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Review

Potential Risks of Microplastic Fomites to Aquatic Organisms with Special Emphasis on Polyethylene-Microplastic-Glyphosate Exposure Case in Aquacultured Shrimp

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Abstract: Plastic litter is increasingly becoming pervasive in aquatic environments, characterized by circulatory patterns between different compartments and continual loading with new debris. Microplastic pollution can cause a variety of effects on aquatic organisms. This review presents the current knowledge of microplastics distribution and sorption capacity, reflecting on possible bioaccumulation and health effects in aquatic organisms. A model case study reveals the fate and toxic effects of glyphosate, focusing on the simultaneous exposure of aquacultured shrimp to polyethylene and glyphosate and their contact route and on the potential effects on their health and the risk for transmission of the contaminants. The toxicity and bioaccumulation of glyphosate-sorbed polyethylene microplastics in shrimp are not well understood, although individual effects have been studied extensively in various organisms. We aim to delineate this knowledge gap by compiling current information regarding the co-exposure to polyethylene microplastic adsorbed with glyphosate to assist in the assessment of the possible health risks to aquacultured shrimp and their consumers.

Keywords: microplastics; sorption capacity; bioaccumulation; combined effects; polyethylene; glyphosate; shrimp

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1. Introduction

Plastic, a versatile and omnipresent organic polymer, is one of most frequently used materials globally. Over the past seven decades, the global production of plastics steadily increased almost 250-fold, from 1.5 to 368 million metric tons, emphasizing the importance of plastic to mankind [1,2]. The consequences of such increase in production have more recently captured the attention of broader audiences, as studies of its environmental impact indicated that plastic litter could disturb ecological relationships and interfere with different ecosystems at multiple scales. For example, plastics can end up in the aquatic environment via direct pathways as the litter coming off shipping or fishing activities and indirectly through improper discharge, runoff, current, wind, and wave action [3,4].

The extended presence of plastic litter and its exposure to the elements in the environment inevitably leads to its degradation into smaller particles with overall dimensions of less than several millimeters, termed "microplastics" [5]. Furthermore, the degradation of primary microplastic particles originally designed for commercial use in personal care and cosmetics products (PCCPs) [6], in industrial applications [7], or originating from synthetic textiles shedding during laundering [8] leads to the formation of smaller secondary microplastic particles [9].

1.1. Distribution, Abundance, and Importance of Microplastics in the Aquatic Environment

There is increasing evidence of ocean-based microplastics present in all marine environments, including the deep seas/trenches [10–15]. One of the most commonly reported polymer types in marine surface water and sediment is polyethylene (PE) [13,16–18]. Moreover, fibers and fragments were noted as prevailing microplastic shapes in the ocean and sediment [13,19–21]. However, the abundance, size, and concentration of observed microplastics can vary significantly across different sampling times and regions of the sampled marine environment [20,22–25].

Although there has been a lot of research on marine microplastic pollution, several studies have revealed the presence and distribution of microplastics in fresh open waters comparable to marine ecosystems [26–29]. The contamination with microplastics has been detected in natural freshwater systems and wastewater treatment plants at various locations around the European, Asian, North American, and South American continents, albeit at very diverse concentrations [30–35]. Such variability suggests that a variety of locations, anthropogenic activities and environments, and sampling strategies were employed [36]. Owing to their long-term persistence and long-range transportation, approximately 1.15 to 2.41 million tons of microplastics are estimated to be annually transported downstream from their initial sources (mainly wastewater treatment plants) by rivers to seas, with consequences for the aquatic organisms and environments along their transport routes [28,34,37,38].

Microplastics are thought to be ubiquitous by now, and besides their physical presence in the environment, other characteristics of these materials such as their toxicity, durability, and persistence could pose a potential threat to the environment and ecosystems [20,39]. Ingestion of or exposure to microplastics could cause negative consequences to organisms. The similarity of microplastics' size, shape, and color to natural sediments and feedstuff can mislead organisms to ingest microplastics instead of their natural diet. This, in turn, can cause malnutrition and, in extreme cases, even starvation effects, observed as the altered growth rate, reduced fitness, and changed behavior of the affected organisms [40–45]. Irregularly shaped and sharp-edged microplastics could cause abrasion and disrupt the integrity of gastrointestinal mucosa in living organisms [46]. Further, ingested microplastics may release different additives used in plastic production due to changes in the digestive tract (pH, enzymatic actions, etc.), and components such as plasticizers, halogen stabilizers, lubricants, and flame-retardants can be introduced to microplastic-eating organisms, causing additional harm [47–49]. More importantly, there is increasing evidence that microplastics can serve as a vehicle or concentrator for certain chemical and biological agents (micropollutants), and their ingestion could facilitate their transfer to organisms and cause adverse health effects or even death [50–52].

1.2. Aim of the Study

The aim of this narrative or traditional review is to provide an overview of the interaction mechanisms between microplastics and environmental micro-pollutants. Moreover, the factors affecting the micro-pollutant sorption on microplastics, including the physicochemical properties of micro-pollutants and microplastics and environmental conditions, are considered. The potential combined toxic effects of microplastics and micro-pollutant mixtures on aquatic organisms are further investigated, and we focus on a summary of the potential adverse outcomes of glyphosate and its commercial formulations' exposure to organisms. The combination effects of polyethylene and glyphosate-based herbicides (GBHs) on aquacultured shrimp are analyzed and applied to discussions in terms of health risks, using the relevant theoretical information.

2. Materials and Methods

The narrative review is undertaken to analyze the current knowledge on the topic; however, the scarcity of available information and evidence presents limitations to the completeness of the analysis.

The scientific paper selection process was carried out over approximately six weeks via the search engines PubMed and Google Scholar. The Boolean operators used were "AND" and "OR". The keywords for the research were: "microplastics", "polyethylene", "interaction", "glyphosate", "joint", "combination", "mixture", "organisms", "aquatic", and "shrimp". The following keywords were used with Boolean operators to combine searches: "microplastics" AND "interaction" OR "joint" OR "combination" OR "mixture" AND "aquatic", with no limitation to the publication year. The second search was made: "glyphosate" AND "organisms" OR "aquatic" OR "shrimp". Moreover, the third search was conducted: "microplastics" OR "polyethylene" AND "joint" OR "combination" OR "mixture" AND "glyphosate" AND "aquatic" OR "shrimp".

Included in the study were systematic reviews, meta-analyses, randomized controlled trials, cohort studies, and studies in English. The exclusion criteria were as follows: articles not related to the topic, full-text not available, and articles in other languages. No time limits were applied during the screening phase of the scientific articles.

