore the MPs accumulation in gills could be influenced by microplastics shape [126], and the entity of accumulation in gills increases with increasing time of exposure [40] and is related to MPs size [129]. It is intriguing to notice that, once taken up, microplastics still exist in gill fishes after depuration for seven days [130]. However, microplastics elimination time seems to be a specie-specific parameter as shown on larvae by Zhang et al. [131]. The effects of microplastics on gills (Table 2) were tested with in vitro and in vivo studies.

**Table 2.** Effects of micro and nano plastics on gills.

Specimens	Micro- and Nano-Plastics Sizes and Type	Concentration	Exposition Time	Effects	References
Japanese medaka ( <i>Oryzias latipes</i> )	10–20 $\mu$ m sized PES and 50–60 $\mu$ m sized PP fiber	10,000 Microplastic fiber/L	21 days	- increased mucus, - denuding of epithelium on arches, - fusion of primary lamellae. - aneurysms in secondary lamellae - epithelial lifting, swellings of inner opercular membrane that altered morphology of rostral most gill lamellae	[132]
Goldfish ( <i>Carassius auratus</i> )	from 0.7 mm to 5.0 mm fiber, ranging between 2.5–3.0 mm and 4.9/5.0 mm, respectively fragments and pellets	0.03 g of fiber/ 15 commercial food pellets; 0.96%, 1.36%, 1.94% and 3.81% (g (food + MPs)/g ww fish).	Six weeks	- fragmentation of gills filaments	[126]

Table 2. Cont.

Specimens	Micro- and Nano-Plastics Sizes and Type	Concentration	Exposition Time	Effects	References
Goldfish ( <i>Carassius auratus</i> )	0.100–1000 µm sized PVC	0.1 or 0.5 mg/L	4 days	- undamaged gills - limited or no effect to antioxidant activity - unaffected ion-regulation function	[133]
Goldfish larvae ( <i>Carassius auratus</i> )	70 nm and 5 µm sized PS	10, 100 and 1000 µg/L	1, 3 and 7 days	- disordered cell arrangement	[134]
European seabass ( <i>Dicentrarchus labrax</i> )	1–5 µm polymer microspheres (an average of 2 µm diameter)	0.26 and 0.69 mg/L	96 h	- increased superoxide dismutase (SOD) activity - induction of CAT, GST and SOD, increasing of LPO levels	[46]
Guppy ( <i>Poecilia reticulata</i> )	32–40 µm sized PS	100 and 1000 µg/L	28 days	weakened Na <sup>+</sup> /K <sup>+</sup> -ATPase activity in gills	[135]
Marine medaka, ( <i>Oryzias melastigma</i> )	10 µm sized PS	20 and 200 mg/L	60 day	- production of ROS - inhibition of SOD, CAT and GST activities - abscission in gill lamellas, loose arrangement of gill filaments, shortening and thickening of gill lamellas	[127]
rainbow trout ( <i>Oncorhynchus mykiss</i> ) gill epithelial cells	220 nm sized polystyrene	0.3 and 3 mM	48 h	- cell viability decrease	[136]
rainbow trout ( <i>Oncorhynchus mykiss</i> ) fingerlings and wildtype Zebrafish ( <i>Danio rerio</i> )	1 µm, 2 µm, 20 µm, 40 µm and 90 µm sized PS	2 × 10 <sup>5</sup> particles/L	2 h	- up or down regulation of some genes related to immune response - 0.2 µm MPs induced ifnγ gene up-regulation in trout - 1 µm MPs enhanced up-regulation of three immune genes in zebrafish	[31]
Zebrafish ( <i>Danio rerio</i> )	~70 µm (mean) sized PA, PE, PP, PVC	0.001–10.0 mg/L	10 days	no histological damage	[137]
Zebrafish ( <i>Danio rerio</i> )			20 days	- alterations of gill epithelium, adhesion and partial fusion of secondary lamellae and mucous hypersecretion, a higher density of neutrophils	[138]
Common carp larvae ( <i>Cyprinus carpio</i> )	90 µm; 50 µm and 25 µm sized HD-PE and PS	100 and 1000 µg/L			
Black rockfish ( <i>Sebastes schlegelii</i> )	0.5 µm and 15 µm polystyrene	190 µg/L	14 days	hypoxia respiratory in gill	[139]
European seabass ( <i>Dicentrarchus labrax</i> )	3 µm; 3–1.2 µm and 1.2–0.45 µm sized EMPs	0.33 mg/g of feed (corresponding to 5 mg of EMPs/150 g of feed)	3 and 5 days	increase in GST and SOD activities	[129]

Table abbreviations: polystyrene (PS), polyester (PES), polypropylene (PP), polyamides (PA), polyethylene (PE), high density polyethylene (HD-PE), polypropylene (PP), polyvinyl chloride (PVC), environmental microplastics (EMPs).

