Aged Polyethylene Microplastics and Glyphosate-based Herbicide Co-exposure Toxicity in Pacific White Shrimp (*Litopenaeus vannamei*)

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Dedication

To all those who believed in me to finish my doctoral degree

and supported me throughout this process.

Without you, this dissertation would never have been possible.

“Failure after long perseverance is much grander than never to have a striving good enough to be called a failure.”

- George Eliot, Penguin Books, ed. 1973, Book II.

Old and Young, Chapter 22, Page 254

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ABBREVIATIONS

Abbreviation	Full name
↑	Increased
↓	Decreased
↕	Altered
AChE	Acetylcholinesterase
ALF3	Anti-lipopolysaccharide factor 3
AMP	Antimicrobial peptide
AMPA	Aminomethylphosphonic acid
ANOVA	Analysis of variance
ATCh	Acetylthiocholine
BC	Behavior change
B-cells	Blister-like or Blastozellen cells
Casp2	Caspase 2
CAT	Catalase
CC	Community composition
cDNA	Complementary deoxyribonucleic acid
Ct	Cycle threshold
CT	Cytotoxicity
CTL4	C-type lectin 4
cytMnSOD	Cytosolic manganese superoxide dismutase
DAMPs	Damage-associated molecular patterns
DDT	Dichlorodiphenyltrichloroethane

Abbreviation	Full name
DEHP	Di-2-ethylhexyl phthalate
dNTP	Deoxyribonucleotide triphosphate
DOM	Dissolved organic matter
dsDNA	Double-stranded deoxyribonucleic acid
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
EC	Effective concentration
EDTA	Ethylenediaminetetraacetic acid
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ET	Endocrine toxicity
GBH	Glyphosate-based herbicide
GBHs	Glyphosate-based herbicides
GBHs–PE	Glyphosate-based herbicides–polyethylene microplastics
GE	Gene expression
GM	Genetically modified
GM	Growth and mortality
GST	Glutathione S-transferase
GT	Genotoxicity
H&E	Hematoxylin and eosin
HOCs	Hydrophobic organic compounds
HP	Hepatopancreatic tissues
HP	Histopathology
HS	High sensitivity
hsp70	Heat shock protein 70
HT	Haemotoxicity

Abbreviation	Full name
IARC	International agency for research on cancer
IFN	Interferon
IL-8	Interleukin-8
IMD	Immune deficiency
IT	Immunotoxicity
LC50	Lethal concentration 50
L-DOPA	L-di-hydroxy-phenylalanine
LPO	Lipid peroxidation
MDA	Malondialdehyde
mg/L a.e.	mg/L acid equivalence
MM	microbiome modulation
mRNA	Messenger ribonucleic acid
MT	Metabolic toxicity
NEI	Neuroendocrine-immune
NF-κB p65	nuclear factor NF-kappa-B p65
NT	Neurotoxicity
OS	Oxidative stress
PAHs	Polycyclic aromatic hydrocarbons
PAMPs	Pathogen-associated molecular patterns
PBDEs	Polybrominated diphenyl ethers
PCB77	3,3′,4,4′-Tetrachlorobiphenyl
PCBs	Polychlorinated biphenyls
PE	Polyethylene
PEN3	Penaeidin 3

Abbreviation	Full name
PET	Polyethylene terephthalate
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PMF	Post-mitochondrial fractions
PO	Phenoloxidase
proPO	Prophenoloxidase
PRRs	Pattern recognition receptors
PS-COOH	Carboxylated polystyrene
PS-NH_2	Aminated polystyrene
qPCR	Quantitative polymerase chain reaction
R-cells	Resorptive cells
RNA	Ribonucleic acid
RNAi	Ribonucleic acid interference
ROS	Reactive oxygen species
RT	Reproductive toxicity
SOD	Superoxide dismutase
STAT	Signal transducers and activators of transcription
THC	Total hemocyte count/mL
TiO_2 NPs	Titanium dioxide nanoparticles
Toll1	Protein toll 1
US EPA	US Environmental Protection Agency
WSSV	White spot syndrome virus
β-actin	Beta-actin

1. LITERATURE REVIEW AND DATA SUMMARY

1.1. Introduction

Plastic, a versatile and omnipresent organic polymer, is one of the 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 (Helmenstine, 2020; Tiseo, 2021). 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 (Kane & Clare, 2019; Jingyi Li, Liu, & Paul Chen, 2018).

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” (L. Lebreton et al., 2018). Furthermore, the degradation of primary microplastic particles originally designed for commercial use in personal care and cosmetics products (Lei et al., 2017), in industrial applications (Auta, Emenike, & Fauziah, 2017), or originating from synthetic textiles shedding during laundering (De Falco, Di Pace, Cocca, & Avella, 2019) leads to the formation of smaller secondary microplastic particles (Rocha-Santos & Duarte, 2015).

1.2. Distribution, Abundance, and Importance of Microplastics in Aquatic environments

There is increasing evidence of ocean-based microplastics present in all marine environments, including the deep seas/trenches (Acosta-Coley et al., 2019; Courtene-Jones, Quinn, Gary, Mogg, & Narayanaswamy, 2017; Cozar et al., 2015; C. J. Moore, Moore, Leecaster, & Weisberg, 2001; Suaria et al., 2016; Vianello et al., 2013). One of the most commonly reported polymer types in marine surface water and sediment is polyethylene (PE) (Frias, Otero, & Sobral, 2014; Gomiero et al., 2019; Ng & Obbard, 2006; Suaria et al., 2016). Moreover, fibers and fragments were noted as prevailing microplastic shapes in the ocean and sediment (Desforges, Galbraith, Dangerfield, & Ross, 2014; A. L. Lusher, Burke, O'Connor, & Officer, 2014; Amy L. Lusher, Tirelli, O'Connor, & Officer, 2015; Suaria et al., 2016). However, the abundance, size, and concentration of observed microplastics can vary significantly across different sampling times and regions of the sampled marine environment (Akarsu, Kumbur, Gökdağ, Kıdeyş, & Sanchez-Vidal, 2020; Claessens, Meester, Landuyt, Clerck, & Janssen, 2011; A. L. Lusher et al., 2014; R. C. Thompson et al., 2004; Van Cauwenberghe, Vanreusel, Mees, & Janssen, 2013).

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 (Klein, Dimzon, Eubeler, & Knepper, 2018; L. C. M. Lebreton et al., 2017; Peng, Wang, & Cai, 2017; van Emmerik & Schwarz, 2020). 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 (Anderson et al., 2017; Lechner et al., 2014; Lima, Costa, & Barletta, 2014; Mason et al., 2016; Mintenig, Int-Veen, Löder, Primpke, & Gerdts, 2017; Su et al., 2016). Such variability suggests that a variety of locations, anthropogenic activities and environments, and sampling strategies were employed (Eerkes-Medrano, Thompson, & Aldridge, 2015). 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 (L. C. M. Lebreton et al., 2017; Magnusson & Norén, 2014; Mintenig et al., 2017; Murphy, Ewins, Carbonnier, & Quinn, 2016).

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 (Hidalgo-Ruz, Gutow, Thompson, & Thiel, 2012; A. L. Lusher et al., 2014). 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 (Mark A. Browne, Dissanayake, Galloway, Lowe, & Thompson, 2008; Graham & Thompson, 2009; Mazurais et al., 2015; Murray & Cowie, 2011; Welden & Cowie, 2016; Wright, Thompson, & Galloway, 2013). Irregularly shaped and sharp-edged

microplastics could cause abrasion and disrupt the integrity of gastrointestinal mucosa in living organisms (Ahrendt et al., 2020). 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 (Lithner, Larsson, & Dave, 2011; Rochman, Hoh, Kurobe, & Teh, 2013; Wardrop et al., 2016). More importantly, there is increasing evidence that microplastics can serve as a vehicle or concentrator for certain chemical and biological agents (micro-pollutants), and their ingestion could facilitate their transfer to organisms and cause adverse health effects or even death (Mark A. Browne, Niven, Galloway, Rowland, & Thompson, 2013; Guzzetti, Sureda, Tejada, & Faggio, 2018; Naik, Naik, D'Costa, & Shaikh, 2019).

1.3. Microplastics: Delivery Vehicles for Micro-pollutants 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 (Kwon, Chang, Hong, & Shim, 2017; W. Wang, Ge, & Yu, 2020; H. Zhang et al., 2020). Some recently observed effects include disruptions to the oceanic carbon cycle due to the increase in dissolved organic carbon (C.-S. Chen, Le, Chiu, & Chin, 2018) or adverse health effects in several organisms (Barnes, Galgani, Thompson, & Barlaz, 2009; Lithner, Damberg, Dave, & Larsson, 2009). 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 (Mark Anthony Browne, Galloway, & Thompson, 2007; R. Thompson, Swan, Moore, & vom Saal, 2009).

1.3.1. 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 (Sørensen et al., 2021). Due to mechanical, chemical, biological, and/or UV ageing processes, the microplastic particles' physicochemical and mechanical properties, texture, and appearance change over time (K. Zhang et al., 2021). These changes were noted as the main causes of the altered sorption capacity of microplastics to micro-pollutants (Göpferich, 1996; J. Liu et al., 2019; J. Wang et al., 2019). 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 affects the number of sorbed micro-pollutants (Fig. 1).