3. Result Statements from the Analysis of the Available Literature

3.1. Microplastics: Delivery Vehicles for Micropollutants in Aquatic Environments

Microplastics can carry and transfer biological and chemical agents from one place to another, effectively acting as a vehicle and increasing the risks of different micro-pollutants to reach otherwise unaffected/less affected compartments within the ecosystems and organisms [53–55]. Some recently observed effects include disruptions to the oceanic carbon cycle due to the increase in dissolved organic carbon (DOC) [56] or adverse health effects in several organisms [57,58]. For example, microplastics could serve as the vehicle of plastic additives including triclosan, polybrominated diphenyl ethers, and nonylphenol added to polymerize or modify the end use properties of plastics [59,60].

3.1.1. Factors Influencing the Sorption Capacity of Microplastics to Micro-Pollutants

There is an increased risk of the release and transfer of additives from plastics to the surrounding environment or organisms during the degradation processes or particle ageing [61]. Due to mechanical, chemical, biological, and/or UV ageing processes, the microplastic particles' physicochemical and mechanical properties, texture, and appearance change over time [62]. These changes were noted as the main causes of the altered sorption capacity of microplastics to micro-pollutants [63–65]. Microplastic sorption capacity depends on the interaction between microplastics and micro-pollutants in an environment that increases or decreases their affinities to each other and influences the number of adsorbed micro-pollutants (Figure 1).

Physical Properties of Microplastics

The physicochemical properties and age of microplastics interrelate in a variety of complex ways that affect sorption capacity. Color, density, specific surface area, and free volume are some of the physical properties of microplastics with the potential to affect their sorption capacity [66]. For instance, lighter-colored microplastics could adsorb lower-molecular-weight polycyclic aromatic hydrocarbons (PAHs) as well as interact with lower concentrations of PAHs and polychlorinated biphenyls (PCBs) when compared to darker-colored microplastics [67,68]. Particle density also plays a role in sorption behavior, as microplastics with a higher density are capable of adsorbing lower concentrations of PAHs, PCBs, and phenanthrene compared to lower-density microplastic particles [69–71]. For example, polyethylene, with its characteristically large specific surface area and free volume, allowed more micro-pollutants to diffuse into the looser network of its polymer structure, suggesting a reason for its higher sorption capacity compared to the other microplastic types [70,72,73].

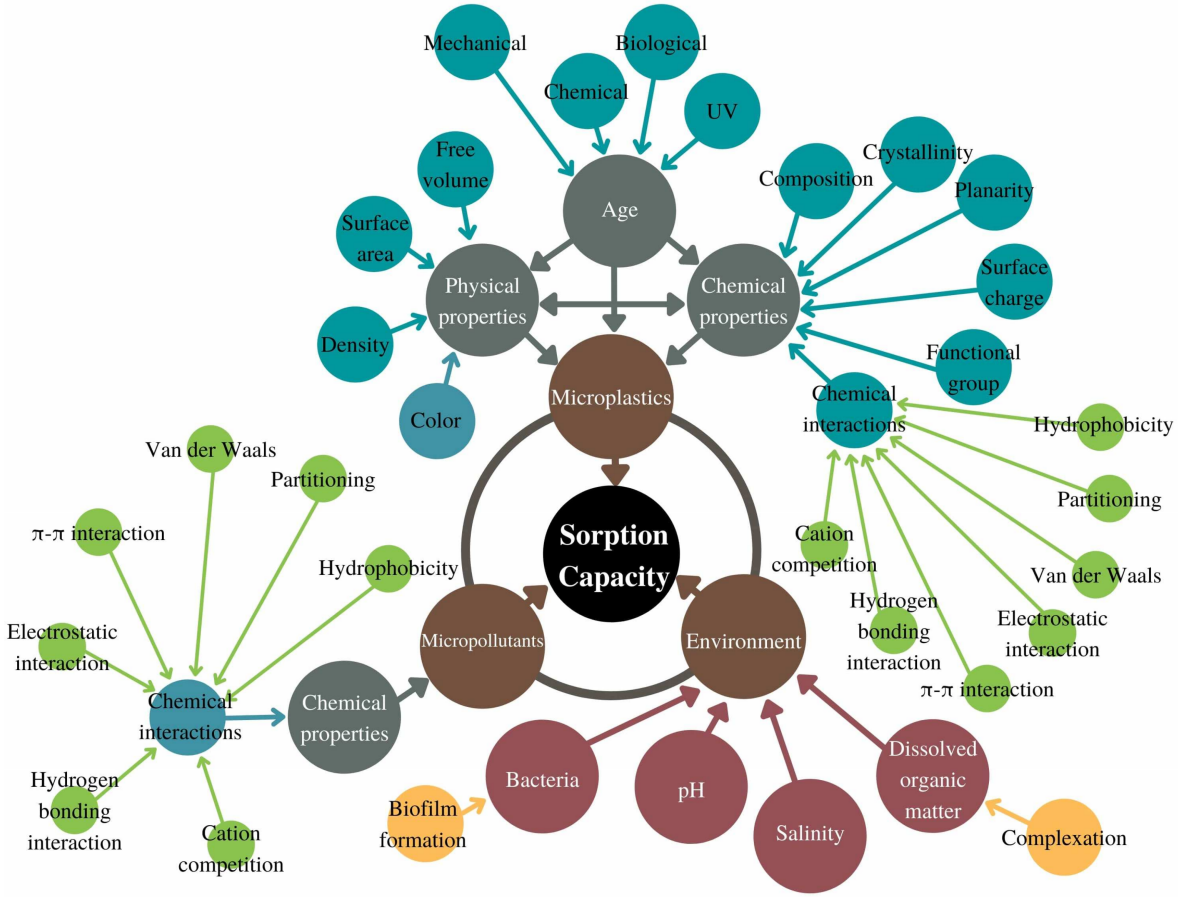

Figure 1. Major factors and sub-factors influencing the sorption capacity of microplastics to micro-pollutants.

Chemical Properties of Microplastics and Micro-Pollutants

Microplastic chemical properties, such as composition, crystallinity, planarity, surface charge, functional group, and chemical interaction potential, could also be considered as factors that have an influence on sorption capacity [74–77]. A decrease in crystallinity found in rubbery plastics with amorphous regions, such as polyethylene, results in increased hydrophobic organic compounds (HOCs) sorption compared to glassy plastics, such as polystyrene [78–80]. Further, the chemical properties of micro-pollutants could also play a significant role in their interactions with microplastics, thereby affecting the sorption capacity. The varying microplastic sorption capacity for micro-pollutants, hydrophobic and hydrophilic compounds, and heavy metals is strongly dependent on type of reaction, including hydrophobicity, partitioning, Van der Waals forces, electrostatic interaction, π-π interaction, and hydrogen bonding interaction [81].