Bussolaro et al. [136] found that polystyrenes NPs decreased the viability of rainbow trout gill epithelial cells in a concentration-dependent manner. On the other hand, Hu et al. [132] conducted an in vivo study by exposing adult Japanese medaka (*O. latipes*) to polyester (PES) and polypropylene (PP) MPs fibers. By means of scanning electron microscopy (SEM), observations of gills increased mucus, denuding of epithelium on arches, and fusion of primary lamellae were observed. Histologic sections revealed: aneurysms in

secondary lamellae, epithelial lifting, swellings of the inner opercular membrane that altered morphology of rostral gill lamellae. SEM and histochemical analyses showed increased mucous cells. Similar results were obtained by Jabeen et al. [126]. They observed that microplastics fibers, retained in gills, cause the filaments fragmentation. According to the authors, this is probably due to the direct contact, and it is correlated to plastic particle size and shape. On zebrafish gills, MPs effects are similar to those induced on Japanese medaka, but Limonta and his team [138] revealed also a higher density of neutrophils in gill tissue of MPs-exposed fish. Microplastics effects on gills also depend on their exposure concentration. Barboza et al. [46] found that lipid oxidative damage in gills occurs after exposure to the higher MPs concentration they tested, but not to the lower. An oxidative stress status induced by microplastics was also detected on larvae gills after dietary exposures [140]. According to Zitouni et al. [129], oxidative alterations were highly correlated with MPs size range. Moreover, the authors emphasized that the toxicity of smaller MPs was closely related to other different factors, including the target tissue, exposure duration, and MPs chemical properties. Oxidative stress induced by MPs leads to consequent gill structural damage and histological changes [127]: abscission in gill lamellae, loose arrangement of gill filaments, shortening and thickening of gill lamellae. As noticed for oxidative stress, also gene expression could be affected by MPs and NPs exposure in different ways based on the plastic sizes. Lu et al. [31] found the smallest MPs particles, used in their study, induced *ifn $\gamma$*  gene up-regulation in *Oncorhynchus mykiss*, *S100a* gene up-regulation in zebrafish gills and up-regulation of three immune genes in zebrafish gills (*ifn $\gamma$* , *il1 $\beta$* , and *igm*). So, the scientists speculated that immune gene expression starts in gill epithelia and associated cells after small microplastics are taken up by them. For Huang et al. [135], another effect of PSMPs exposure was a weakened  $\text{Na}^+/\text{K}^+$ -ATPase activity in gills that could affect the osmotic balance of these organs. Besides the above-mentioned damages on the gills, a changed oxygen consumption that is an indicator of respiration stress, can occur. Therefore, bigger microplastics have a greater impact than smaller ones, as Yin et al. [139] demonstrated. On the other hand, Yang et al. [134] argue that nanoplastics are more dangerous for gills than microplastics. According to these authors, NPs effects on histological damage have an entity that is proportional to the exposure concentration. Interestingly, the authors found that, by detecting gill at both microscopic and ultra-micro levels, no enrichment of MPs or NPs was found within their tissues. Despite this, the gill tissues of larvae showed damage phenomenon. Differently from what reported above, some authors found no damage or alteration on gills after MPs and NPs exposure [133,137]. The inconsistencies in previous studies may be due to the different experimental concentrations employed (Table 2). In addition to MPs and NPs effects on gills, there are other issues. One of the concerns about MPs and NPs is represented by the evidence that, once absorbed by the gill, they can be internalized and spread in fish body [43,141]. Another growing concern is represented by the effects of the co-exposure to microplastics with other contaminants. This is due to the evidence that MPs and NPs, not only can increase the bioavailability and uptake of contaminants but can also enhance their toxic effect on gills [46,61,142–144]. For example, Karami and his team [145], demonstrated that low-density polyethylene (LDPE) fragments can cause toxicity and modulate the adverse impacts of phenanthrene (Phe), by influencing among other parameters, the degree of tissue changes (DTC) in the gills. In the gill tissues, histological alterations were observed, e.g., some basal cell hyperplasia, sloughing, necrosis in the connective tissue. By increasing the concentration of MPs, the following phenomena were observed: epithelial lifting, hyperplasia, necrosis of the connective tissue, extensive cell sloughing, desquamation and consequently blood vessel exposure and, in some instances, total loss of the secondary lamellae. This corroborates the concentration-dependent impact of MPs that other scientists [46,136] encountered too. Besides the oxidative stress induction, microplastics associated with other substances can enhance the expression of immune-related genes in gills as shown by Xu et al. [144] with phenanthrene on zebrafish.



