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Toxicological assessment of nanoparticles and microplastics

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Abstract

According to National Oceanic and Atmospheric Administration (NOAA), microplastics are small plastic particles of less than 0.2 inches (5 millimeters) long and considered as sesame seeds. The microplastics (MPs) reach to the rivers, lakes, and oceans after being flushed into sewers. Nanoparticles (NPs) are colloidal particles within the size range of 1 to 100 nanometers (nm). Secondary NPs occurred naturally by the degradation of macro and microplastic waste materials and are more heterogenous than primary NPs. The damaged biomolecules lead to the occurrence of numerous reactions including inflammatory response, cell death, tissue damage, and DNA damage. Free radical generation (non-enzymatic antioxidants) and antioxidant defenses helps to balance the excess ROS and repair the damaged cells to reduce the oxidative stress. NPs and MPs eventually move to the aquatic environment followed by the entrance of NPs and MPs into the bodies of aquatic animals to cause harmful effects. NPs may also dramatically increase the CAT and SOD activities. MPs and NPs are very harmful to the organisms however combined with other chemical components. These components are cause serious harmful to the fish and damage the nervous system of fish. NPs and MPs can also cross the blood brain barriers in the brains of fish.

Introduction

Microplastic

Microplastics (MPs) are created by the weathering and breakdown of plastic objects, cartires, clothing, paint coatings, and leakage of preproduction pellets and powders. They may also be intentionally added to daily life products (e.g., cosmetics and abrasive cleaners) [1]. According to National Oceanic and Atmospheric Administration (NOAA), MPs are small plastic particles less than 0.2 inches (5 millimeters) long, consider as a sesame seed. In 2014, up to 51 trillion bits of MPs were recorded in the ocean. The recorded number was 500 times higher than the number of stars in the Milky Way [2]. Plankton nets with mesh netting measuring 0.004 to 0.02 inches (0.1 to 0.5 mm) and are small enough to catch plastic particles to analyze MPs [2]. MPs are classified into two types as primary MPs and secondary MPs. Cosmetics and industrial raw materials are the main sources of primary MPs. Plastic microbeads are used in everyday cosmetics such as facial cleansers and toothpaste to increase friction and improve cleansing [3].

MPs reach rivers, lakes, and oceans after being flushed into sewers. Secondary MPs are mostly produced as a result of large-scale plastic physical smashing and biological breakdown, tyre wear and sewage treatment facilities [4]. MPs always cause chronic toxicity due to their accumulation in organisms [5].

Nanoparticles

NPs are colloidal particles within the size range of 1 to 100 nanometers (nm) [6]. More than 100 nm size of nanoparticles has also been reported [7]. Primary NPs are formed for specific purposes for inclusion into cosmetics, paints and electronics. Secondary NPs occurred naturally by the degradation of macro and microplastic waste and are more heterogenous than primary NPs.

Secondary NPs are negatively charged and highly polydisperse with different shapes [8]. In an aquatic habitat primary NPs are present in low quantity; however the concentration of secondary NPs may increase due to their release by the degradation and fragmentation of MPs. [9]. In an aquatic environment, polystyrene (PS) NPs show more acute toxicity to *Daphnia magna* than PS MPs [10].

Naturally occurring NPs are found in the North Sea, are the mixture of Polystyrene (PS), polyethene (PE), polyvinylchloride (PVC) and PE terephthalate (PET) [11]. In the aquatic environment, NPs are formed from plastic waste, accelerated abrasion of expanded PS with glass beads and sand in the laboratory produced a mixture of NPs and MPs [12].

PS NPs were produced from coffee cup lids after exposure to ultraviolet radiation (UV) in weathering chambers and PE-NPs were formed after digestion (and egestion) by Antarctic krill [13] and PE NPs formed after digestion by Antarctic krill [14]. Plastics have various group of synthetic polymer materials to become a typical sign of artificial waste and environmental pollution [15]. Plastics are widely used in all aspects of our everyday life due to their low price, durability, light weight and good ductility [16]. Most of the plastics waste generated in industrial processes such as non-ferrous industries and small workshops are directly discharged into the surrounding environment without treatment [17]. Atmospheric agents, such as waves, abrasion, ultraviolet radiation and photo-oxidation in combination with bacteria degrade plastic fragments into micro and nanosized particles [18].