Age of Microplastics

Microplastic age can be a pivotal attribute of sorption capacity alteration. Ageing represents the accumulation of changes in microplastics over time, including changes in physicochemical properties [81]. The increased frequency or induction of oxygen-containing functional groups (e.g., hydroxyl, carbonyl, and formyl groups) occurs after the particles' exposure to ageing [82–84]. It has been reported that oxygen-containing functional groups, emerging during the microplastic ageing process, could interact with hydrophilic micro-pollutants by forming hydrogen bonds and increase the affinity between these chemicals [81,82,85]. The relatively high concentration of heavy metals found on aged polyethylene compared with the pristine polyethylene terephthalate (PET) can be

related to the higher partition coefficient, resulting from the different chemical properties and biofilms [86,87].

Environmental Factors

In the process of determining microplastic sorption capacity, it is important to consider environmental factors such as surrounding bacteria, pH, salinity, and dissolved organic matter (DOM). Microplastics may interact with natural organic materials and form a biomolecular corona, which leads to changes in the microplastics' surface, thereby affecting surface charge, aggregation tendency, and sorption capacity [88]. The ability of bacteria to produce extracellular polymers during biofilm formation in order to facilitate the attachment and matrix development could result in an alteration in the microplastic sorption capacity with respect to physicochemical properties per se. For example, similar metal adsorption capacities of different types of microplastics might be related to similar biofilm distributions on the microplastics, regardless of the salinity conditions of microplastic deployment sites and the biofilm formation time [89].

In addition to influencing differences in bacterial populations in terms of biofilm formation, the pH and salinity of marine waters can result in both positive and negative effects on the micro-pollutant sorption capacity of microplastics. A decrease in seawater pH in marine environments suffering from increased acidification caused by the uptake of increasing carbon dioxide from the atmosphere [90] may play the role of proton donor to the microplastic surface and the increasing cationic characteristics of microplastics. This change, in turn, can lead to the higher adsorption of anionic micro-pollutants, including tylosin and perfluorooctanesulfonic acid (PFOS), on cationic-enriched microplastics through electrostatic interactions [76,91]. pH affects the sorption capacity of trace metals, such as Cadmium (Cd), Cobalt (Co), Nickle (Ni), Lead (Pb), and Chromium (Cr), on high-density polyethylene in saltwater via increased competition, decreased chromate ion activity, as well as complexation and free ion interaction with saltwater cations [92].

Salinity is an environmental factor of considerable importance, influencing the microplastic sorption capacity in marine systems, as it can affect micro-pollutant solubility in aqueous phases and micro-pollutant partitioning in other phases [93]. The increased amount of salt dissolved in a body of water enhances the sorption capacity of microplastics to 3,3′,4,4′-Tetrachlorobiphenyl (PCB77), lubrication oil, and other micro pollutants [76,94–96]. Conversely, increased salinity was shown to decrease the sorption capacity of dichlorodiphenyltrichloroethane (DDT) and ciprofloxacin on microplastics due to cation competition for sorption sites [74,83,97]. However, there were no observed salinity-related effects on phenanthrene (Phe), as the salt content in water did not affect the aqueous solubility and pore-filling mechanism of Phe [97].

Dissolved organic matter (DOM) is an important factor in determining microplastics' sorption capacity due to its diversity of chemical components leading to complex interactions. DOM components such as humic and fulvic acids have been reported to both increase and decrease sorption capacity in several different studies, indicating complexities of the interactions that are yet be fully described [98–100]. Fulvic acid negatively affected the sorption capacity of tetracycline on microplastics, as tetracycline was deemed to sorb onto DOM rather than microplastics [98]. Likewise, microplastics could adsorb fewer hydrophobic organic compounds (HOCs) due to the increasing desorption from the presence of dissolved organic matter [99]. Yet, the positive effect of the role of dissolved organic matter bridge formation in the micro-pollutant-microplastic surface complex might be a reason for the increased sorption capacity of oxytetracycline on aged microplastics [100].

3.2. Microplastics: The Potential for Microplastic-Sorbed Micropollutant Bioaccumulation in Aquatic Organisms

The term bioaccumulation refers to the net result of processes by which organisms uptake substances both directly from the abiotic environment (e.g., air, water, and soil or sediment) as well as indirectly from dietary sources and then transform and ultimately eliminate them [101]. In this framework, substances can be various micro-pollutants adsorbed on microplastics that act as their delivery vehicle to organisms. Once microplastics have entered the organism, we describe five possible scenarios of interaction between micro-pollutants and microplastics in terms of sorption and desorption related to micro-pollutant bioaccumulation in organisms [77,102].

The first scenario assumes that the sorption ability under aquatic conditions and the desorption ability under the gut conditions of organisms are high. This possibility could allow microplastics to act as a vehicle with the ability to transfer micro-pollutants in the organism. The presence of microplastics with high sorption and desorption abilities could enhance the bioaccumulation of venlafaxine in the hepatic tissue of Oriental weatherfish, *Misgurnus anguillicaudatus* [103]. Moreover, the rapid desorption of organic micro-pollutants under gut conditions could increase the bioaccumulation of phenanthrene, DDT, perfluorooctanoic acid (PFOA), and di-2-ethylhexyl phthalate (DEHP) [104].

The second scenario assumes a high sorption ability in aquatic environments and a low desorption ability in the body of organisms that could lead to low bioaccumulation. However, the high sorption of micro-pollutants on microplastics may nevertheless adversely affect the health of organisms. For example, mussels exposed to relatively high concentrations of fluoranthene sorbed on polyethylene showed low bioaccumulation levels but an increase in tissue alterations and antioxidant biomarker levels [105].

In the third scenario, the occurrence of low sorption ability in the environment coupled with high desorption ability in the body could also lead to low bioaccumulation. For example, despite the low PE adsorption capacity and subsequent lower concentrations of polybrominated diphenyl ethers (PBDEs) and bifenthrin on PE, these chemicals bioaccumulate in amphipods and midge *Chironomus tepperi*, respectively. This contradiction is explained by PBDEs and bifenthrin having low sorption on polyethylene in aqueous phases but the ability to be almost completely desorbed from PE microplastics inside the organisms, therefore allowing for significant bioaccumulation [106,107].

The fourth scenario assumes that the sorption and desorption abilities are low in both the environment and organism (digestive tract) conditions. This combination is likely to result in low bioaccumulation, as illustrated by the studies of the exposure of fluoranthene-sorbed microplastic in mussels (*Mytilus* spp.) [105].