## 5. Toxicity of Microplastics and Nanoplastics in Digestive Tract: Intestinal Retention Time, Uptake and Elimination

The food-borne route is the major contamination by MP/NPs pathway, with the gut being the target organ [146]. Plastic fragments, in the form of microfibers or microbeads, may accumulate in digestive tract of fish via food and water [147,148]. The bioavailability of MP/NPs and consequently the effects at different levels depend mainly on their size, shape, concentration, and the presence of chemical pollutants [18,149]. The contaminants, species, developmental stage are considered as co-factors for plastic particle toxicity (PPT) [18]. As evidenced by numerous studies carried out under experimental conditions, the ingestion of microplastics can generate two different types of impacts on marine organisms: physical injuries to the organs where accumulation occurs and chemical damage by transfer and accumulation of pollutants with an enhancement of toxicant uptake or an increase in their bioavailability [17]. Conflicting results have been reported in literature regarding the effects of MPs and NPs accumulation in the gut. It has been demonstrated that the physical presence alone of MPs in the gut may be toxic due to their intrinsic ability to induce intestinal blockages or tissue abrasions, which may cause injury of the gut lining, morbidity, and mortality. Experiments carried out on *Dicentrarchus labrax* fed with microplastics for 90 days showed physical damage in the intestinal tract, both in specimens treated with contaminated plastics and in those treated with virgin microplastics [46]. Grigorakis et al. [146] reported that MPs might scratch gut tissues. While Jovanovic et al. [150] reported that MPs did not cause imminent harm in gilt-head seabream after 45 days of exposure, other studies reported abrasions, ulcers, false satiation, blockages in the digestive tract [151], dysbiosis in the gut [30,31], inflammation, bowel wall thinning, villi and epithelial damage, and oxidative stress in the gut tissues [152–154]. Histopathological changes in intestine were observed in goldfish (*Carassius auratus*) larvae by Yang et al. [134] using PS MP/NPs of size < 5 µm with negative repercussions on larval growth and elevated oxidative stress markers. A reduction in weight and body length was also observed in larvae of *Cyprinus carpio* treated with PVC MPs [140]. MPs were found in gastrointestinal tract of wild fishes also, with higher lipid peroxidation levels in the different organs, including the gut [46]. Oxidative stress and structural damages by PS nanoparticles (10 µm at 2–200 µg/L) accumulation in intestine were observed in adult *Oryzias melastigma* [127] as well as in *O. latipes* treated with PS MPs, 10 µm. In this species, histopathological alterations with swollen enterocytes were observed [122]. In the literature there are also different opinions about the crossing of the intestinal wall by micro-nano plastics. Some authors claim that MPs in fish remain in the gastrointestinal tract and expelled with the feces [146,155]. Other authors found instead MPNs in fish filets assuming rather that smallest plastic particles (<0.1 µm) can transfer from the intestinal tract to the circulatory system and thus spread to the muscle as to all other organs and tissues [145]. In this regard, severe toxicity usually arise from smaller plastic particles with potential impacts on living organisms due to crossing biological barriers such as cell walls or intestinal barrier [145]. MPs intake during 90 days to *Sparus aurata* induced an increase in oxidative damage and a pro-inflammatory response in gut of *S. aurata*, and were able to recover after the exposure to MPs was removed [156]. Ohkubo et al. [157] have estimated the uptake and gut retention of microplastics in juvenile stage of *Fundulus heteroclitus* and red seabream, *Pagrus major* [157]. Various factors affect MPs bioavailability and level of risks at cellular and molecular level on aquatic organisms. Biomarkers for antioxidant response (superoxide dismutase, catalase, glutathione peroxidase, reductase, and glutathione S-transferase), neurotoxic impairment (acetylcholinesterase), lysosomal activity alteration, and genotoxicity have been analyzed by Suman et al. [158]. Virgin microplastics are not causing imminent harm to fish after dietary exposure in *S. aurata* [150] and *O. mykiss* [28], and did not present evident tissue damage in *Barbodes gonionotus* exposed to PVC fragments (0.2, 0.5 and 1.0 mg/L) for 96 h [159]. Little is known about their uptake, translocation, and accumulation within fish organs [23]. MP/NPs showed that oxidative stress and its responding pathways, including inflammatory responses, could