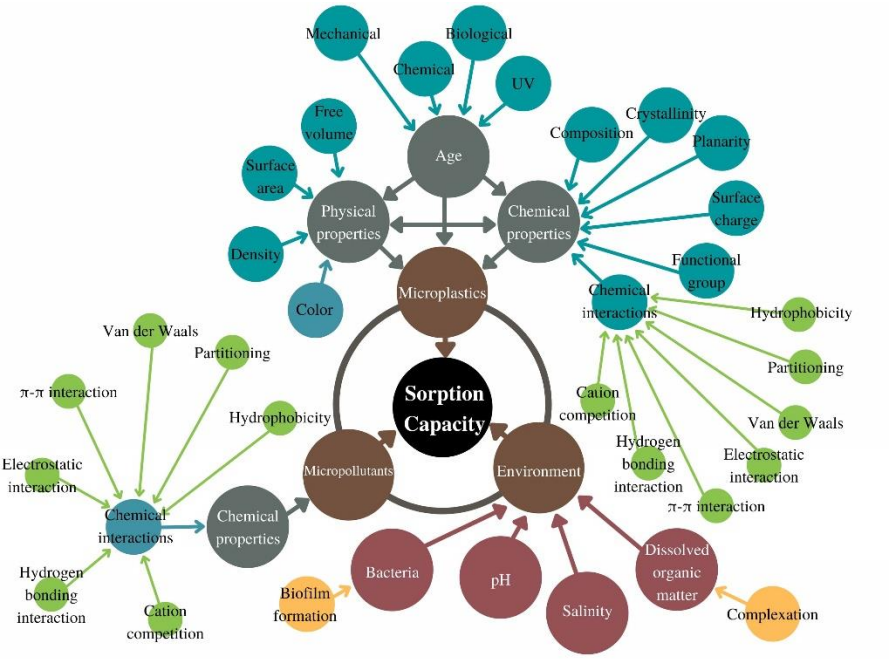


Fig. 1. Major factors and sub-factors influencing the sorption capacity of microplastics to micro-pollutants.

1.3.1.1. 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 (Agboola & Benson, 2021). 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 (Antunes, Frias, Micaelo, & Sobral, 2013; Fisner et al., 2017). 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 (Fries & Zarfl, 2012; Karapanagioti & Klontza, 2008; Mato et al., 2001). 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 (Karapanagioti & Klontza, 2008; Pascall, Zabik, Zabik, & Hernandez, 2005; Teuten, Rowland, Galloway, & Thompson, 2007).

1.3.1.2. 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 (Amelia et al., 2021; Jia Li, Zhang, & Zhang, 2018; Llorca, Schirinzi, Martínez, Barceló, & Farré, 2018; Fei Wang, Shih, & Li, 2015). 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 (Xiaoying Guo et al., 2012; Rochman, Hoh, Hentschel, & Kaye, 2013; Wu, Taylor, Knappe, Nanny, & Barlaz, 2001). 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 (F. Yu, Yang, Zhu, Bai, & Ma, 2019).

1.3.1.3. 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 (F. Yu et al., 2019). The increased frequency or induction of oxygen-containing functional groups (e.g., hydroxyl, carbonyl, and formyl groups) occurs after the particles' exposure to ageing (Hüffer, Weniger, & Hofmann, 2018; G. Liu et al., 2019; Mylläri, Ruoko, & Syrjälä, 2015). 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 (Hüffer et al., 2018; Shen, Li, Sima, Cheng, & Jiang, 2018; F. Yu et al., 2019). 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 (Brennecke, Duarte, Paiva, Caçador, & Canning-Clode, 2016; Turner & Holmes, 2015).

1.3.1.4. 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 (Mattsson, Hansson, & Cedervall, 2015). 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 (Johansen, Prentice, Cresswell, & Howell, 2018).

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 (Takahashi et al., 2014) 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 (Xuetao Guo, Pang, Chen, & Jia, 2018; Fei Wang et al., 2015). pH affects the sorption capacity of trace metals, such as cadmium, cobalt, nickel, lead, and chromium, on high-density polyethylene in saltwater via increased competition, decreased chromate ion activity, as well as complexation and free ion interaction with saltwater cations (Holmes, Turner, & Thompson, 2014).

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 (Borrirukwisitsak, Keenan, & Gauchotte-Lindsay, 2012). 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 (Hu et al., 2017; Velzeboer, Kwadijk, & Koelmans, 2014; Fei Wang et al., 2015; Zhan et al., 2016). Conversely, increased salinity was shown to decrease the sorption capacity of dichlorodiphenyltrichloroethane (DDT) and ciprofloxacin on microplastics due to cation competition for sorption sites (Bakir, Rowland, & Thompson, 2014b; Jia Li et al., 2018; G. Liu et al., 2019). However, there were no observed salinity-related effects on phenanthrene, as the salt content in water did not affect the aqueous solubility and pore-filling mechanism of Phe (Bakir et al., 2014b).

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 (Seidensticker, Zarfl, Cirpka, Fellenberg, & Grathwohl, 2017; Xu, Liu, Brookes, & xu, 2018; H. Zhang et al., 2018). Fulvic acid negatively affected the sorption capacity of tetracycline on microplastics, as tetracycline was deemed to sorb onto DOM rather than microplastics (Xu et al., 2018). Likewise, microplastics could adsorb fewer HOCs due to the increasing desorption from the presence of dissolved organic matter (Seidensticker et al., 2017). Yet, the positive effect of the role of dissolved organic matter bridge formation in the micropollutant-microplastic surface complex might be a reason for the increased sorption capacity of oxytetracycline on aged microplastics (H. Zhang et al., 2018).

1.3.2. Microplastics: The Potential for Microplastics-Sorbed Micro-pollutant 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 (UN GHS, 2015). 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 (Amelia et al., 2021; Tourinho, Kočí, Loureiro, & van Gestel, 2019).

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* (Qu et al., 2018). 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) (Bakir, Rowland, & Thompson, 2014a).

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 (Paul-Pont et al., 2016).

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 polyethylene (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 PE in aqueous phases but the ability to be almost completely desorbed from PE microplastics inside the organisms, therefore allowing for significant bioaccumulation (Chua, Shimeta, Nugegoda, Morrison, & Clarke, 2014; Ziajahromi, Kumar, Neale, & Leusch, 2019).

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.) (Paul-Pont et al., 2016).

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 PCBs in feed were adsorbed to and transferred by virgin polyethylene in simulated gut conditions (Mohamed Nor & Koelmans, 2019).

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 (Bakir et al., 2014b; Barboza, Vieira, Branco, Figueiredo, et al., 2018; H. Ma et al., 2020; Servos, 1999) (Fig. 2).

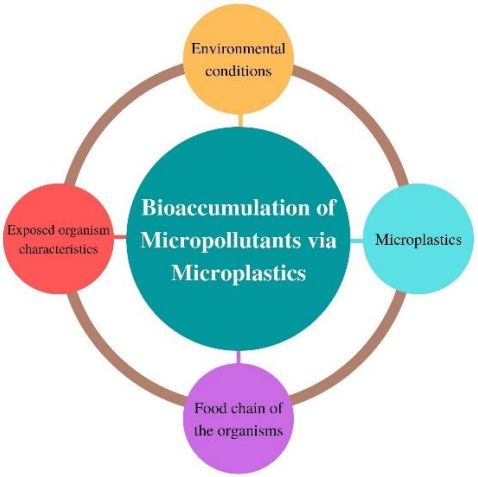


Fig. 2. Factors influencing the bioaccumulation of micro-pollutants in an organism from exposure to microplastics as a vector.

1.3.3. Joint Effects of Microplastics and Micro-pollutants

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 (Q. Zhang et al., 2018; Zhu et al., 2019). 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 (McLaughlin & Lanno, 2014). 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 (Bhagat, Nishimura, & Shimada, 2021; Mattsson et al., 2015). 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 (Bhagat et al., 2021).

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 (Roell, Reif, & Motsinger-Reif, 2017), indicating no direct connection between the two substances or actions, and defined as non-interaction or inertism (Greco, Bravo, & Parsons, 1995). Deviations from the additive effect can be synergistic or antagonistic (Roell et al., 2017). 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 (Roell et al., 2017). 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 (Levy, 1965). 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 (Barboza, Vieira, Branco, Carvalho, & Guilhermino, 2018). 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 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* (Guilhermino et al., 2018).

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. (Thiagarajan et al., 2019). 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* (Kim, Chae, & An, 2017). 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 (Q. Zhang et al., 2018).

1.3.4. 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 (Aktar, Sengupta, & Chowdhury, 2009; Oliver, 2014). However, the introduction of GM crops is also causing an increase in herbicide use, leading to chemical pollution in soil, water, and air (Prado et al., 2014). Moreover, adaptive evolutionary processes act in weeds develop 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 (Baucom, 2019; Duke & Powles, 2008; Powles, 2008). 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 (Benbrook, 2016; Grandcoin, Piel, & Baurès, 2017). 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 (Alonso, Demetrio, Agustina Etchegoyen, & Marino, 2018; Battaglin, Meyer, Kuivila, & Dietze, 2014; Bonansea, Filippi, Wunderlin, Marino, & Amé, 2017; Castro Berman, Marino, Quiroga, & Zagarese, 2018; Primost, Marino, Aparicio, Costa, & Carriquiriborde, 2017; Van Stempvoort et al., 2016). 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 (FAO, 2005; Then, 2013). Additionally, a glyphosate residue concentration of 1481 ± 73 µg/L—exceeding the legally permitted maximum contaminant level in drinking water regulated by the European Union, 0.1 µg/L, by approximately 15,000-fold—was reported in natural water in Brazil (EC, 1998; Tzaskos, Marcovicz, Dias, & Rosso, 2012). 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 (Kanissery, Gairhe, Kadyampakeni, Batuman, & Alferez, 2019).