The fifth scenario considers the high sorption and low desorption abilities of microplastics, but only inside an organism (internally). Such a variant could assist in the depuration or removal of micro-pollutants from the affected organism. High sorption could trap and remove micro-pollutants via excretion, leading to the lower bioaccumulation of micro-pollutants. Such processes were observed when contaminated polychlorinated biphenyls (PCBs) in feed were adsorbed to and transferred by virgin polyethylene in simulated gut conditions [108].

Therefore, it is highly likely that a range of potential bioaccumulation outcomes can be attributed to the physicochemical properties of microplastics (such as sorption and desorption abilities), the environmental conditions, the exposed organism characteristics, and the position of an organism in a food chain [97,109–111] (Figure 2).

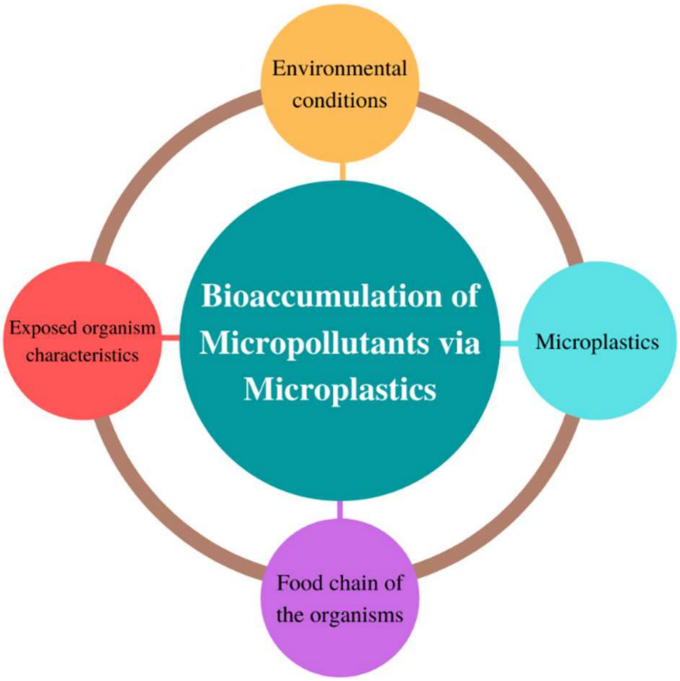

Figure 2. Factors influencing the bioaccumulation of micropollutants in an organism from exposure to microplastics as a vector.

3.3. Joint Effects of Microplastics and Micropollutants

The extent of the accumulation of micro-pollutants by sorption onto microplastics not only affects their bioaccumulation in aquatic organisms but also influences their bioavailability and toxicity [112,113]. The bioavailability of micro-pollutants is a measure of their accessibility to biota in the environment, and it is one of the key factors controlling the uptake of the micro-pollutants adsorbed on microplastics in the bodies of organisms, the transfer of these micro-pollutants, and the magnitude of the toxic effects on exposed organisms [114]. In addition to sorption, biomolecular corona formation, the entrapment of micro-pollutants in flocs, and aggregates of microplastics can cause further alteration of their bioavailability, resulting in altered toxicity, particularly antagonistic effects, in affected organisms [88,115]. Different interactive toxic effects occurring in the mixtures of microplastics and micro-pollutants in organisms were observed (additive, synergistic, antagonistic, and potentiating), dependent both on the chemical combination and the measured endpoint [115].

An additive effect is generally considered as the interaction in which two or more chemicals or actions used in combination produce a total effect equal to the sum of the individual effects [116], indicating no direct connection between the two substances or actions, and defined as non-interaction or inertism [117]. Deviations from the additive effect can be synergistic or antagonistic [116]. Synergistic interaction between chemicals is indicated by a significantly stronger observed effect of the chemical mixture than that predicted from a single chemical, whereas an antagonistic interaction is indicated by a significantly weaker effect of a mixture than that expected from a single compound [116]. Similar to the synergistic effect, a potentiating effect occurs when the combined effects of two or more chemicals are significantly greater than the sum of the effects of individual chemicals. In addition, potentiation also includes a situation where a chemical that typically has no observed effects per se could enhance the effects of another chemical, leading to an increase in the observed effects of the second chemical [118]. The combination of microplastics and micro-pollutants can exhibit distinct effects under varying conditions and endpoints.

The additive effect on oxidative stress and cellular damage was reported in the gills of *Dicentrarchus labrax* juveniles exposed to a combination of microplastics and mercury at a low concentration. However, different tissues with different physiologic systems and

functions show heterogeneity in response to the same chemical exposure, and in this study, synergistic effects could be observed in hepatic tissue [119]. Likewise, the diverse effects from the exposure to florfenicol and microplastic mixtures in different concentrations could be detected in *Corbicula fluminea*. Additive, synergistic, and potentiating effects, i.e., the inhibition of acetylcholinesterase (AChE) activity, the inhibition of feeding, and the reduction of isocitrate dehydrogenase (IDH) activity, respectively, were documented. Furthermore, the toxic synergism of the mixtures was observed in the increase in gill glutathione S-transferase (GST) activity and the foot lipid peroxidation (LPO) level in *C. fluminea* [120].

The chemistry of functional groups in microplastics influences their micro-pollutant sorption capacity, which in turn affects the bioavailability and toxicity of micro-pollutants and implicitly links the altered functional groups of microplastics with changes in micro-pollutant bioavailability and toxicity. The combination of titanium dioxide nanoparticles (TiO_2 NPs) with neutral and positively charged microplastics, virgin polystyrene, and aminated polystyrene (PS-NH_2) displayed the additive toxicity, while the negatively charged microplastics, carboxylated polystyrene (PS-COOH), exhibited antagonistic toxicity towards *Chlorella* sp. [121]. On the contrary, another study indicated that PS-NH_2 could be attributed to the antagonistic toxicity of nickel, while PS-COOH could be associated with the synergistic toxicity of nickel on *Daphnia magna* [122]. PS-NH_2 could also play a role in the reported antagonism of glyphosate toxicity on *Microcystis aeruginosa*, as the NH_2-functional group could affect the sorption ability, resulting in the decreased concentration and bioavailability of glyphosate in the exposure medium [112].

3.4. Fate of Glyphosate in the Environment and Its Toxic Effects on Organisms

As the global population continues to expand, the growing demand for food production needs to be supported by an efficient and sustainable agricultural system. The innovations of efficient herbicides and herbicide-tolerant genetically modified (GM) crops represent one of the solutions that not only protect plants from weeds but also increase worldwide crop production, leading to both economic and labor benefits [123,124]. However, the introduction of GM crops is also causing an increase in herbicide use, leading to chemical pollution in soil, water, and air [125]. Moreover, an adaptive evolutionary processes act in weeds develops resistance against herbicides over time, thereby requiring higher amounts to control resistant weed strains. This implies that having herbicide-tolerant GM crops nearby would increase the risk of exposure to the higher levels of herbicides [126–128]. A typical example of herbicide-tolerant GM crops is genetically modified glyphosate-tolerant plants, which are designed to tolerate glyphosate, an active ingredient of a broad-spectrum and non-selective organophosphate herbicide.