play the role of key events [132]. Several different types of effects have been observed in both freshwater [40,82,125,160] and saltwater species [127] in the larval and adult stage. Polystyrene nanoparticles (PS NPs) were found accumulated in larvae or adult gut of Medaka (*O. melastigma*) with increased mortality and decrease in average lengths and weights [161]. Because of their microscopic size, micro- and nanoparticles could find in phagocytosis or endocytosis the preferred route of absorption. The occurrence of microplastics (<5 mm in diameter) and mesoplastics (5–20 mm in diameter) in the gastrointestinal tract of some commercially pelagic, demersal and reef fishes collected from Southeast coast of the Bay of Bengal was observed by Karuppasamy et al. [162]. According to the custom of the Indian population to consume dry fish, the presence of microplastics in the fish gut is, in this case, a potential serious human health concern, as they are directly consumed. The transfer of microplastics of various types and sizes, through the intestine, to the lymphatic system has also been demonstrated. Some researchers have observed that the mechanism transporting MNPs through physiological barriers might be influenced by para-physiological conditions of the animal. In this regard, an increase in MPs transport in the colon of patients with inflammatory bowel disease related to an increase in intestinal permeability was observed [17], whereas a reduction of NPs transport in diabetes condition was seen [163]. NPs (100–1000 nm) stimulated the secretion of cytokines such as IL-6 and IL-8 from monocytes and they act as stressors to the innate immune system of fish [164]. Larger particles at about 100 µm or above were proven to have no significant effect [28]. NPs can negatively affect fish at different stages of development, accumulating in tissues, decreasing locomotor and foraging activities. However, mortality, malformation or effects on hatching related to NPs have not been reported. High concentration of MPs/NPs are usually highly cytotoxic. ROS production and pro-inflammatory responses are the most frequently encountered. MPs/NPs toxicity is evaluated based on the response of gut lipid peroxidation biomarkers, as an indication of the response to oxidative stress, inflammation, epithelial barrier integrity and changes in gut microbiota [60,61]. Therefore, the key role of exposure routes in the uptake, localization, and subsequent distribution of nanoparticles was highlighted by Zhang et al. [131]. The ingestion and effects of MPs in fish are largely dependent on their shape and size [126].

## 6. Toxicity of Microplastics and Nanoplastics in Digestive Tract of Zebrafish: An Emerging Model to Study the Bioaccumulation and Toxicity of Environmental Contaminants

Zebrafish represents an ideal animal model to study microplastic and nanoplastic toxicity. Due to the transparency of the eggs, embryos and post-hatch larvae, the localization of fluorescent-labelled MPs/NPs particles may be followed to determine the distribution, uptake, trafficking, degradation, and translocation using confocal microscopy [78,165]. On Medline from 2015 to 2021, using key words “microplastics” and “zebrafish”, we have n.78 available results, concerning the effects of micro/nanoplastics in different organs and tissues. Typing the keyword “gut” to get only 10 results. In general, the negative effect of microplastics on zebrafish was related to the concentration and particle size. Intestinal toxicity of different length of microplastic fibers ( $50 \pm 26$  µm and  $200 \pm 90$  µm) has been studied in both larvae and adults of zebrafish proving that longer microfibers caused very serious intestinal damage compared to shorter microfibers causing a significant reduction in food intake [166]. Studies of metabolomics were also carried out to unravel the underlying mechanisms of microplastics toxicity through the study of chemical processes involving all the endogenous and exogenous metabolites of enteric cells that cause oxidative stress and inflammation [166]. The impact of polystyrene (PS) NPs was studied at the cellular, molecular, and systemic levels by Sendra and colleagues [165]. It has been demonstrated by the same authors that the absorption of PS NPs takes place by endocytosis and phagocytosis respectively for 50-nm and 1-micron nanoparticles. Regardless of the pathway of internalization, the particles will be degraded by lysosomes resulting in alkalization and increased of reactive oxygen species (ROS) that increase their susceptibility to pathogens [165,167]. Oxidative damage and inflammation induced by co-exposure to MPs and cadmium have been studied in zebrafish by Lu et al. [11,31]