AMPA, the main metabolite of glyphosate, can present toxic effects similar to original compounds. 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 (Bonnet, Bonnemoy, Dusser, & Bohatier, 2007). 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 (Mercurio, Flores, Mueller, Carter, & Negri, 2014).

When sprayed GBHs and AMPA enter soils, several transport mechanisms are present that reduce the spreading of the contamination (Fig. 3). GBHs' molecules could be taken up by plant tissues, resulting in biotransformation products, particularly AMPA, and their accumulation in the tissues (Botten, Wood, & Werner, 2021). 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 (Quaghebeur, De Smet, De Wulf, & Steurbaut, 2004; Silva et al., 2018). 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 (Rampazzo Todorovic et al., 2014; Rendon-von Osten & Dzul-Caamal, 2017; X. Yang et al., 2015). Strongly bound GBH and AMPA molecules could also be moved off site to waters by water erosion (Silva et al., 2018). 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 (Glozier et al., 2012; J. Moore, 2021). 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.

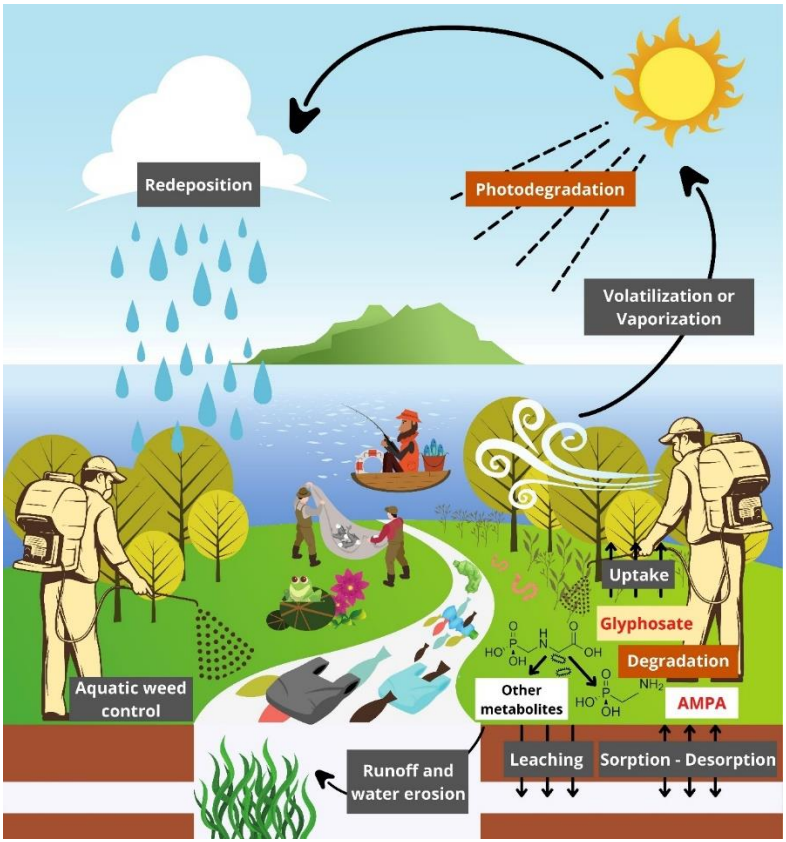


Fig. 3. Glyphosate fate and transport.

In general, glyphosate's mode of action after the absorption of GBHs through foliage is to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) 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 (Comai, Sen, & Stalker, 1983). This pathway is absent in animals and

is considered to contribute to the nonexistent or low toxicity of glyphosate toward animals (Dost, 2003; Duke et al., 2012; OECD, 2006). 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 (Gutierrez, Battauz, & Caisso, 2017; Piccini et al., 2020); decreased acetylcholinesterase activity in mussels, shrimp, and fish (Hong, Yang, Huang, Yan, & Cheng, 2018; Matozzo, Munari, Masiero, Finos, & Marin, 2019; P. Mensah, Muller, & Palmer, 2012a; Rossi, Fantón, Michlig, Repetti, & Cazenave, 2020); excessive reactive oxygen species (ROS) formation or impaired antioxidant capacity in plankton, worms, mussels, shrimp, prawns, and fish (Contardo-Jara, Klingelmann, & Wiegand, 2009; de Melo, Nazari, Müller, & Gismondi, 2020; Fantón, Bacchetta, Rossi, & Gutierrez, 2020; Hong et al., 2018; M.-H. Li et al., 2017; Matozzo et al., 2019; P. Mensah, Palmer, & Muller, 2012; Rossi et al., 2020; Topal et al., 2015). Studies also showed histopathological changes in fish and frogs (M.-H. Li et al., 2017; J. Ma & Li, 2015; Riaño, Ortiz-Ruiz, Pinto-Sánchez, & Gómez-Ramírez, 2020; Topal et al., 2015); endocrine disruption and reproductive impairment in plankton and fish (Gutierrez et al., 2017; Reno, Doyle, Momo, Regaldo, & Gagneten, 2018; Uren Webster, Laing, Florance, & Santos, 2014); metabolic alterations in fish and frogs (M.-H. Li et al., 2017; X. Wang et al., 2019), behavioral changes in shrimp, fish, and frogs (P. K. Mensah, Muller, & Palmer, 2011; Mikó, Ujszegi, Gál, & Hettyey, 2017; Topal et al., 2015; X. Wang et al., 2019); growth and development inhibition and increased mortality in plankton, shrimp, fish, and frogs (Babalola, Truter, & van Wyk, 2019; Daam, Moutinho, Espíndola, & Schiesari, 2019; Gustinasari, Sługocki, Czerniawski,

Pandebesie, & Hermana, 2021; Ikeogu, 2020; P. Mensah, Muller, & Palmer, 2012b). This information is summarized in Table 1.

In human cells, glyphosate and GBHs can act as endocrine disruptors, affecting the estrogen and androgen pathways (Gasnier et al., 2009; Richard, Moslemi, Sipahutar, Benachour, & Seralini, 2005; Thongprakaisang, Thiantanawat, Rangkadilok, Suriyo, & Satayavivad, 2013), damaging the neural system, genetic materials, and ultrastructure of the cells (Martinez & Al-Ahmad, 2019; Martínez et al., 2020; Nagy, Tessema, Budnik, & Ádám, 2019), and enhancing the proliferation of the breast cancer cell line (Thongprakaisang et al., 2013). 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 (International Agency for Research on Cancer (IARC), 2017). 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 (Richard et al., 2005; Uren Webster et al., 2014). 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 (Contardo-Jara et al., 2009; Ikeogu, 2020; Rossi et al., 2020), in spite of its high-water solubility and moderate bioaccumulation potential (Mottier et al., 2015).

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↓	Piccini et al. (2020)
Zooplankton	*Daphnia magna*	Sumin Atut 360 SL	12, 24, 48 h	LC50-12 h: 76.67 mg/L LC50-24 h: 36.2 mg/L LC50-48 h: 21.34 mg/L	GM		Mortality↑, head width↓	Gustinasari et al. (2021)
	Cyclops vicinus			LC50-12 h: 207.89 mg/L LC50-24 h: 159.8 mg/L LC50-48 h: 92.93 mg/L			Mortality↑, body length↓	

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Zooplankton	*D. magna*	Eskoba®	15 days	LC50-48 h: 29.48 mg a.e./L	GM, CC, RT		Mortality↑, growth and fecundity ↓	Reno et al. (2018)
		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 ↕	Gutierrez et al. (2017)

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Zooplankton	*Notodiaptomus carteri*	Sulfosato Touchdown®	10 days	EC: 0.81 mg/L	MT, OS		Superoxide dismutase (SOD) and glutathione-S-transferase (GST) activities↑	Fantón et al. (2020)
			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↑	Contardo-Jara et al. (2009)
		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↓	Iummato et al. (2018)

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 ↕	Milan et al. (2018)
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 ↕	Iori et al. (2020)
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↓	Matozzo et al. (2019)

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Shrimp	*Caridina nilotica*	Roundup®	48 and 96 h	LC50-48 h (Neonate): 4.5 mg/L a.e. 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.	GM, BC		Mortality↑, behavior↕	P. K. Mensah et al. (2011)
Shrimp	*C. nilotica*	Roundup®	25 days	EC: 2.2 mg/L	GM		Growth rate and feed utilization↓, molting frequency↑	P. Mensah, Muller, et al. (2012b)

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Shrimp	*C. nilotica*	Roundup®	96 h and 21 days	EC-96 h: 4.3 mg/L EC-21 d: 2.2 mg/L	NT		Acetylcholinesterase (AChE) activity↓	P. Mensah, Muller, et al. (2012a)
Shrimp	*C. nilotica*	Roundup®	96 h and 21 days	EC-96 h: 4.3 mg/L EC-21 d: 2.2 mg/L	OS		Lipid peroxidation (LPO)↑	P. Mensah, Palmer, et al. (2012)
Shrimp	*Macrobrachium nipponensis*	Roundup®	48 and 96 h	LC50-48 h: 57.684 mg/L LC50-96 h: 11.237 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↑	Hong et al. (2018)