The increased amount of plastic litter in oceans has become a serious worldwide issue [19]. The degree of contamination in both environments, are not only related to the marine environment but also to the freshwater [20] [21]. In the past 70 years, a significant increase in the production of plastics to about 368 million tons until 2019 were generally used in health, household, food sector and in almost every field of life [22]. The management of plastic waste has emerged a major issue as about 40% of plastic are discarded in the form of bags, bottles and packages [23]. About 79% of the plastic that is disposed in the land falls between 4.8 to 12.7million tons to marine systems.

Through the process of weathering the plastics are fragmented into smaller pieces [24]. The maximum amount of this marine water is <5 mm fragments [25]. These fragments are also called to be the MPs [26]. MPs cause serious diseases in marine animals because of their small size these MPs are easily ingested [27]. In turtles and seabirds, it causes the blockage and reduction of their gut [28]. In fishes, it affects the metabolism and causes some reproductive disorders [29]. The toxic damage of NPs and MPs neglect the variations in toxicity. The toxicity differences between MPs and NPs in the digestive system, reproductive system, and nervous system investigated the potential causes of differences in toxicity [30]. It is essential to examine the distinct toxicity mechanism of MPs/NPs in bioaccumulation and pathway activation [31].

The risks of MPs are mostly due to their large specific surface area, which allows organic contaminants, heavy metals, pathogenic bacteria, and plastic additives produced during the cracking process to easily adsorb [32]. Under the action of fish gastric juice, polyethylene fragments will leach out a complex mixture and sub lethal endocrine effects on fish. Plastic items can be photodegraded by sunlight [33] [34].

MPs are mistaken for food by animals with low nutritional levels, and these particles are transferred to animals with greater nutritional levels [35]. MPs frequently enter the esophagus, stomach, and intestines through the mouth, therefore their harmful effect is reported in the digestive tract [36].

MPs can disrupt intestinal flora, destroying the ratio of probiotics to pathogenic bacteria to reduce intestinal mucus secretion, damage the intestinal mucosal epithelium, and eventually lead to intestinal barrier destruction; and cause fatty acid [37] and amino acid metabolism disorders, resulting in lipid deposition [4]. It was also reported that the reproduction of gametes, embryos, and offspring, and microplastics produce substantial reproductive damage. MPs interfere with gamete plasma membrane fluidity and hinder gamete binding; coat the embryo's surface, creating hypoxia, and collect in the yolk sac, affecting nutrient absorption; induce aberrant growth and development of children, as well as metabolic diseases [38].

MPs induce widespread neurotoxicity in animals, as well as aberrant behavior and depression. MPs build up in the brain and impede the function of Acetyl cholinesterase (AchE); they permeate the epidermis and into the muscular tissue, causing nerve fiber atrophy [39]. Pollutants in the environment including MPs often have harmful consequences due to oxidative stress. Furthermore, they can disrupt the circulatory system and impair immunological function [40].

The smaller size MPs have a higher bioavailability and a longer retention time in the body, making it more harmful to the biota [41].

MPs are most abundant in remote areas of the world [42]. Bakelite is one of the earliest plastics that was made for use commercially [43] but till 2018 the production of plastics has reached up to 357 million tons globally [44]. It has been estimated that about 8 million tons of plastics have been thrown into the aquatic environment out of which about 80% comes from land resources and 20 from marine sources [45]. The number of MPs may also increase due to aquaculture practices, the tear of accessories like nets and cleaning facilities may also increase the number of MPs in the environment [46]. NPs are used in consumer and industrial products to meet the needs [47], on the other hand MPs are deliberately used to serve a specific function [48].