demonstrating that MPs enhanced the toxicity of heavy metals in fish tissues, including the gut. Using zebrafish, the influence of different polymer types of MPs was evaluated to understand the toxicology of microplastics (MPs) in combination with other organic pollutants (biocidal compounds or polycyclic aromatic hydrocarbons) with accumulation and consequent increasing serious toxic effects even further leading to oxidative stress and lipid peroxidation also in the liver as well as enhanced neurotoxicity in the brain and metabolic disorders [144,168]. Uptake and tissue accumulation of polystyrene microplastics (PS-MPs) studies in zebrafish have been conducted. Both 5  $\mu\text{m}$  and 20  $\mu\text{m}$  diameter MPs were found in the intestine after seven days of exposure, associated with inflammation, lipid accumulation and alterations of metabolic profiles in fish liver [125]. Inflammation and oxidative stress were observed in the zebrafish gut exposed to polystyrene MPs 5- $\mu\text{m}$  beads in size, at different concentration (50  $\mu\text{g/L}$  and 500  $\mu\text{g/L}$ ), for 21 days, associated with alterations in the gut microbiome and tissue metabolic profiles [154]. The shape-dependent effects cannot be underestimated. The recent scientific studies have shown that microplastic fibers resulted in more severe intestinal toxicity than microplastic fragments and beads did. Their accumulation caused mucosal damage and increased permeability, inflammation, and metabolism disruption as well as gut microbiota dysbiosis and specific bacteria alterations interfere with the immune system deteriorating host health [154,169]. PS particles < 5  $\mu\text{m}$  in size, polyamides, polyethylene, polypropylene, and polyvinyl chloride cause intestinal damage of adult zebrafish gut [137] although the most serious effects were found in the larval stage [111,125]. In this regard polyethylene (PE) particles 25 nm and 50 nm in sizes were found in cells of the intestinal epithelium in embryos of 0, 24, and 72 hpf [141]. Changes in gut microbiota with disorders of the metabolome and microbiome, as well as changes in the expression of genes correlated with epithelium integrity, were observed in both larval [55] and adult zebrafish [154,165,170]. Upregulation of intestinal Cytochrome P450 gene (*cyp1a*) was shown by Mak et al. [69] in adult zebrafish. In zebrafish, Limonta et al. [138] reported transcriptomic and histological alterations on the mucosal epithelium of zebrafish although it recognizes plastic particles as inedible materials but ingests them when mixed with food or for accidental ingestion due to small particles (1–5  $\mu\text{m}$ ) [23]. Fluorescent MPs were observed in the villi, in the apical surface of the enterocytes, in the lamina propria and very frequently inside the goblet cells. Microfragments and microfibrils did not cross the intestinal barrier. Based on the histological analysis, significant changes in the MPs-treated gut tissues were observed: epithelial and villi damage has been described in zebrafish [154,171,172] as bowel wall thinning, cracking of villi, epithelial damage with splitting of enterocytes and increased volume of mucus [30,137]. In contrast, De Sales-Ribeiro et al. [23] argue that the ingestion of microplastics does not induce any histopathological changes in zebrafish.

## 7. Translocation of Micro and Nanoplastics to Liver

MP/NPs cross the intestinal barrier, travels through the bloodstream to the organs such as liver and kidney [125,173]. In the liver, oxidative stress determines enzymatic and metabolic changes [125]. Histologically, in the liver, MPs particles were detected in the cytoplasm of the hepatocytes ranged between 1416 and 1634  $\mu\text{m}$ , with moderate lipid-like vacuolar degeneration translated into an enlargement of the hepatocyte cytoplasm due to the presence of one or several vacuoles that displaced the nuclei to the periphery. Congestion, sinusoidal dilatation, glycogen-depletion [107] and cellular necrosis were also found [107,126]. Signs of inflammation and lipid accumulation or lipid profile changes were found in African catfish, *C. gariepinus* by Karami et al. [145]. MPs has been observed into the livers of *Engraulis encrasicolus*, *Sardina pilchardus* and *Clupea harengus* after degradation of the hepatic tissue and in the livers cryosections observed in polarized light microscopy [174]. The livers contained relatively large MPs that ranged from 124  $\mu\text{m}$  to 438  $\mu\text{m}$ . High levels of bisphenol A and analogous compounds in numerous fish species of commercial interest were found. The potential relationship between MPs and bisphenol contamination of fish was investigated [175]. Higher concentrations of bisphenols were found in fish with

microplastics while they were absent in liver samples of fish without microplastics. It has been established that a large part of the quantities of microplastics ingested are translocated to fish liver [176]. Different opinion about the size of the microplastics that would be able to move into the intestine, from gut to circulatory system before further translocation to liver bare present in literature. Some authors refer that 20 µm microplastics cannot translocate to fish liver in contrast to microplastics less than 5 µm [125]. In zebrafish, 5 µm diameter MPs accumulated in fish liver were found while 20 µm diameter MPs were not found in the liver but only in the gills and intestines. Histopathological analysis showed inflammation and lipid accumulation in zebrafish liver also. Alterations of metabolic profiles in fish liver and alteration of lipid and energy metabolism were also found. However, the exact route through which MPs reach the liver is still unknown.