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Prawn	*Macrobrachium potiuna*	Roundup WG®	7 and 14 days	EC: 0.0065 mg/L	CT		Altered ultrastructure of hepatopancreas and impaired R cells	de Melo et al. (2019)
Prawn	*M. potiuna*	Roundup WG®	7 and 14 days	EC: 0.0065 mg/L	OS, GE		Antioxidant gene expression in hepatopancreas ↕	de Melo et al. (2020)
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↑	Ikeogu (2020)
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↓	Rossi et al. (2020)
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↓	Uren Webster et al. (2014)

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Fish	*Oncorhynchus mykiss*	Commercial formulation	6, 12, 24, 48, 96 h	EC: 2.5 mg/L	HP, BC, OS		Glutathione peroxidase and CAT activities↑, antioxidant gene expression↕, swimming performance↓, histopathological liver damage	Topal et al. (2015)
			21 days	EC: 5 mg/L				
Fish	*Carassius auratis*	Nongteshi®	90 days	EC: 0.2 mmol/L	HT, HP, OS, MT		Blood biochemistry ↕, renal tissue↓, oxidative stress mechanisms ↕, metabolisms ↕	M.-H. Li et al. (2017)
Fish	*Cyprinus carpio* L.	Commercial formulation	168 h	LC50-96 h: 520.77 mg/L	IT, HP		Contents of cytokines ↕, histopathological damage	J. Ma and Li (2015)
Frog	*Rana dalmatina*	Glyphogan®	21 days	EC: 2 mg a.e./L	BC		Anti-predator behaviors ↕	Mikó et al. (2017)
Frog	*Dendropsophus molitor*	Roundup Active®	30 days	EC: 325 µg a.e./L	HP		Hepatic tissue injuries	Riaño et al. (2020)
Frog	*Physalaemus cuvieri*	Glyphosate analytical standard	96 h	LC50-96 h: 115 mg a.e./L	GM		Mortality↑	Daam et al. (2019)
	Hypsiboas pardalis			LC50-96 h: 106 mg a.e./L				

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
Frog	*Xenopus laevis*	Roundup®	96 h	LC50-96 h: 1.05 mg a.e/L	GM		Mortality↑, malformation↑, growth↓	Babalola et al. (2019)
		Kilo Max®		LC50-96 h: 207 mg a.e./L				
		Enviro Glyphosate®		LC50-96 h: 466 mg a.e./L				
Frog	*Microhyla fissipes*	KISSUN®	10 days	LC50-10 d: 77.5 mg/L	GM, BC, MT		Mortality↑, growth↓, swimming behavior ↕, metabolism↕	X. Wang et al. (2019)
Human	*Homo sapiens*	Roundup®	24 h	EC: 2%	ET	+/-	Aromatase activity and messenger ribonucleic acid (mRNA) levels ↕	Richard et al. (2005)
		Glyphosate analytical standard		EC: 2%				
Human	*H. sapiens*	Glyphosate and AMPA analytical standards	24 h	EC: 100 µM	NT		Neurological damage, glucose metabolism ↕	Martinez and Al-Ahmad (2019)

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	Gasnier et al. (2009)
		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 ↕	Thongprakaisang et al. (2013)
Human	*H. sapiens*	Glyphosate analytical standards	4 h	Exposure concentration: 1000 µM	CT, GT		No significant cytotoxicity and genotoxicity	Nagy et al. (2019)
		Roundup Mega®		EC: 250 µM			Cell death and DNA damage	
		Fozat 480®		EC: 500 µM				
		Glyfos®		EC: 250 µM				

Table 1. *Cont.*

Organism		Formulation	Duration	Concentration	Endpoints Studied	Bioaccumulation	Effect	References
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 ↕	Martínez et al. (2020)
		AMPA analytical standards		EC: 10 mM				

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

1.4. 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 (Fletcher, 2021). Intensive shrimp production in ponds lined with polyethylene or polyvinyl chloride liners is commonly used in shrimp culture (A. Ranjan & C.E. Boyd, 2018). 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 (Pruder, Duerr, Walsh, Lawrence, & Bray, 1992). Polyethylene microplastic, a polymer commonly found in surface seawater and sediment (Frias et al., 2014; Gomiero et al., 2019; Ng & Obbard, 2006), 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 (Gouin et al., 2019).

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 (Curren, Leaw, Lim, & Leong, 2020). 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 (Alonso et al., 2018; Battaglin et al., 2014; Bonansea et al., 2017; Bonnet et al., 2007; Castro Berman et al., 2018; FAO, 2005; Glozier et al., 2012; Mercurio et al., 2014; J. Moore, 2021; Primost et al., 2017; Quaghebeur et al., 2004; Rampazzo Todorovic et al., 2014; Rendon-von Osten & Dzul-Caamal, 2017; Silva et al., 2018; Then, 2013; Van Stempvoort et al., 2016; X. Yang et al., 2015), 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 (Abby-Kalio NJ., 1990; Menon) 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 (Corteel et al., 2009). Shrimp during the molting process (ecdysis) have the highest mortality rate, followed by the animals in the post-molt and pre-molt stages, respectively (Van Thuong et al., 2016).

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 glyphosate-based herbicides–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 (P. Kungvankij & T.E. Chua, 1986). 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* (Zocchi & Sommaruga, 2019). The interaction between polyethylene microplastics and GBH decreased lethal concentration 50 (LC50) and increased GST activity compared with the direct negative effects of individual polyethylene microplastics and GBH additions in *Scinax squalirostris* (Lajmanovich et al., 2022). 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 (J. Chen et al., 2021).

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.

1.5. Objectives of the Study

Global plastic production increased rapidly, and continues to do so, with dramatic impacts on ecosystems due to 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. Moreover, the case of co-exposure and the combination effects of polyethylene, a plastic type commonly used in aquaculture, and GBHs on aquatic organisms are considered. Nevertheless, the investigation of deleterious effects from the exposure of aged polyethylene microplastics and a GBH contaminated in feed to aquacultured shrimp, i.e., Pacific white shrimp (*Litopenaeus vannamei*) is still scarce. Based on existing gaps in this information, we have developed 2 major study objectives:

1.5.1. Objective 1

The first objective was to determine toxicological impacts of 96-hour exposures to feed attached with a GBH at an environmentally relevant concentration in soybean, or at an unexpectedly high concentration in case of GBH misuse, and with mechanically and biologically aged PE microplastics, either alone or in combination, on oxidative stress, antioxidant and immune responses, and histology of *L. vannamei*.

1.5.2. Objective 2

The second objective was to assess whether the co-existence of PE microplastics can affect the responses provoked by the GBH toxicity. Moreover, AChE activity and concentration were also measured in this context as a sensitive biomarker in GBH exposure in shrimp (P. Mensah, Muller, et al., 2012a).

2. MATERIALS AND METHODS

2.1. Animals and Experimental Design

A total of 180 juvenile Pacific white shrimp (*L. vannamei*; 4.5 ± 0.9 g, 8.1 ± 0.6 cm, American Penaeid Inc., Florida, United States) from Crusta Nova farm (Munich, Germany) were randomly and equally divided into 6 treatment groups: A) Negative control (NC; basic feed without additional contaminants except with background contaminants); B) Only microplastic (MP; basic feed contaminated with 408 ± 128 microplastic pieces/g feed, Supplementary data); C) MP+Lo-GBH (MP + 20 mg glyphosate/kg feed); D) Lo-GBH (basic feed contaminated with 20 mg glyphosate/kg feed); E) MP+Hi-GBH (MP + 200 mg glyphosate/kg feed); and F) Hi-GBH (basic feed contaminated with 200 mg glyphosate/kg feed). A required sample size of 30 shrimp per treatment group was determined using the G*power software (version 3.1). Each of these groups was further divided into 3 replicates of 10 shrimp. To mimic the environment of shrimp farming with pond liners, 2 side walls of independent aquariums (50 cm long x 26.5 cm wide x 31.5 cm high) where 10-day acclimation and 96-hour toxicity test took place were lined with PE pond liner (NaturaGart Vertriebs GmbH, Ibbenbüren, Germany) and filled with 33 L of artificial saltwater (AB Aqua Medic GmbH, Bissendorf, Germany). The average loading rate of the present study was 1.4 ± 0.9 g wet weight/L. Although this loading rate exceeded the US Environmental Protection Agency (US EPA) loading rate guideline for penaeid shrimp (0.8 g wet weight/L test solution, (OPPTS 850.1045; US EPA, 2016)), the density used in the present study was within the stocking density applied to *L.*

vannamei culture (Briggs, Funge-Smith, Subasinghe, & Phillips, 2004). The shrimp were reared in aquariums at 30 ± 1°C and on a photoperiod of 12-hour light: 12-hour dark. Total artificial saltwater was renewed and debris, i.e., shrimp exoskeleton and feces were removed once a day to maintain water quality as follows: 22 ± 2 ppt salinity, > 6 mg/L dissolved oxygen, pH 7.8 ± 0.2, < 0.05 ppm total ammonia nitrogen, < 0.2 ppm nitrite, and < 10 ppm nitrate. Temperature, salinity, pH, total ammonia nitrogen, nitrite, clinical signs, and death were monitored daily. Dissolved oxygen and nitrate were checked weekly. The shrimp were manually fed with 3.54% of biomass/day three times a day (8.00 – 9.00, 12.00 – 13.00, and 16.00 – 17.00) based on recommendation of the feed manufacturer for entire experimental period. The experimental design and protocol were approved by the local animal welfare committee (Regierung von Oberbayern; reference number ROB-55.2-2532.Vet_03-22-2).