The attachments of MPs are dependent on their compositions [49], while some remain inert like cerium oxide NPs [45]. MPs are made up of polymeric materials affected by the ageing process exposed to sunlight and dissolve in the environment. The ageing of MPs may break into very small particles [13].

MPs toxicity pathways in aquatic environments

The primary suggested mechanisms for the environmental toxicity of contaminants and ecotoxicity in organisms are characterized as oxidative stress [50]. Oxidative stress can disrupt the ability of organism to deal with excessive reactive oxygen species (ROS), inducing antioxidant defense and causing oxidative damage to molecules [51].

Excessive ROS produced polyunsaturated fatty acid peroxidation, decreased cell membrane permeability, and altered the structure of other components such as DNA, proteins, and lipids. The damaged biomolecules leads to generate chain of events including an inflammatory response, cell death, tissue damage, and DNA damage [52]. Free radical generation (non-enzymatic antioxidants) and antioxidant defenses, which balance excess ROS and repair damaged cells to reduce oxidative stress [53]. The toxicity processes underlie the oxidative stress generated by MPs in aquatic species (free radical formation, antioxidant defense regulation, certain signal pathways, and gene expression).

Ribeiro observed that exposing Scrobicularia plana to 1 mg/L (20 mm) MPs for 14 days and then depurating for 7 days produced DNA damage, neurotoxicity, and oxidative damage. Oxidative stress indicators were found in organism tissues [54]. Furthermore, MPs may dramatically boost SOD and CAT activities, showing that oxidative stress was created following MP therapy [29]. Lei proposed that MPs produced oxidative stress in *Danio rerio* and *Caenorhabditis elegans* by causing free radical generation since overproduction can change the homeostasis of cellular components by reducing antioxidant system activity [55].

Some researchers discovered the stress response stimulation linked to specific signal pathways. Nano polystyrene particles dysregulated the expression of genes involved in the regulation of oxidative stress and triggered the expression of the Nrf signaling pathway [56]. MPs triggered oxidative stress by suppressing detoxification and the immune system through JNK and ERK signaling pathways [57].

Impact of MPs on the toxicity of other chemical contaminants, in the aquatic environment

Pollutants adhering to the surface of MPs may enhance the characteristics of MP and alter toxicity consequences in organisms. MPs can act as a vector for contaminants to enter an organism. Polybrominated diphenyl solubility and Kow are two critical parameters in predicting pollutant sorption to MPs [58]. Rochman predicted that MPs coupled with a variety of chemical contaminants could damage endocrine system function, alter gene expression, and impair germ cell proliferation. Individual chemicals toxicity is influenced by the contact of MPs and compounds. It is vital to evaluate MP bioavailability and associated pollutants [59].

For aquatic species, there are three types of complicated toxicity effects on coupled pollutants, antagonistic, additive, and synergistic. Microalgae, Daphnia magna, and fish are common aquatic species studied in toxicity research. The herbicide chlorpyrifos was observed to adsorb onto MPs surfaces, reducing bioavailability in algal cells and suppressing microalgae development [60]. Davarpanah and Guilhermino (2019) observed that a combination comprising 3 mg/L gold nanoparticles + 4 mg/L MPs lowered the average specific growth rate of *Tetraselmis chuii* [61].

The combined toxicity of two types of MPs in association with triclosan was lower than that of single MPs [62]. Bellingeri observed that combining Cu with MPs exposure resulted in no major difference in algal growth suppression when compared to a single exposure in both short-term and long-term testing [63]. Zhang proposed that co-exposure of *Daphnia magna* to MPs and roxithromycin may trigger CAT and GST activities as well as MDA levels [64].

Rainier observed that combining MPs with methylmercury dramatically changed organ homeostasis, such as in the liver, intestine, muscle tissue, and brain, when compared to MPs exposure individually. However, the adsorption properties were also affected by the physicochemical properties of the pollutant (composition, size, shape, and color) [65].