### 8. Translocation of Micro and Nanoplastics to Kidney

As in other organs, micro and nanoplastics were found in fish kidney too [177]. They were found in kidney of 11 commercial fish species collected from the marine fish market [178], but also in fish exposed to them in laboratory [43]. On the other hand, Choi and his team [179] did not detect microplastics in kidney fish after exposure in their laboratory. This inconsistency may be due to the different plastic sizes employed by Kashiwada [43] and Choi's teams [179]: respectively, 39.4 nm and 150–180 µm (Table 3).

**Table 3.** Micro and nanoplastics effect on kidney fish.

Specimens	Micro- and Nano-Plastics Sizes and Type	Concentration	Exposition Time	Effects	References
sheepshead minnow ( <i>Cyprinodon variegatus</i> ) larvae	150–180 µm PE microspheres	50 and 250 mg/L	4 days	- No effects	[179]
Japanese medaka ( <i>Oryzias latipes</i> )	10 µm PS	500, 1000 and 2000 µg/g	10 weeks	- glomerulopathy - nephrogenesis - glomerulomegaly	[180]
Japanese medaka ( <i>Oryzias latipes</i> )	10–20 µm sized PES and 50–60 µm sized PP fiber	10,000 Microplastic fiber/L	21 days	- No alteration	[181]
Gilthead seabream ( <i>Sparus aurata</i> )	From 40 to 150 µm PVC-MPs	100 mg/kg and 500 mg/kg	15 and 30 days	- alteration of creatine kinase, albumin and globulin in serum	[182]
Zebrafish ( <i>Danio rerio</i> )	~70 µm (mean) sized PA, PE, PP, PVC	0.001–10.0 mg/L	10 days	- No histological damage	[137]
Zebrafish Larvae ( <i>Danio rerio</i> )	Fluorescent PSNPs (25 nm; 1.05 g cm <sup>-3</sup> ) internally dyed with FirefliTM Fluorescent Green (468/508 nm)	0.2, 2, and 20 mg/L	120 hpf	- transcriptional alterations of pck1	[115]

Table abbreviations. PVC-MPs: polyvinylchloride microparticles, polystyrene (PS), polyester (PES), polypropylene (PP), polyamides (PA), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene nanoplastic (PSNPs).

The literature reports that after MPs and NPs exposure, a damage in kidney can occur and this can also involve the immunogenic part of this organ: the head kidney. For instance,