At the end of the 96-hour toxicity test, after cold-induced anesthesia, 100-µL hemolymph was withdrawn from ventral sinus of each individual using a sterilized 1-mL syringe with 26-guage (3/8") needle containing 100-µL of an ethylenediaminetetraacetic acid (EDTA)-free anticoagulant (27 mM trisodium citrate (Diagonal, Münster, Germany), 385 mM sodium chloride (AppliChem GmbH, Darmstadt, Germany), 115 mM glucose (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), pH 7.5) (Huang, Yang, & Wang, 2010). The obtained hemolymph-anticoagulant mixture was used for determination of total hemocyte count/mL (THC) and phenoloxidase (PO) activity. Subsequently, the shrimp were euthanized and measured the total weight and length (rostrum tip - telson end). The three measured shrimp from each treatment group were fixed with Davidson's solution (30 mL 95% ethanol (Carl Roth GmbH, Karlsruhe, Germany), 20 mL 37% formaldehyde (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), 10 mL glacial

acetic acid (Alfa Aesar, Lancashire, United Kingdom), and 30 mL distilled water (Walter Schmidt Chemie GmbH, Berlin, Germany)), and then processed for histological examination (Bell & Lightner, 1988; Cervellione, McGurk, Silva, Owen, & Van Den Broeck, 2017). A whole hepatopancreas and a muscle portion were extracted from the rest of the euthanized shrimp and snap-frozen in a bath containing dry ice (Cleangas GmbH & Co. KG, Willingen, Germany) and denatured absolute ethanol (Carl Roth GmbH, Karlsruhe, Germany) for determining the malondialdehyde (MDA) content, total protein, and mRNA expression in hepatopancreas as well as for measuring the AChE activity and concentration, and total protein in muscular tissue.

2.2. Preparation of Aged Microplastics and Treatment Feeds

Polyethylene (PE) microplastic fragments were prepared and aged mechanically by grinding a clean black commercial PE cutting board (Metaltex Deutschland GmbH, Mahlberg-Orschweier, Germany) with an angle grinder (Makita® 9558NBR, Makita Werkzeug GmbH, Ratingen, Germany). The PE microplastic fragments were sieved twice through a 500-µm stainless sieve, collected with a 32-µm stainless steel sieve (Joachim Edinger Industrial agencies, Leinburg, Germany), and then flushed into glass petri dishes using pre-filtered absolute ethanol (Fisher Scientific GmbH, Schwerte, Germany) in a laminar flow hood to obtain PE fragment sizes in a range frequently found in marine sediment (30 - 500 µm) (Vianello et al., 2013). The petri dishes loosely covered with aluminum foil were placed under the hood at room temperature for 24 hours to evaporate the ethanol.

The contamination and number of the dried PE fragments were observed and approximately enumerated in microplastic pieces/g under a light microscope (Olympus® CX31, Japan).

Processes of biofilm formation and GBH sorption were conducted in metal screw-capped glass bottles with minor modifications of existing protocols (OECD, 2005; Lobelle & Cunliffe, 2011; Mercurio et al., 2014; Smedes & Booij, 2012). To simulate environmental conditions where biofilm formation and biological degradation are occurred on microplastics in shrimp culture, 6 glass bottles rinsed with Milli-Q water (Ultra Clear® electronic dispenser, Evoqua Water Technologies GmbH, Günzburg, Germany) and filtered absolute ethanol 3 times were prepared for NC, MP, MP+Lo-GBH, Lo-GBH, MP+Hi-GBH, and Hi-GBH treatment groups. The mechanically degraded PE fragments were added into the 3 glass bottles for groups MP, MP+Lo-GBH, and MP+Hi-GBH. Thereafter, the all 6 bottles were added with 50-mL shrimp culture saltwater and placed on a shaking water bath (Stuart® SBS40, Cole-Parmer Ltd, Staffordshire, United Kingdom) in the dark at shrimp culture temperature (30°C) with 100 RPM of rotational speed for 5 months. The bottle position was rotated once a week. After a period of 5 months, the 4 bottles corresponding to groups MP+Lo-GBH, Lo-GBH, MP+Hi-GBH, and Hi-GBH were spiked with GBH containing 480 g/L glyphosate (Roundup® Flex 480, Monsanto Poland, Warsaw, Poland) and subsequently placed on the shaking water bath with the previous condition for 96 hours allowing the GBH to sorb to the mechanically and biologically aged PE microplastics. The calculation of spiked GBH was based on feed amount, environmentally relevant glyphosate residue level of 100 mg/kg in soybean (Then, 2013), soybean percentage of 20% in typical 35%-protein shrimp feed (FAO, 2017), glyphosate concentration in the used GBH product, balanced saltwater volume coated on feed, saltwater volume in spiking

process, and 30% expected loss of the chemical to yield final glyphosate concentrations of 20, 20, 200, 200 mg glyphosate/kg in experimental feeds of groups MP+Lo-GBH, Lo-GBH, MP+Hi-GBH, and Hi-GBH, respectively. The selection of a commercial GBH product over the pure glyphosate was to simulate the glyphosate exposure form including adjuvants in natural environments. This naturalistic study was designed to avoid the distortion of results, however, the possible effects would be considered as the combination of the chemicals, not only the active ingredient, i.e., glyphosate or adjuvants alone.

A commercially manufactured 1.6-mm shrimp feed (Aquafeed, Spezialfuttermittelwerk Beeskow GmbH, Beeskow, Germany) was used as basic component of 6 experimental feeds. Feed preparation was carried out in glass petri dishes according to an existing protocol with some modifications (J. Guo, Davis, Starkey, & Davis, 2021). Six portions of basic feed were coated with the first layer of 3% v/w of corresponding balanced saltwater suspensions and the second layer of 3% v/w of sardine oil (Dynamite Baits Ltd, Nottingham, United Kingdom). Afterwards, the experimental feeds were spray-coated with 5% w/v solution of gelatin powder (OSNA Nährmittel GmbH, Osnabrück, Germany) dissolved in autoclaved Milli-Q water and then dried with loosely covered aluminum foil to prevent the feeds from unintentional microplastic contamination under the hood overnight. All the experimental feeds were vacuum sealed and stored at -20°C until needed.

2.3. Acetylcholinesterase

Acetylcholinesterase (AChE) activity, a biomarker of GBH exposure (P. Mensah, Muller, et al., 2012a), was measured in triplicate in pooled muscle samples in accordance with procedures described by Ellman, Courtney, Andres, and Featherstone (1961), Wilson and Henderson (2007), and Tu et al. (2009). A pooled muscle sample composed of 40-mg muscles from 3 individual shrimp in same treatment group was completely homogenized in ice-cold solubilization buffer (50 mM KH_2PO_4/K_2HPO_4, pH 7.5 and 1X protease inhibitor cocktail (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany)) in a ratio of 1 to 7.5 (muscle to buffer) by a motorized tissue grinder and pestle. The clear supernatant (post-mitochondrial fractions, PMF) was obtained from centrifuging the homogenate at 10,000 g for 10 minutes at 4°C. The final 320-µL reaction mixture in each well of a 96-well plate included PMF sample, cholinesterase assay buffer (0.05 M Na_2HPO_4/NaH_2PO_4, pH 7.4), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) color reagent (Carl Roth GmbH, Karlsruhe, Germany) with a final concentration of 0.32 mM, and acetylthiocholine (ATCh) substrate (Carl Roth GmbH, Karlsruhe, Germany) with a final concentration of 1.5 mM. The measurement was performed photometrically using a multi-mode microplate reader (SpectraMax® M5, Molecular Devices, United States) at 412 nm for 10 minutes at 25°C. The AChE activity unit was converted from absorbance/min to nmol hydrolyzed ATCh/min/ml PMF. Additionally, the AChE concentration at minute 10 was calculated in the unit of nmol hydrolyzed ATCh/ml PMF.

Total protein concentration in PMF samples were determined using Coomassie (Bradford) protein assay kit (Thermo Scientific®, Illinois, United

States) according to the manufacturer's instruction. Prior to the measurement, the obtained PMF samples were diluted 1:10 with ice-cold solubilization buffer. The measured total protein concentration was used to normalize and calculate the AChE activity and concentration as nmol hydrolyzed ATCh/min/mg protein and nmol hydrolyzed ATCh/mg protein, respectively.