Glyphosate-based herbicides (GBHs) have become one of most widely applied herbicides worldwide in terms of volume due to their various advantages in terms of utility and economy [129,130]. Their extensive application in agriculture received considerable attention in many countries such as Argentina, Brazil, Canada, and the United States of America due to the intense cultivation of glyphosate-tolerant GM plants [131–136]. GBHs' overuse could be confirmed by the residue concentrations of glyphosate and its primary microbial metabolite product (aminomethylphosphonic acid; AMPA) in Argentina—this being up to almost 100 mg/kg, which is almost a fivefold increase compared to the maximum residue limit for soybeans used in feed and food [137,138]. Additionally, a glyphosate residue concentration of 1481 $\pm$ 73 µg/L—exceeding the legally permitted maximum contaminant level (MCL) in drinking water regulated by the European Union, 0.1 µg/L, by approximately 15,000-fold—was reported in natural water in Brazil [139,140]. GBHs are intentionally applied to the foliar part of undesired plants; however, the uncontrolled application of GBHs could contaminate soils in and around the treated areas and be transformed into metabolites by biodegradation, photo-degradation, and complex chemical reactions [141].

AMPA, the main metabolite of glyphosate, can present toxic effects similar to original compounds [142]. The half-life of glyphosate depends on the physical and chemical properties of the external environment. In natural freshwater, it is estimated to be more

than 60 days [143]. In seawater, it could persist for at least 47 days under low-light conditions at 25 °C, increase to 267 days in dark environments at 25 °C, and to 315 days in the dark at 31 °C prior to being metabolized by seawater microorganisms [144].

When sprayed GBHs and AMPA enter soils, several transport mechanisms are present that reduce the spreading of the contamination (Figure 3). GBHs' molecules could be taken up by plant tissues, resulting in biotransformation products, particularly AMPA, and their accumulation in the tissues [145]. In addition, the molecules could be immobilized through sorption onto soil particles, organic matter, and clay minerals or lost to the atmosphere through volatilization and vaporization and ultimately redeposited into environments via drops of rain [146,147]. The molecules that could not bind strongly with, or desorb from, soil particles tend to leach into groundwater basins or dissolve and suspend in runoff waters [148–150]. Strongly bound GBH and AMPA molecules could also be moved off site to waters by water erosion [147]. Aside from the above, the occurrence of GBHs in water could result from aquatic weed control efforts by which GBHs are intentionally applied directly to the water, as well as from the disposal of GBH waste into water sources [151,152]. Hence, the continual use, release, and transfer of GBHs and AMPA into aquatic environments could result in the prolonged exposure of aquatic organisms to the chemicals through contaminated feed intake and polluted aquatic habitats, thereby increasing the risk of the induction of detrimental effects.

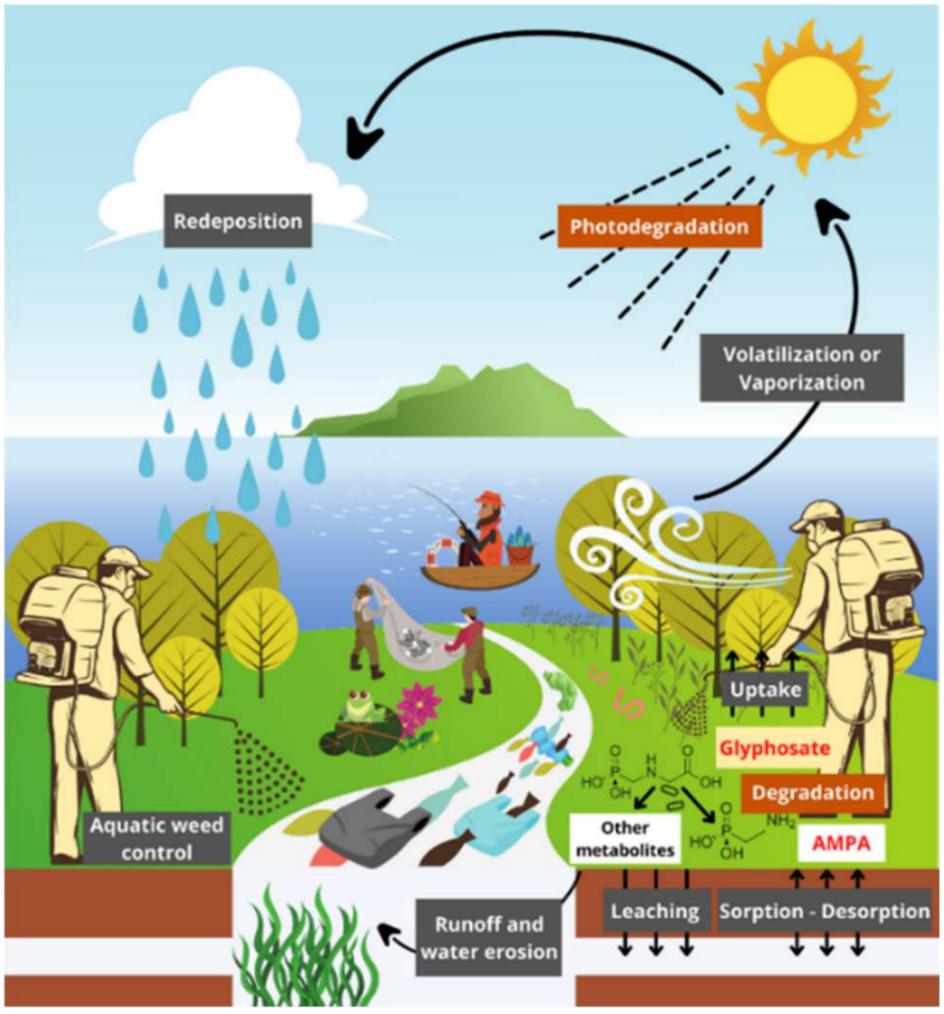

Figure 3. Glyphosate fate and transport.