Zhu et al. [183] found, through the histological analysis, alterations in kidney after MPs exposure, like glomerulopathy and nephrogenesis. Glomerulopathy was showed as expansion and congestion of glomerular capillaries, with severe cases with increased glomerular size (i.e., glomerulomegaly) and expansion of Bowman's space while nephrogenesis was evident as tubular and/or glomerular generation or regeneration. The authors found that glomerulopathy and nephrogenesis, in exposed fish, increases in severity with exposure level. Kidney health status can be also evaluated by assessing creatinine and uric acid that can be considered as an index of the glomerular filtration rate and as a biomarker for kidney dysfunction. So, Hamed et al. [184], after 15 days exposure to MPs, detected a significant increase of fish kidney function (creatinine and uric acid). MPs-dependent kidney damage was also demonstrated by Espinosa et al. [182], testing creatine kinase, albumin, and globulin in fish. These parameters were significantly increased in serum from fish fed with PVC-MPs at 100 mg/kg for 15 days and 30 days. Alterations in plasma globulin and albumin were also obtained on juvenile *C. gariepinus* by Karami et al. [145], but with different kind and size of MPs, time of exposure, and administered doses (Table 3). In accordance with the results of Brun et al. [115], another effect of NPs could be the disruption of processes related to energy metabolism. The authors demonstrated that NPs affect *pck1* transcription in kidney by inducing alterations in glucose homeostasis. According to Zhu et al. [183], head kidney is one of the most altered sites when fish kidney is exposed to MPs and NPs and head-kidney leucocytes are affected too. Brandts et al. [185] found that NPs altered the expression of target genes related to the immune function, cellular stress, and stress-related hormone secretion in *D. labrax* head kidney. Among these genes *tnfa* (tumour necrosis factor alpha-like, Gene ID: 100136034), *tgfb* (transforming growth factor beta 1, Gene ID: 7040), *hsp70* (heat shock protein 8, Gene ID: 115594641), *mc2r* (melanocortin 2 receptor, Gene ID: 115567821), and *gr* (glutathione reductase, Gene ID: 115568704) were interested. With regard to head-kidney leucocytes, Espinosa et al. [186] found that their phagocytosis and respiratory burst respectively decrease and increase on gilthead seabream (*S. aurata*) and European sea bass (*D. labrax*) after polyvinylchloride (PVC) and polyethylene (PE) microplastics exposure. Moreover, according to the authors, these microplastics induced *nrf2* (nuclear factor erythroid 2-related factor 2-like, Gene ID: 109102552) up-regulation in seabream HKLs. In addition, Espinosa et al. [182] tested the expression of stress genes in head kidney. The gene expression of *prdx5* (peroxiredoxin 5, Gene ID: 115593974) decreased already after 15 days of exposure while the expression of *prdx1* (peroxiredoxin 1, Gene ID: 115573364) and *prdx3* (peroxiredoxin 3, Gene ID: 115596707) increased after 30 days of PVC-MPs. As for other organs (i.g. gills), micro and nanoplastics can exacerbate other compounds toxicity in kidney. For example, Brandts et al. [185] found that NPs can amplify humic acid effects. The combination of this compound and nanoplastics induces an alteration of target genes involved in stress-related functions such as *il10* (interleukin 10, Gene ID: 100136835), *tgfb*, *mc2r*, and *gr* gene expression in head kidney of *D. labrax*. Microplastics can affect paraquat effects too [187] and influence blood biochemical parameters in common carp (*C. carpio*). For example, exposure to microplastics was followed by an increase in creatine phosphokinase (CPK) activity in blood that may signify among, other things, renal failure [188]. As reported for gills, Lei et al. [137] found no histological damage to kidneys of zebrafish after microplastics exposure. In the same way, Hu et al. [132] wrote that hematoxylin-eosin staining showed no alterations in internal organs (among the others, kidney) of their samples of exposed individuals to MPs. However, all the mentioned studies reported above employed different species, MPs type and sizes, concentrations, and exposure times (see Table 3). This could explain the different effects obtained by the different authors.

## 9. Impact of Micro and Nano Plastics Bioaccumulation on Blood Response in Fish

In recent years, the investigation of blood parameters in fish have become of considerable importance and aroused enormous interest not only in aquaculture, but also in the evaluation of fish as organisms sensitive to anthropogenic pollutant load. In fish,

the hematological and hematochemical parameters represent important laboratory indexes useful in the diagnosis of many diseases and therefore for the evaluation of the state of health [189,190], physiological state of fish, food conditions, and quality of the water in which they live. The values of blood parameters provide useful information in various body processes and are of great importance in evaluating the harmful effects of anthropogenic pollution on aquatic environments. Fish live in intimate contact with the aquatic environment that significantly influences their blood homeostasis. In the past few years, there has been a significant increase of the experimental studies conducted in the hope of achieving a better understanding of the impact of micro- and nano-plastics (MP/NPs) on diverse organisms including fish. The fish homeostasis is altered by the exposure to polluting substances and materials of various kinds (including micro-nano plastics), causing a series of protective mechanisms such as changes in blood parameters [191,192]. Therefore, the hematological and hematochemical parameters are useful bioindicators in evaluating the effect of different pollutants in fish [192]. In fish, the complete blood count or CBC (complete blood count), is a laboratory test of the blood (fast, inexpensive and practical) which contains all the information necessary for the evaluation of hematopoiesis [193]. The parameters evaluated are the following: WBC (white blood cells), RBC (red blood cells), Hb (hemoglobin), Hct (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), and TC (thrombocytes). Currently, an improvement in the techniques of haematological investigations in fish has been achieved. New methods of blood analysis are now used routinely and applied in the study of fish species. Two of these new techniques are represented by the hematological determination by means of an automated electronic system and by flow cytometry. The hematological determination in fish by automated method uses an electronic blood cell counter with an impedance analysis system. This apparatus was previously used for mammals and subsequently modified in the software to carry out the hematological analysis also in fish. For this purpose, a suitable fish lysing solution is used in the instrument. This method has been validated for various fish species [189,194–197]. Flow cytometry is one of the most modern analysis techniques for the detection and quantification of fish blood cells. It allows to identify, count, and characterize individual cells in mixed populations with rapidity and high precision [190]. These new diagnostic methods have brought great benefits to the veterinary medicine and to the study of fish haematology. Previous studies on various fish species, have shown a marked variation in haematological and hematochemical parameters due to exposure to various pollutants [13,184,190,198,199]. Among the various polluting substances and materials, MPs represent the most plentiful pollutants on Earth [200] due to their ubiquitous presence within the aquatic environment [201]. In the last years, the studies conducted to monitor the impact of MP/NPs on aquatic organisms have widely increased. A large variety of effects in various fish species were reported [128,202]. Due to their small size, microplastics and nanoplastics can be ingested (directly or through the food chain) by fish. Once inside the body, the plastic particles mainly accumulate in the digestive tract and transferred to the circulatory system or to adjacent tissues [36], and can persist in the organism for a long time [203–205]. The harmful effects caused by microplastics are due to additives and chemical substances used during their production or utilization of pollutants absorbed from the environment [51,206,207]. MP/NPs induce physical and chemical toxic effects in various organisms including fish. These effects have an impact on the blood response of the exposed organism. In this regard, a study conducted by Hamed et al. [184] on Nile Tilapia (*O. niloticus*) showed variations in hematological and hematochemical parameters of this species after exposure to MPs. Regarding to the hematological profile, the obtained results revealed a significant reduction in red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular hemoglobin concentration (MCHC), thrombocytes (PT), white blood cell (WBC), and percent of monocytes after exposure to MPs. Percent of lymphocytes, eosinophils and neutrophils showed fluctuation after exposure to MPs. On the contrary, mean corpuscular volume (MCV) and mean corpuscular hemoglobin