2.4. Malondialdehyde Content

Quantification of malondialdehyde (MDA) content was performed as previously described protocols (Fatima, Ahmad, Sayeed, Athar, & Raisuddin, 2000; P. Mensah, Palmer, et al., 2012; Tu et al., 2008; Utley, Bernheim, & Hochstein, 1967) with some modifications. Briefly, a pooled sample of 40-mg hepatopancreas from each 3 individuals was homogenized in ice-cold solubilization buffer (50 mM KH_2PO_4/K_2HPO_4, pH 7.5 and 1X protease inhibitor cocktail (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany)) to obtained 10% w/v homogenate. The homogenate was diluted 1:5 with the ice-cold solubilization buffer and dispensed in 2 microcentrifuge tubes as MDA sample and tissue blank control. The aliquots were incubated in an incubator (Binder GmbH, Tuttlingen, Germany) at 37°C for an hour. Afterwards, the MDA sample aliquot was added to 600 µL of 5% chilled trichloroacetic acid (Carl Roth GmbH, Karlsruhe, Germany) and 600 µL of 0.67% thiobarbituric acid (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) while 1200 µL of ice-cold solubilization buffer was added in the tissue blank control aliquot. The filtrates in microcentrifuge tubes with screwed caps were obtained from the filtration of 1-mL syringes loaded with the well-mixed aliquots through 0.2-µm syringe filters (Sarstedt AG & Co.

KG, Nümbrecht, Germany) and then placed in a boiling water bath (GFL® 1002-1013, Burgwedel, Germany) for 15 minutes. After 15 minutes of boiling, the filtrates were cooled down at room temperature for 1 minute, placed in 3 replicates in a 96-well microtiter plate, and measured at 535 nm.

Total protein concentration was quantified using a Coomassie-based protein assay kit (Thermo Scientific®, Illinois, United States). The hepatopancreas homogenate was centrifuged at 10,000 g for 10 minutes at 4°C to obtain the

supernatant as post-mitochondrial fractions (PMF) sample. Then, the PMF sample was diluted 1:10 with ice-cold solubilization buffer and assayed as total protein sample of hepatopancreas according to the procedure specified by the manufacturer. Final MDA contents normalized with total protein concentrations were expressed as nmol MDA produced/mg protein.

2.5. Total Hemocyte Count

Thirty µL of the obtained hemolymph-anticoagulant mixture was immediately mixed with the equal volume of 10% formal saline with the preparation of 10 mL 37% formaldehyde (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), 0.9 g sodium chloride (AppliChem GmbH, Darmstadt, Germany), 1.2 g Na_2HPO_4 (Carl Roth GmbH, Karlsruhe, Germany), and 90 mL distilled water (Walter Schmidt Chemie GmbH, Berlin, Germany) and kept at 4°C. The 3 separately fixed hemolymph samples from the same treatment group were pooled together, mixed with an equal proportion of 0.25% Rose Bengal Solution (S3 Chemicals®, Bad Oeynhausen, Germany), and incubated for 10 minutes at room temperature.

After 10-minute incubation, 10 µL of the stained hemolymph pool was placed in a hemacytometer (Bright-Line®, American Optical Company, New York, United States) and settled for 2 minutes to obtain a single layer for total hemocyte count (THC) determination using a light microscope (Olympus® CX31, Tokyo, Japan). The THC was represented as hemocyte numbers per milliliter of hemolymph (Burge, Madigan, Burnett, & Burnett, 2007).

2.6. Phenoloxidase Activity

The obtained hemolymph-anticoagulant mixture was further centrifuged in a refrigerated centrifuge (4°C) at 800 g for 10 minutes to separate the supernatant and freeze at -80°C as plasma sample until phenoloxidase (PO) activity assayed. The PO activity was investigated as previously described with a little modification (Huang et al., 2010; Song et al., 2017). In brief, 6.7 µL one-time thawed pool of 3 plasma samples was pipetted directly into a 96-well microtiter plate, followed by 293.3 µL of freshly made 3 mg/mL L-di-hydroxy-phenylalanine (L-DOPA) solution (Cayman Chemical Company, Michigan, United States) in 0.1 M potassium phosphate (K_2HPO_4/KH_2PO_4) buffer, pH 6.6. The mixture was thoroughly mixed and incubated at room temperature for 6 minutes. Afterwards, the optical density at 490 nm of the pooled plasma PO activity was measured using a spectrophotometer (SpectraMax® M5, Molecular Devices, United States) and expressed as unit of PO activity per mL hemolymph. An increase in absorbance of 0.001 per minute is defined as one unit of PO activity. The control solution which consisted of 6.7 µL of EDTA-free anticoagulant and 293.3 µL of 3 mg/mL L-DOPA solution was used for the background PO

activity in all test conditions. Each plasma pool and blank control was performed in triplicate.

2.7. qRT-PCR Analysis of Gene Expression

Ninety mg of snap-frozen pooled hepatopancreas was homogenized directly in 1 mL of TRI Reagent® (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and followed the procedure described by TRI Reagent® manufacturer. One hepatopancreas pool consisted of 30 mg hepatopancreas from each 3 individuals. The total isolated ribonucleic acid (RNA) dissolved in RNase-free water was quantified spectrophotometrically with Qubit™ 4 Fluorometer (Invitrogen™, Thermo Fisher Scientific, Waltham, USA) using a Qubit™ RNA High Sensitivity (HS) assay kit (Invitrogen™, Thermo Fisher Scientific, Waltham, USA) and stored at -80 °C.

One µg of total RNA from each pooled hepatopancreas was primed with 0.5 µg of oligo$(dT)_{15}$ primer (Promega GmBH, Walldorf, Germany), incubated at 70°C for 5 minutes, and chilled at 4°C for 5 minutes in a 5-µL reaction mixture. Afterwards, 15 µL of the reverse transcription reaction mix including 1 µL of ImProm-II™ Reverse Transcriptase (Promega GmBH, Walldorf, Germany) was added to the reaction mixture for final concentrations of 1X reaction buffer (Promega GmBH, Walldorf, Germany), 3 mM $MgCl_2$ (Promega GmBH, Walldorf, Germany), and 0.5 mM deoxyribonucleotide triphosphate (dNTP) (Promega GmBH, Walldorf, Germany). The reaction tube was incubated at 25°C for 5 minutes, then at 37°C for 60 minutes for reverse transcription, and finally at 70°C for 15

minutes for reverse transcriptase inactivation. The complementary deoxyribonucleic acid (cDNA) was quantified with Qubit™ 4 Fluorometer (Invitrogen™, Thermo Fisher Scientific, Waltham, USA) using a Qubit™ 1X double-stranded deoxyribonucleic acid (dsDNA) HS assay kit (Invitrogen™, Thermo Fisher Scientific, Waltham, USA) to determine the variation of obtained cDNA concentrations and then stored at -20 °C until further use.

Quantitative polymerase chain reaction (qPCR) was performed on several genes. Beta-actin (*β-actin*; housekeeping gene) was employed as a reference gene for cDNA normalization. Heat shock protein 70 (*hsp70*), catalase (*CAT*), cytosolic manganese superoxide dismutase (*cytMnSOD*), protein toll 1 (*Toll1*), caspase 2 (*Casp2*), anti-lipopolysaccharide factor 3 (*ALF3*), immune deficiency (*IMD*), signal transducers and activators of transcription (*STAT*), penaeidin 3 (*PEN3*), and C-type lectin 4 (*CTL4*) were genes of interest. The characteristics of used primer sets including name, respective forward and reverse sequences, and reference were summarized in Table 2.

Table 2. Details of primers for the investigated genes.

Gene	Forward and reverse primer sequence	Reference
β-actin	5′-TGGACTTCGAGCAGGAGATG-3′	L. Zhang, Pan, Xu, and Si (2018)
	5′-GGAATGAGGGCTGGAACAGG-3′	
hsp70	5′-CTCCTGCGTGGGTGTGTT-3′	Qian et al. (2012)
	5′-GCGGCGTCACCAATCAGA-3′	
CAT	5′-GGCTATGGTTCTCGTACTTCCAAGC-3′	Y.-Y. Chen et al. (2014)
	5′-GCATTGTATAGGTCCCTTGTTGCA-3′	
cytMnSOD	5′-TGACGAGAGCTTTGGATCATTCC-3′	
	5′-TGATTTGCAAGGGATCCTGGTT-3′	
Toll1	5′-GACCATCCCTTTTACACCAGACT-3′	Q. Yu et al. (2020)
	5′-CCTCGCACATCCAGGACTTTTA-3′	
Casp2	5′-CGTGGTTCATTCAGTCGCT-3′	Janewanthanakul, Supungul, Tang, and Tassanakajon (2020)
	5′-AACCTTTCGCATCAGGGTTG-3′	
ALF3	5′-CAACACCCGCAGCAAATCC-3′	Yin et al. (2023)
	5′-GGTTTGGCTTCTTCCTCGGT-3′	
IMD	5′-ATCGAGGAACGAGACAAGGT-3′	M. Yan, Wang, Huang, Wang, and Wang (2020)
	5′-CGTACACTCGGTCGACATTC-3′	
STAT	5′-CTTCGCCATCCGTCCTCTAG-3′	L. Yang et al. (2019)
	5′-GGCTTGATCCTTAGGCACATTC-3′	
PEN3	5′-CTCCTGCGTCCGCCATG-3′	
	5′-GTGTAACCGCCCTTGTACAC-3′	
CTL4	5′-CTTGGACGCTTATGTCACCTAC-3′	
	5′-CATCCTTGCTCTTGATGTAGTCG-3′	

The qPCR primer efficiencies were investigated to ensure the comparability of the genes of interest and of the reference gene. A cDNA template was diluted in 10-fold dilutions to create a standard curve with 7 concentration points. A linear line of the fluorescent signals converted to cycle threshold (Ct) values was plotted and the slope of the linear equation was applied to calculate the primer efficiency according to the equation Efficiency (%) = $(10^{[-1/\text{slope}]}-1)\times 100$.