In general, glyphosate's mode of action after the absorption of GBHs through foliage is to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in the shikimic acid pathway. This inhibition results in the deficiency of essential aromatic amino acids responsible for protein synthesis, such as phenylalanine, tryptophan, and tyrosine, ultimately leading to the stunted growth, leaf malformation, desiccation, and death of target plants [153]. This pathway is absent in animals and is considered to contribute to the nonexistent or low toxicity of glyphosate toward animals [154–156]. Nonetheless, comprehensive toxicology investigations in different animal species have illustrated the hazard potential in various organisms following acute and chronic exposure to glyphosate/GBHs. Multiple studies confirmed different effects on environmental and animal health such as: changes in the community structure and diversity in plankton [157,158]; decreased acetylcholinesterase activity in mussels, shrimp, and fish [159–162]; excessive reactive oxygen species formation or impaired antioxidant capacity in plankton, worms, mussels, shrimp, prawns, and fish [159,160,162–168]. Studies also showed histopathological changes in fish and frogs [166,168–170]; endocrine disruption and reproductive impairment in plankton and fish [157,171,172]; metabolic alterations in fish and frogs [166,173], behavioral changes in shrimp, fish, and frogs [168,173–175]; growth and development inhibition and increased mortality in plankton, shrimp, fish, and frogs [176–180]. This information is summarized in Table 1.

In human cells, glyphosate and GBHs can act as endocrine disruptors, affecting the estrogen and androgen pathways [181–183], damaging the neural system, genetic materials, and ultrastructure of the cells [184–186], and enhancing the proliferation of the breast cancer cell line [183]. Considering multiple deleterious effects of glyphosate and GBHs, the International Agency for Research on Cancer (IARC) classified glyphosate into Group 2A, "probably carcinogenic to humans", in 2017 [187]. Furthermore, adjuvants added in commercial GBH formulations to increase the efficacy of GBHs could also enhance the toxicity and/or bioaccumulation of glyphosate, in some cases displaying increased toxicity compared to the parent material [172,182,188]. In addition, the detection of glyphosate in worm and fish tissues indicates that there is a risk of glyphosate bioaccumulation at multiple trophic levels, from producers to consumers [162,163,179], in spite of its high-water solubility and moderate bioaccumulation potential [189].

Table 1. Studies of the toxic effects of glyphosate and its commercial formulations on organisms from different trophic levels. CC = community composition, GM = growth and mortality, RT = reproductive toxicity, MT = metabolic toxicity, OS = oxidative stress, NT = neurotoxicity, GE = gene expression, MM = microbiome modulation, HT = haemotoxicity, BC = behavior change, GT = genotoxicity, CT = cytotoxicity, HP = histopathology, IT = Immunotoxicity, ET = endocrine toxicity, ↑= Increased, ↓= Decreased, ↕ = Altered.

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Producers								
Bacterioplankton	Bacterioplankton community	96% pure 2-^{13}C-glyphosate	6 days	EC: 100 µg/L	CC		Bacterial richness and diversity ↓	[158]
Zooplankton	*Daphnia magna*	Sumin Atut 360 SL	12, 24, 48 h	LC50-12 h: 76.67 mg/L	GM		Mortality ↑, head width ↓	[178]
				LC50-24 h: 36.2 mg/L				
				LC50-48 h: 21.34 mg/L				
	Cyclops vicinus			LC50-12 h: 207.89 mg/L			Mortality ↑, body length ↓	
				LC50-24 h: 159.8 mg/L				
				LC50-48 h: 92.93 mg/L				
Zooplankton	*D. magna*	Eskoba®	15 days	LC50-48 h: 29.48 mg a.e./L	GM, CC, RT		Mortality ↑, growth and fecundity ↓	[171]
		Panzer Gold®		LC50-48 h: 2.12 mg a.e./L				
		Roundup® Ultramax		LC50-48 h: 11.68 mg a.e./L				
		Sulfosato Touchdown®		LC50-48 h: 1.62 mg a.e./L				
	Ceriodaphnia dubia	Eskoba®		LC50-48 h: 14.49 mg a.e./L				
		Panzer Gold®		LC50-48 h: 0.54 mg a.e./L				
		Roundup® Ultramax		LC50-48 h: 4.84 mg a.e./L				
		Sulfosato Touchdown®		LC50-48 h: 0.31 mg a.e./L				
Zooplankton	Zooplankton community	Sulfosato Touchdown®	30 days	EC: 2.7 mg/L	CC, RT		Diversity ↓, time of the first hatching ↕, time of the maximum hatching ↕, frequency of the hatchings ↕	[157]
Zooplankton	*Notodiaptomus carteri*	Sulfosato Touchdown®	10 days	EC: 0.81 mg/L	MT, OS		Superoxide dismutase (SOD) and glutathione-S-transferase (GST) activities ↑	[165]
			30 days	EC: 0.38 mg/L	GM		Growth and development ↓	
Consumers								
Worm	*Lumbriculus variegatus*	Glyphosate analytical standard	4 days	EC: 0.05 mg/L	OS	+	SOD and biotransformation enzyme soluble GST ↑	[163]
		Roundup Ultra®		EC: 0.05 mg/L				
Mussel	*Limnoperna fortunei*	Glifosato Atanor®	28 days	EC: 6 mg/L active principle and 2.5% surfactant Impacto®	OS, MT, NT		GST and alkaline phosphatase activities ↑, carboxylesterase activity ↓	[190]

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Mussel	*Mytilus galloprovincialis*	Glyphosate analytical standard	21 days	EC: 10 µg/L	GE		Energy metabolism and $Ca2^{+}$ homeostasis ↕, cell signaling ↕, endoplasmic reticulum stress response ↕	[191]
Mussel	*M. galloprovincialis*	Glyphosate and AMPA analytical standards	7 and 21 days	EC: 100 µg/L	GE, MM		Physiological homeostasis and dysbiosis of gut microbiota ↕	[192]
Mussel	*M. galloprovincialis*	Glyphosate and AMPA analytical standards	7, 14 and 21 days	EC: 100 µg/L	OS, NT, HT		Hemocyte parameters ↕, antioxidant enzyme activity ↕, acetylcholinesterase (AChE) activity ↓	[160]
Shrimp	*Caridina nilotica*	Roundup®	48 and 96 h	LC50-48 h (Neonate): 4.5 mg/L a.e.	GM, BC		Mortality ↑, behavior ↕	[174]
				LC50-48 h (Juvenile): 9.4 mg/L a.e.				
				LC50-48 h (Adult): 37.1 mg/L a.e.				
				LC50-96 h (Neonate): 2.5 mg/L a.e.				
				LC50-96 h (Juvenile): 7.0 mg/L a.e.				
				LC50-96 h (Adult): 25.3 mg/L a.e.				
Shrimp	*C. nilotica*	Roundup®	25 days	EC: 2.2 mg/L	GM		Growth rate and feed utilization ↓, molting frequency ↑	[180]
Shrimp	*C. nilotica*	Roundup®	96 h and 21 days	EC-96 h: 4.3 mg/L	NT		AChE activity ↓	[161]
				EC-21 d: 2.2 mg/L				
Shrimp	*C. nilotica*	Roundup®	96 h and 21 days	EC-96 h: 4.3 mg/L	OS		Lipid peroxidation (LPO) ↑	[167]
				EC-21 d: 2.2 mg/L				
Shrimp	*Macrobrachium nipponensis*	Roundup®	48 and 96 h	LC50-48 h: 57.684 mg/L	GM, HT, OS, NT, GT		Mortality ↑, total hemocyte count ↓, SOD and catalase (CAT) levels ↓, total antioxidant capacity ↓, malondialdehyde (MDA) ↑, hydrogen peroxide ↑, protein carbonyl ↑, AChE activity ↓, MN frequency of hemocyte ↑, comet ratio and %DNA in the tails ↑	[159]
				LC50-96 h: 11.237 mg/L				
Prawn	*Macrobrachium potiuna*	Roundup WG®	7 and 14 days	EC: 0.0065 mg/L	CT		Altered ultrastructure of hepatopancreas and impaired R cells	[193]