Toxicity of NPs on CNS of Fishes

The bad effects generated by metallic nanoparticles are one of the most vulnerable organs to the negative effects induced by metallic nanoparticles (MNPs)to decay and release metal ions in water [66]. This includes their distribution throughout the body, penetration through cell membranes, and transit by the blood-brain barrier, among other things [67]. When it comes to metallic NPs, the most common form are found dispersed in water reservoirs [68].

Sub-chronic exposure to low levels of TiO_2 NPs resulted in brain damage, reduced spatial recognition recall, and impaired behavioral responsiveness in zebrafish (*Danio rerio*). Zebrafish larvae were exposed to TiO_2 nanoparticles at concentrations of 1, 10, and 100 grams per milliliter of water, and they demonstrated a 50-70 percent loss of dopaminergic neurons, as well as symptoms that were comparable to Parkinson's disease [69].

Undertaken *in vitro* tests on $PC1_2$ cells validated their findings, TiO_2 NPs did not penetrate very far into the brains of zebrafish [70]. The exposure to high concentrations of ZnO nanoparticles (2000 g/L) and Ag NPs (4 mg/l), the brain antioxidant system was found to be negatively affected, whereas exposure to low concentrations of ZnO nanoparticles (500 g/L) and Ag NPs (2 mg/L) had the complete opposite effect, promoting the antioxidant activity of the brain [71]. The zebrafish were unable to grow their nervous and circulatory systems properly because of the presence of ZnO nanoparticles in their environment [72].

Effects of nanoplastics on fish nervous system

Since the early 1990s, the chemical features of NPs have little information regarding the impact of NPs on the growth and development of aquatic animals. A variety of animals, including sea hellions and mussels, have been reported for the adverse effects caused by leachate from patches, however the behavioral and physical repercussions of leachate from patches have remained largely unexplored [73].

The benefits of ecologically relevant attention to NPs on adult zebrafish gestation and biochemistry were computed to analyze the ecological toxicity of polystyrene NPs (**Fig. 1**).

Polystyrene is a form of plastic frequently utilized and creates a significant amount of product. As a result, it is one of the most major contributors to the

accumulation of plastic trash in the environment, which has been linked to global warming [74].
According to the researchers, there is no evidence to support the hypothesis that polystyrene NPs have an implicit effect on the gestation of adult zebrafish at this time.

An adult zebrafish was used to investigate the neurobehavioral changes caused by colored napkins to analyze the neurobehavioral changes induced by polystyrene NPs and their dispersion and accumulation in colored napkins.

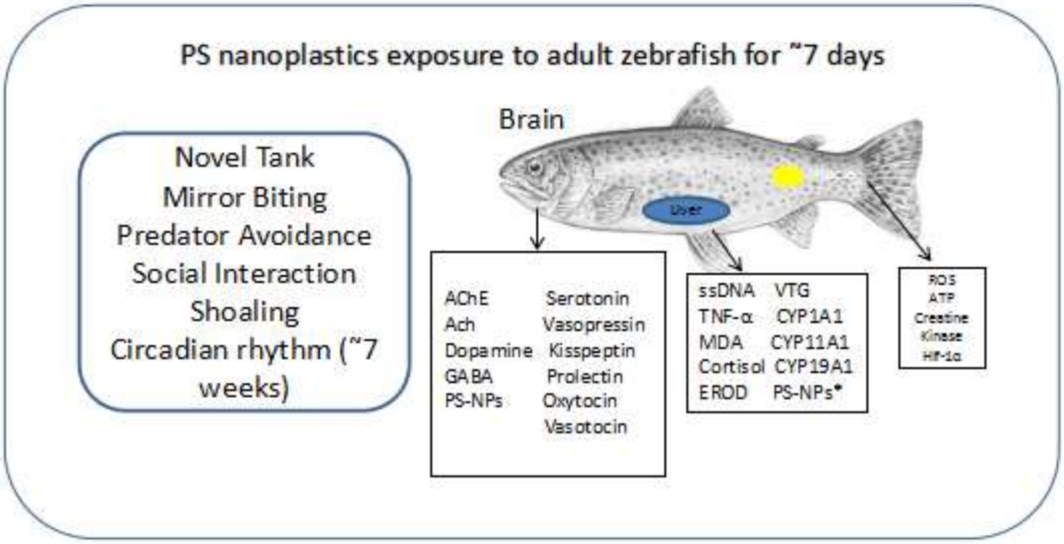

Fig. 1: Elaborating the biochemical assays and the effect of NPs on the nervous system of fishes.