(MCH) exhibited significant increase. These changes in hematological parameters represent a defense mechanism as a response to MPs toxicity [208]. Similar results were previously found by Mukherjee and Shiha [209] in a study on Indian freshwater major carp exposed to cadmium. Moreover, the immune system may be influenced by toxic chemicals in the micro and nano plastics [107]. The immune system of fish is a useful indicator of negative response to environmental stressors [210]. Effects of MP/NPs on chemokines, cytokines and phagocytes, and increased levels of globulins and immunoglobulins [145] were demonstrated in a previous study by Jacob et al. [13]. Changes in hematochemical parameters due to exposure to micro- and nano-plastics were detected in different species of fish [73,182,184,187,211]. A significant increment of creatinine, uric acid, AST, ALT, ALP, glucose, cholesterol, total protein, albumin, globulin, and A/G ratio was found in Nile Tilapia *O. niloticus* [184] and common goby *P. microps* [73,211] after exposure to MPs. Increased levels of ALT, AST, ALP, creatinine, glucose, also occur in common carp (*C. carpio*) exposed to sublethal concentrations of micro-plastic [187]. Furthermore, it was shown that nano-plastic intake in fish can alter the proportion of triglycerides/cholesterol of blood [212]. Contrary to what was observed in Nile tilapia and common goby the exposure to MPs causes a decrease of the cholesterol, high-density lipoprotein and triglyceride levels in African catfish and common carp [145,187]. The increased albumin levels observed in fish after MPs exposure indicate a liver or kidney damage [213] caused by the harmful effects of micro-plastics [182]. All the variations of blood parameters that occur in fish exposed to MP/NPs, represent an effective physiological response of the organism to toxic effects of plastic particles. These blood changes are important biomarkers in toxicological research, environmental monitoring, and prediction of fish health conditions. In the future, it will be important to refine the diagnostic techniques of fish hematology in order to define the potential blood biomarkers related to micro and nano plastics bioaccumulation.

## 10. Conclusions

This review provides crucial multidimensional characterization of NPs impacts on human and animal health, suggesting the need for deeper investigations following longer exposure times. The current scientific evidence shows the main factors determining the toxicity of plastic particles, including particle size, concentration, exposure time, particle condition, and shape.

Plastic particles smaller than 10  $\mu\text{m}$  cause more toxic effects than larger plastic particles with negative effects, such as decreased survival [214], decreased activity of a neurotransmission biomarker, AChE [10,73,91,215], decreased energy storage of glycogen [79,107,216], aberration of liver energy metabolism [145], effects on heart and lipid tissues [125,217], effects on heart rate [48,118], increased feeding time [35,62,212], inflammation [125], oxidative damage [61], necrosis [61], effects on body length [60], intestinal bacterial composition [30], and texture of brain and muscle including impact on the water balance in the brain [35,62,212]. Recently, MPs/NPs effects on fish started to be investigated through transcriptomic approaches [138,166,218,219]. Nevertheless, further investigations are needed to understand MPs and NPs effects on omics fish.

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