The volume of qPCR reaction was 20 µL, which contained 10 µL of 2X GoTaq® qPCR Master Mix (Promega GmBH, Walldorf, Germany), 1.6 µL of cDNA template, 0.8 µL of 10 µM sense primer, 0.8 µL of 10 µM anti-sense primer, and 6.8 µL of RNase-free water. The amplification conditions were as follows: 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. At the end of the reaction, a dissociation curve analysis was performed from 55 – 95°C. All samples and negative control were amplified in triplicate in 96-well tube plates (Agilent Technology, California, USA) on an Agilent Mx3000P QPCR System (Agilent Technology, California, USA). The obtained Ct values were used for relative quantification of the mRNA expression level according to the $2^{-\Delta\Delta Ct}$ method.

2.8. Histological Examination

A cephalothorax of each representative shrimp (3 shrimp/treatment group) preserved in Davidson's solution for a minimum of 48 hours was cut into 4 sections with 2-3 mm thickness from anterior to posterior part, placed in a tissue processing cassette (Labomedic GmbH, Bonn, Germany), and

submitted to the Institute of Veterinary Pathology (Center for Clinical Veterinary Medicine, Ludwig-Maximilians-University, Munich, Germany) for hematoxylin and eosin (H&E) staining. Each section was evaluated for the hepatopancreas morphology and the presence of hepatopancreatic cells and hemocytes using an optical microscope (Leica DM750) with a digital camera (Leica ICC50 HD).

2.9. Statistical Analysis

All the numerical data are presented as the mean ± standard deviation. The data were analyzed and visualized using RStudio program. Data were tested for homogeneity of variance (Bartlett's test) and normality of distribution (Shapiro-Wilk test) prior to the analysis. The data which did not meet the assumptions of equal variance and normality were analyzed by a non-parametric test (Kruskal-Wallis test) followed by Wilcoxon rank sum test (Mann Whitney U test) with continuity correction. While the data which satisfied the assumptions were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test to identify statistically significant differences between means of treatment groups. Significant differences were considered at *P*-value of less than 0.05.

3. RESULTS

3.1. Influence of PE Microplastics and GBH in Single or Combination on AChE

A significant decrease in AChE concentration was observed in shrimp exposed to GBH alone, where concentrations relative to the NC were significantly reduced by 24.87% and 31.36% after exposure to low and high GBH concentrations, respectively. Following the co-exposure to PE microplastics and GBH, reduction in the AChE concentration showed a similar trend, but less pronounced and not statistically significant, at approximately 13.17% and 19.44% at the same concentrations of GBH. Similar to shrimp exposed to PE microplastics alone, although not significantly different, there was an 18.65% decrease in AChE concentration in the muscle tissue (Fig. 4A). Neither single nor combined exposures to PE microplastics and GBH significantly affected the AChE activity in the shrimp muscle (Fig. 4B).

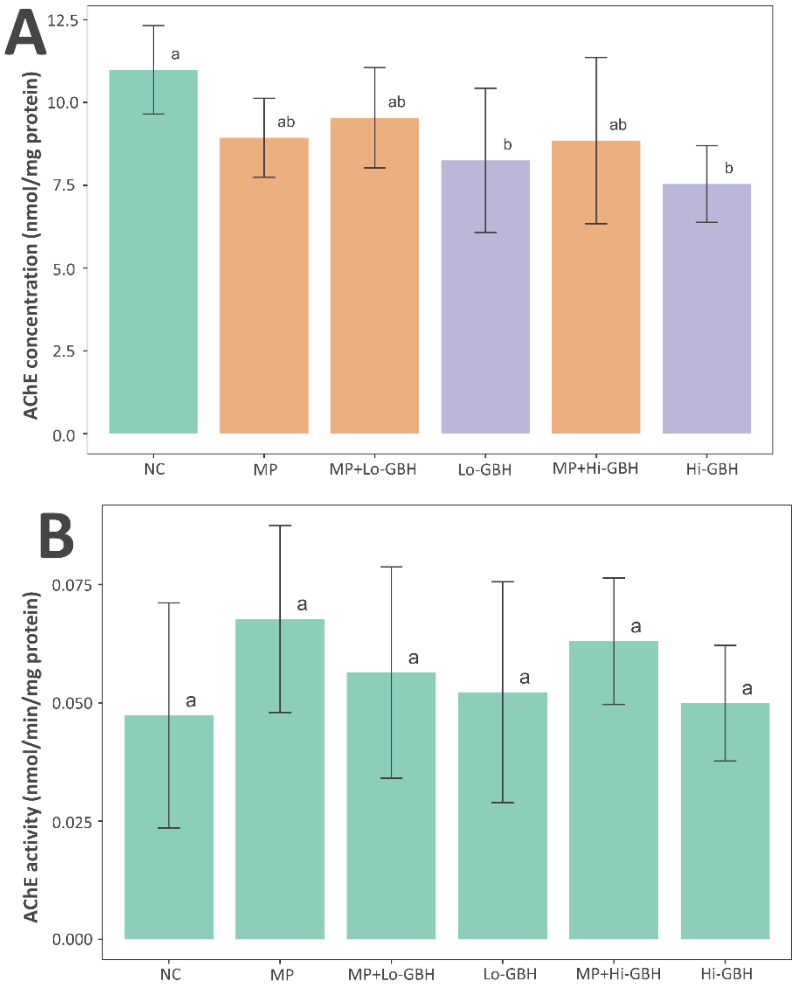


Fig. 4. Acetylcholinesterase (AChE) concentration (A) and activity (B) in the muscle of Pacific white shrimp (*Litopenaeus vannamei*) exposed to PE microplastics and GBH alone or in combination for 96 hours. Data are expressed as mean ± SD ($n = 9$). Different letters denote significant difference ($P < 0.05$, Tukey multiple comparison test).

3.2. Influence of PE Microplastics and GBH in Single or Combination on Oxidative Stress

3.2.1. Oxidative damage: malondialdehyde (MDA) content

After 96 hours of exposure, there were significant increases of 91.41% and 103.93% in MDA content in the hepatopancreas of shrimp in groups MP+Lo-GBH and Hi-GBH compared to negative control, respectively. The insignificant increases of the MDA content compared with negative control were observed in groups MP (31.72%), Lo-GBH (77.86%), and MP+Hi-GBH (81.26%). However, these differences between the individual and combined treatments at low and Hi-GBH concentrations were not statistically significant (Fig. 5A).

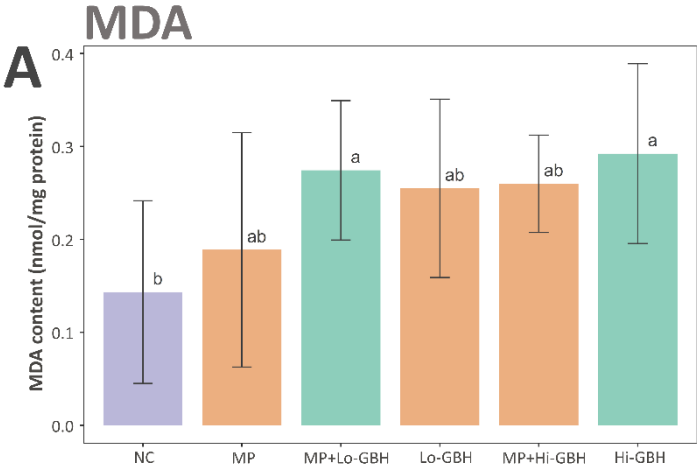
3.2.2. Antioxidant system: superoxide dismutase (SOD) and catalase (CAT)

A group MP showed non-significant difference compared to negative control and inverse trend in transcript expressions of between genes encoding SOD and CAT. There was a 1.16-fold increase in gene encoding SOD expression, while 0.87-fold decrease in the one encoding CAT expression was observed (Fig. 5B and C).

The group Hi-GBH displayed a significantly 1.97-fold increase in transcript encoding SOD (*cytMnSOD*) compared to negative control (Fig. 5B). Moreover, although not statistically significant, the other alteration of *cytMnSOD* and *CAT* expression levels were observed in groups Lo-GBH and

Hi-GBH. The opposite trend of 0.65-fold decreased *cytMnSOD* and 1.65-fold increased *CAT* expressions was presented in group Lo-GBH, whereas the similarly increasing trend of 1.97-fold *cytMnSOD* and 1.31-fold *CAT* expressions was observed in group Hi-GBH (Fig. 5B and C).

Under the combination treatments, no significant differences were recognized for the expressions of *cytMnSOD* and *CAT* in groups MP+Lo-GBH and MP+Hi-GBH when compared to negative control. The group MP+Lo-GBH exhibited increased trend of slight *cytMnSOD* up-regulation (1.06-fold) and *CAT* up-regulation (1.65-fold), while the group MP+Hi-GBH showed reverse trend of slight *cytMnSOD* down-regulation (0.97-fold) and *CAT* up-regulation (1.89-fold) compared to negative control (Fig. 5B and C). Moreover, the transcript encoding SOD was significantly lower in group MP+Hi-GBH compared to group Hi-GBH (Fig. 5B).