Micro and Nano Plastics in Aquatic Ecosystem Can Be Taken-Up by Fish and Reside in Their Brain

Although several laboratory and field studies have evidenced that the MPs consumption by subaquatic organisms, including corporate species [46], remains unclear whether MPs and NPs consumed by fish can be absorbed by the gastrointestinal (GI) tract and spread to other body sections of the fish after being swallowed [75].
In light of the fact that micro and nanoplastics are capable of penetrating and shattering the blood-brain barrier, once the gastrointestinal barrier has been breached, the brain may be exposed (BBB). The fish Oreochromis niloticus was the subject of Ding's research, which found that MPs increased both the size of the fish's brain and the circulation of its blood [76]. Similarly, Sökmen and his team believe that because NPs may enter red blood cells [77], as indicated by Geiser's *in vitro* work, they can have an influence on the brain rather than the bloodstream, and that this is consistent with the findings of Geiser's *in vitro* study [78].

It has been reported that the circular NPs particles have been found in the brains of fish after they have been exposed to water or food, suggesting that these particles are capable of passing a highly selective permeability barrier such as the blood-brain barrier [79]. Ding [38] discovered that 0.3, 5, and truly 70 x 90 mm polystyrene MPs could be accreted in the brain of red tilapia, showing that the MPs could be transported into the brain through the blood-brain barrier (*O. niloticus*). MPs having the diameter of 10 microns or less can penetrate organs and cross the blood-brain barrier, ultimately reaching the brain [80]. Micro and nanoplastics can penetrate the blood-brain barrier (BBB) before the BBB has been proved, which could have an impact on the uptake of NPs by the brain during the early stages of the life of a fish [81]. Sokmen [77] reported that NPs with a diameter of 20 nm fitted to the yolk sac can pass across the blood-brain barrier and bioaccumulate in the brain of mice. NPs have been shown to cause bone accumulation in the thraldom sac of zebrafish embryos and to cause them to move to the brain after only a little exposure time in laboratory conditions. MPs and NPs travel to the brain by several different pathways, which adds up to a significant amount of time [82] found that young zebrafish that have recently experienced an in-water vulnerability can have their NPs reach their heads, as well as MPs that cross the gills and collect in the fish head. Consequently, it is critical to explore the effects that micro and nanoplastics have on the brain and other organs.

Neurotoxicity of micro and Nano plastics in fishes

In the brains of fish that had been exposed to NPs patches through water or food, researchers identified patches that indicated the NPs were capable of passing the blood-brain barrier. Additionally, in subsea simulations, the brain is required to assess the harm that nanoparticles provide to the surrounding environment (NPs). NPs accumulate in the tissues of growing fish and perform a variety of harmful actions, including those that affect the nervous system, as they accumulate. When it comes to the influence of NPs and MPs on the neurological system, it is necessary to consider not only the quantity and length of exposure but also cyclic processes similar to those identified during oxidative stress, such as those shown after chronic exposure [38].

Dicentrachus labrax, *Trachurus*, and *Scomber colias* were exposed to plastics for a while, Barboza [80] discovered advanced LPO conditions and decreased Pang exertion in their brains compared to unexposed samples of the same species, this was presumably because advanced LPO attention results in the rupture of acetylcholine-containing vesicles, resulting in increased neurotransmitter released in synaptic checks and increased. Using zebrafish naiads exposed to polystyrene microplastics (PS-MPs), researchers [83] revealed that neurotoxicity in zebrafish naiads may be connected with certain metabolic alterations.