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Prawn	*M. potiuna*	Roundup WG®	7 and 14 days	EC: 0.0065 mg/L	OS, GE		Antioxidant gene expression in hepatopancreas ↕	[164]
Fish	*Clarias gariepinus*	Delsate®	48 h and 91 days	LC50-48 h: 75 mg/L EC-91d: 5, 10, 15 mg/L	GM	+	Mortality and residues in muscles ↑	[179]
Fish	*Markiana nigripinnis* *Astyanax lacustris*	Mixture of pesticides including glyphosate (Roundup®)	21 days	Field pesticide application	OS, NT	+	Biometric parameters and organismic indices ↕, antioxidation enzyme activities ↕, oxidative damage, AChE activity ↓	[162]
Fish	*Danio rerio*	Roundup® GC liquid glyphosate concentrate Glyphosate analytical standard	21 days	EC: 10 mg/L a.e. EC: 10 mg/L	RT		Embryo mortality ↑, premature hatching ↑, reproductive gene expression ↕, egg ↓	[172]
Fish	*Oncorhynchus mykiss*	Commercial formulation	6, 12, 24, 48, 96 h 21 days	EC: 2.5 mg/L EC: 5 mg/L	HP, BC, OS		Glutathione peroxidase and CAT activities ↑, antioxidant gene expression ↕, swimming performance ↓, histopathological liver damage	[168]
Fish	*Carassius auratis*	Nongteshi®	90 days	EC: 0.2 mmol/L	HT, HP, OS, MT		Blood biochemistry ↕, renal tissue ↓, oxidative stress mechanisms ↕, metabolisms ↕	[166]
Fish	*Cyprinus carpio* L.	Commercial formulation	168 h	LC50-96 h: 520.77 mg/L	IT, HP		Contents of cytokines ↕, histopathological damage	[169]
Frog	*Rana dalmatina*	Glyphogan®	21 days	EC: 2 mg a.e./L	BC		Anti-predator behaviors ↕	[175]
Frog	*Dendropsophus molitor*	Roundup Active®	30 days	EC: 325 µg a.e./L	HP		Hepatic tissue injuries	[170]
Frog	*Physalaemus cuvieri* *Hypsiboas pardalis*	Glyphosate analytical standard	96 h	LC50-96 h: 115 mg a.e./L LC50-96 h: 106 mg a.e./L	GM		Mortality ↑	[177]
Frog	*Xenopus laevis*	Roundup® Kilo Max® Enviro Glyphosate®	96 h	LC50-96 h: 1.05 mg a.e/L LC50-96 h: 207 mg a.e./L LC50-96 h: 466 mg a.e./L	GM		Mortality ↑, malformation ↑, growth ↓	[176]
Frog	*Microhyla fissipes*	KISSUN®	10 days	LC50-10 d: 77.5 mg/L	GM, BC, MT		Mortality ↑, growth ↓, swimming behavior ↕, metabolism ↕	[173]
Human	*Homo sapiens*	Roundup® Glyphosate analytical standard	24 h	EC: 2% EC: 2%	ET	+/-	Aromatase activity and mRNA levels ↕	[182]
Human	*H. sapiens*	Glyphosate and AMPA analytical standards	24 h	EC: 100 µM	NT		Neurological damage, glucose metabolism ↕	[184]

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Human	*H. sapiens*	Glyphosate analytical standards	24 h	EC: 0.5 ppm	ET, CT		Disruption of the androgen receptor and estrogen receptors, aromatase transcription and activity ↕, DNA damages	[181]
		Roundup Express®						
		Bioforce®						
		Grands Travaux®						
		Grands Travaux plus®						
Human	*H. sapiens*	Glyphosate analytical standards	6 and 24 h	EC: 10^{-12} M	ET, GE		Human hormone-dependent breast cancer ↑, expression of the estrogen receptors α and β ↕	[183]
Human	*H. sapiens*	Glyphosate analytical standards	4 h	Exposure concentration: 1000 µM	CT, GT		No significant cytotoxicity and genotoxicity	[186]
		Roundup Mega®		EC: 250 µM			Cell death and DNA damage	
		Fozat 480®		EC: 500 µM				
		Glyfos®		EC: 250 µM				
Human	*H. sapiens*	Glyphosate analytical standards	48 h	EC: 5 mM	NT, GE, OS		MDA levels ↑, nitric oxide and reactive oxygen species production ↑, caspase 3/7 activity ↑, neurological and apoptotic gene expressions ↕	[185]
		AMPA analytical standards		EC: 10 mM				

mg/L a.e. = mg/L acid equivalence. EC = effective concentration. LC50 = lethal concentration 50.

4. A Case Study of Glyphosate Transport via Polyethylene Microplastic Fomites

Shrimp is an economically important aquaculture species, and global farmed shrimp production was estimated to be over 4.5 million tons in 2021, with a growth rate of almost 9% globally [194]. Intensive shrimp production in ponds lined with polyethylene or polyvinyl chloride liners is commonly used in shrimp culture [195]. The use of liners supports the effective removal of settled organics during shrimp grow out, the reduction of pond cleaning and preparation time, and the prevention of acid-sulfate soils contamination [196]. Polyethylene microplastic, a polymer commonly found in surface seawater and sediment [16–18], may enter shrimp farming and the environment through water runoff, flooding, and winds. The quantification of the release of microplastics into shrimp environments, however, is uncertain, and qualitative assessment assigning relative ratings of high, medium, or low is needed to address the environmentally heterogenous levels [197].