MPs exposure in fish activates cellular oxidative stress pathways, resulting in the peroxidation of cell membranes in the affected fish [86]. In the brain, lipid peroxidation can induce the membranes of neurotransmitter-containing vesicles to rupture, resulting in an increase in neurotransmitter attention at synaptic synapses. To confirm that microplastics are neurotoxic, researchers evaluated an increase in the exertion of the enzyme acetylcholinesterase.

Since cholinesterase (ChE) enzyme activity is essential for cholinergic neurotransmission at neuromuscular junctions and cholinergic synapses, the ramifications of these results are significant. Accumulation of acetylcholinesterase (Pang) exertion has been shown to affect brain function and is regarded to be a key indicator for neurotoxicity in both zebrafish and humans [87]. Zebrafish exposed to NPs showed decreased Pang exertion and decreased levels of neurotransmitters like dopamine, melatonin, aminobutyric acid, serotonin, vasopressin, kisspeptin and oxytocin in the presence of the plant, suggesting that the plant has neuroprotective properties.

The suppression of acetylcholinesterase (Pang) enzymatic conditioning in Medaka, latipes, and *Pomatoschistus microps* following exposure to micro and nanoplastics has been documented in a paper [59]. The reduction of Pang effort has also been observed in zebrafish naiads, where it has been shown to impair the neural system's function, resulting in growth retardation, paralysis, and death [88]. EE2 (17-ethinylestradiol) was used as a positive control in the assessment of neurotoxicity because it can alter the development of the neuroendocrine system during pregnancy. It was found that Pang's exertion was lowered among groups of naiads who were treated to MPP or MPP combined with EE2.

Similarly, in the same study, polyethene microplastics (1–5 m) were found to reduce the workload of P microps during angioplasty [89]. In addition to causing neurotoxicity, the tiny patches may have negative effects on the cholinergic system as a result of the Pang effort. The reduction in locomotor capability observed in zebrafish exposed to NPs and NPs can therefore be explained by a restriction in the amount of acetylcholinesterase that can be exerted [89]. The GFAP gene and its associated protein are highly conserved in zebrafish, and they carry out functions that are similar to those seen in mammals, including reproduction [90].

Conclusion

MPs are small particles that are as small as 0.2 inches or 5 mm. NPs are collide particles with size range of 1 to 100 mm. NPs are purposely made for using in the paints, electronics and other domestic and industrial materials. NPs and MPs divide and reach the aquatic medium where these are eventually taken up by the aquatic organisms. These are very harmful not only to fish but also to the other aquatic organisms as well. The major effect of these particles is seen in the esophagus, intestines and stomach as these enter the body through the mouth easily. Majorly MPs induce neurotoxicity and aberrent behavior to the organisms. It is observed that when organism ls exposed to MPs for 14 days it gives DNA damage at a large extent as well. The primary suggested mechanisms for the environmental toxicity of contaminants and ecotoxicity in organisms are characterized as oxidative stress. When biomolecules are damaged, a chain of events occurs, including an inflammatory response, cell death, tissue damage, and DNA damage. Furthermore, MPs may dramatically boost SOD and CAT activities, showing that oxidative stress was created following MP therapy. Pollutants adhering to the surface of MPs may enhance some MP characteristics and alter toxicity consequences in organisms. Because of their sorption properties, MPs can act as a vector for contaminants to enter an organism. Polybrominated diphenyl solubility and Kow, for example, are two critical parameters in predicting pollutant sorption to MPs. When it comes to metallic NPs, the most common form in which they are found is as dispersed or emulsified particles in water reservoirs. In the brains of fish that had been exposed to NPs patches through water or food, researchers identified patches that indicated the NPs were capable of passing the blood-brain barrier. Additionally, in subsea simulations, the brain is required in order to assess the harm that nanoparticles provide to the surrounding environment (NPs).

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Conflict of interest

The authors declare no conflict of interest.

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