Regardless of the characterization of exposure routes, there is solid evidence that the digestive tracts of commercially harvested shrimp species are contaminated with microplastics, suggesting that shrimp are routinely exposed to microplastics during their production cycle [198]. Routine use of the plastic pond liners in farmed shrimp production and their subsequent degradation into microplastic particles, taken together with environmental microplastics exposure, also suggest a high likelihood of microplastic–shrimp interactions and therefore an increase in the associated risks of a downstream production chain. In addition to microplastics, the likelihood of shrimp exposure to GBHs is also high due to their widespread intensive use and environmental persistence, especially in agricultural areas surrounding shrimp pond farming operations. It has been reported that GBHs can reach aquatic environments through contaminated feed sources, rainfall, leaching, runoff, and intentional introduction [131–138,143,144,146–152], and this can include aquaculture establishments.

Consequently, the simultaneous exposure of shrimp to both microplastics and GBHs may occur in shrimp aquaculture pond environments and lead to toxicological interactions altering the bioavailability, effects, and bioaccumulation of glyphosate and its metabolites in aquacultured shrimp. Furthermore, shrimp behavior and physiology play an important role in the exposure and intoxication of GBHs-sorbed polyethylene microplastics. The feeding behavior of shrimp as non-selective opportunistic benthic feeders leads to the ingestion of various particles that accumulate at the bottom of their habitats [199,200] and can contribute to the risk of consuming GBHs-sorbed polyethylene microplastics in ponds with polyethylene liners. Furthermore, the physiological processes of shrimp growth and development, such as the molting cycle, can also be critically linked to GBHs-sorbed polyethylene microplastics toxicity, as only the intact exoskeleton is acting as a defense barrier. As demonstrated during the pathogenesis of a white spot syndrome virus (WSSV), molting can influence the disease susceptibility, and shrimp in the post-molt stages are more susceptible to WSSV infection via immersion than those in the pre-molt stage [201]. Shrimp during the molting process (ecdysis) have the highest mortality rate, followed by the animals in the post-molt and pre-molt stages, respectively [202].

When the concurrent presence of polyethylene microplastics and glyphosate in a shrimp production environment is considered together with the shrimp feeding behavior and molting cycle processes, two potential exposure routes of shrimp to GBHs–polyethylene microplastics (GBHs–PE) fomites emerge: oral and water. Oral exposure to GBHs–PE microplastics from the ingestion of contaminated feed and polluted water is considered an important hazard. Following the inadequate application of GBHs on plants, glyphosate residues and their adjuvants in feed ingredients can end up in commercial or self-prepared shrimp feed. During feeding, those pesticide residues can interact with ubiquitous microplastics or fragmented pond liners in the water and at the bottom of a pond. Furthermore, runoff from a field can contain both microplastic polyethylene and GBH residues either separately or already adsorbed. Their passage through the water column already infiltrated with microplastics from other sources, including pond liners, further allows for interactions

and the adsorption of GBHs on PE microplastics. This situation results in the increased availability of GBHs–PE for shrimp to ingest during their normal feeding behavior [203]. Besides the direct consequences and toxic effects of GBHs–PE microplastics in shrimp, possible indirect effects along the gastrointestinal tract can also be of significance, such as interference with the natural shrimp intestinal microbiome and the disturbance of the microbiome roles in disease protection, improved feed energy utilization via microbial digestion, and the production of vitamins.

The other possible exposure route is through the water, i.e., the waterborne route. The waterborne GBHs–PE microplastics in a shrimp farm could originate from different sources, including the fragmentation and weathering of the deteriorating pond liner, wind, runoff, and flooding, in combination with glyphosate residues, its metabolites, adjuvants released from the contaminated feed in water, and GBHs polluted from soil surface runoff or by leaching. Such GBHs–PE particles may enter shrimp via their gill filaments as well as their susceptible epidermis during the molting process and spread via the hemolymph and other tissues, causing alterations in different body systems. Despite the poorly understood adverse effects of GBHs–PE microplastics on shrimp, many studies illustrating their adverse effects on other organisms have been reported. Polyethylene microbeads combined with glyphosate showed a modified toxicological effect on the mortality rate of *Daphnia magna*. The different sorption capacities of PE to various glyphosate formulations significantly increased or reduced the mortality rate of *Daphnia magna* [204]. The interaction between polyethylene microplastics and GBH decreased lethal concentration 50 (LC50) and increased glutathione S-transferases (GST) activity compared with the direct negative effects of individual polyethylene microplastics and GBH additions in *Scinax squalirostris* [205]. The chronic co-exposure of polyethylene microplastics and glyphosate to *Cyprinus carpio* L. caused a decrease in swimming activity, changed the morphological integrity and dysfunction of the intestinal barriers, altered gut microbiota abundance and diversity, and modified the metabolic profiles associated with an altered amino acid and lipid metabolism [206].

Due to our limited knowledge about GBHs–polyethylene microplastics' fate and transport in a real environmental matrix and the gaps in our understanding regarding different adverse outcome pathways in shrimp bodies, it is still difficult to confirm this new contaminant class as being a serious hazard for shrimp in aquaculture. The lack of information concerning the toxicity and bioaccumulation of GBHs–PE microplastics in shrimp requires additional time and effort to assess the health risks to shrimp and consumers, interfering with providing guidelines for sustainable shrimp production to reduce the risks of introducing microplastic-pesticide contaminants in the food supply.

5. Conclusions

Global plastic production increased rapidly, and continues to do so, with dramatic impacts on ecosystems due the uncontrolled deposition of plastic waste and ineffective plastic waste management. Microplastic-sized fragments of any type of plastic can act as vehicles for surrounding micro-pollutants and threaten aquatic ecosystems and organisms. An overview of the reported and potential interactions between microplastics and environmental micro-pollutants, as well as combinations of their toxic effects on aquatic organisms, is presented. The sorption of micro-pollutants on microplastics and their interaction mechanisms, such as the physicochemical properties of micro-pollutants and microplastics and the environmental conditions, can change their environmental behaviors, bioavailability, and effects, resulting in altered outcomes. The possible adverse outcomes of glyphosate and its commercial formulations (GBHs) are summarized. Moreover, the case of co-exposure and the combination effects of polyethylene (plastic that is commonly used as a pond liner in shrimp farms) and GBHs on aquacultured shrimp are analyzed. Nevertheless, the evaluation of actual risks from the co-exposure of GBHs and polyethylene to aquacultured shrimp is difficult due to the limited number of non-standardized studies on the repercussions of GBHs–PE microplastics. Their effects on shrimp are currently

uncertain. Further research regarding GBHs–PE microplastic toxicity and bioaccumulation in shrimp is needed to assess the health risks to shrimp and consumers.

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