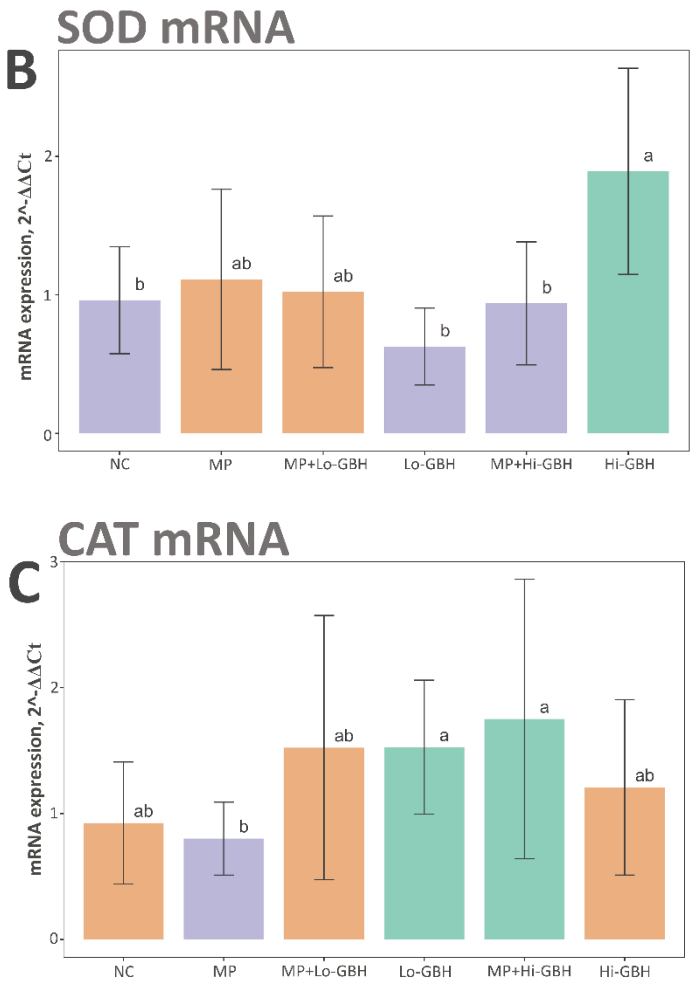


Fig. 5. Oxidative stress biomarkers: malondialdehyde (MDA) content (A) - and expression of mRNA encoding antioxidant enzymes (B, C) in hepatopancreas of Pacific white shrimp (*L. vannamei*) after 96 hours of exposure to PE microplastics and GBH. Data are expressed as mean ± SD (n = 9). Different letters denote significant difference ($P < 0.05$, Tukey multiple comparison test (A) and Wilcoxon rank sum test (B, C)).

3.3. Influence of PE Microplastics and GBH in Single or Combination on Immune Responses

No mortality during the 96-hour exposure period was observed, and the shrimp in all study groups showed no signs of abnormality in their behavior and general health.

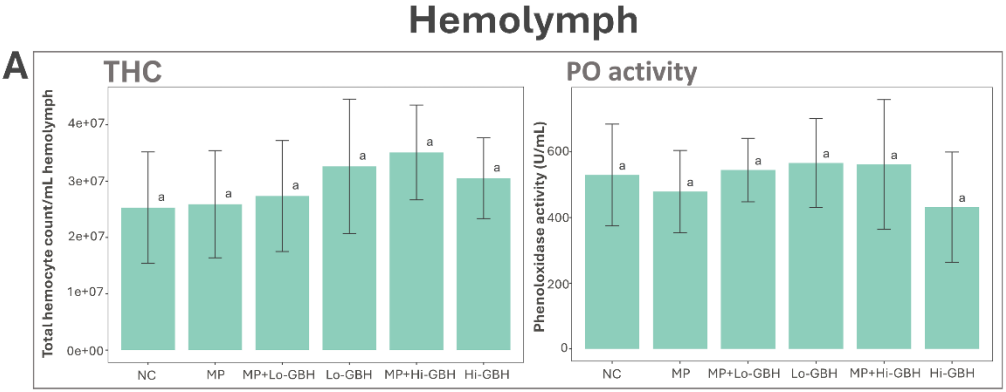
3.3.1. Hemolymph: Total hemocyte count (THC) and phenoloxidase (PO) activity

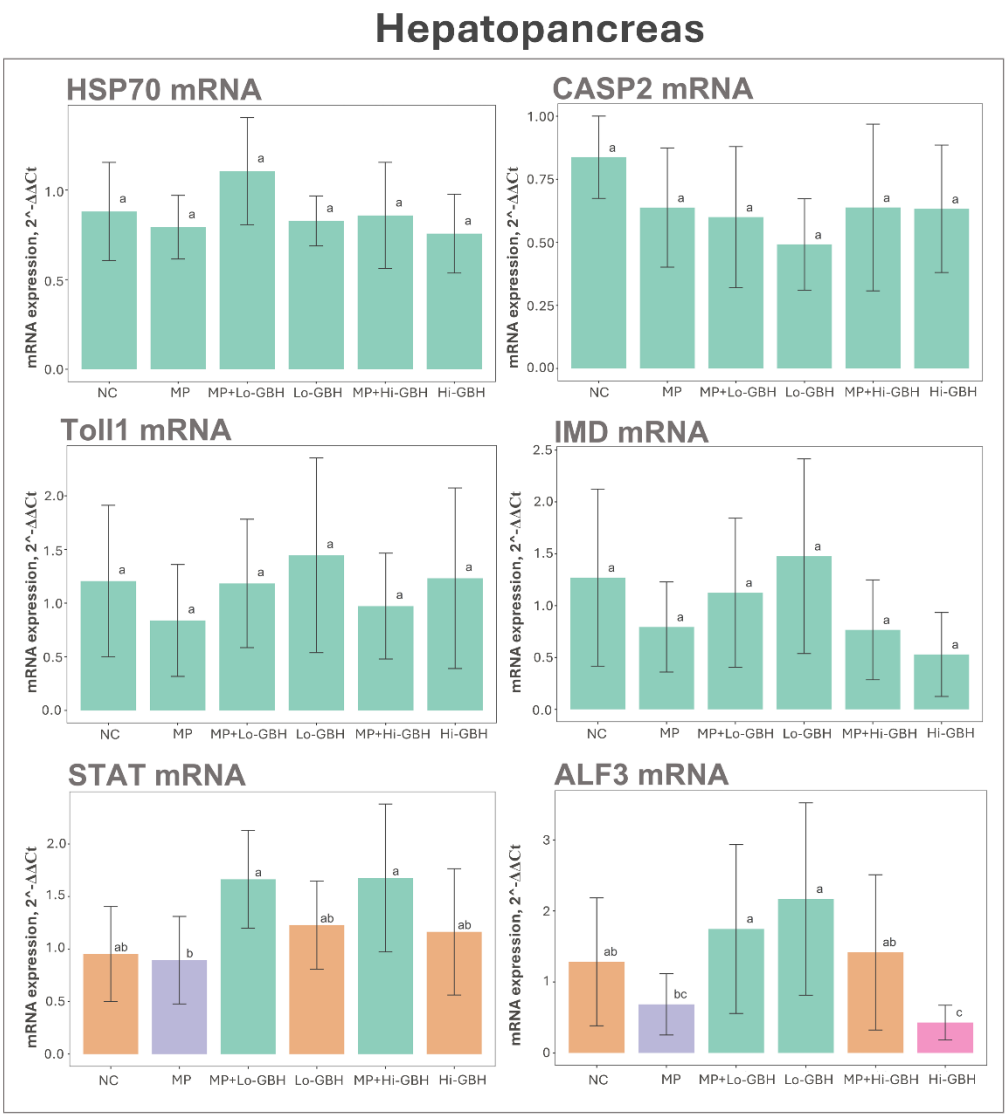
THC and PO activity were all not significantly affected after 96-hour dietary exposure to PE microplastics and GBH, either in single or combination. Yet, the THC was increased across all treatments by 2.40%, 8.15%, 29.00%, 38.74%, and 20.63% in groups MP, MP+Lo-GBH, Lo-GBH, MP+Hi-GBH, and Hi-GBH, respectively. In group Hi-GBH, the PO activity was the most decreased by 18.50%, while the PO activity was slightly decreased by 9.59% in group MP and increased by 2.85%, 6.91%, and 6.07% in groups MP+Lo-GBH, Lo-GBH, and MP+Hi-GBH, respectively (Fig. 6A).

3.3.2. Hepatopancreas: immune-related gene expression

Exposure of shrimp to dietary PE microplastics for 96 hours did not significantly alter the transcript profiling of all immune-related genes of interest compared to negative control. While in individual GBH exposure, the transcript encoding anti-lipopolysaccharide factor 3 (*ALF3*) was significantly down-regulated in group Hi-GBH compared to groups NC and MP+Hi-GBH.

Penaeidin-3 (*PEN3*) in groups Lo-GBH and Hi-GBH were significantly up-regulated compared to negative control. Combination exposure to PE microplastics and GBH also resulted in significant upregulation of *PEN3* compared to negative control. Furthermore, statistically significant difference between groups MP+Lo-GBH and Lo-GBH was seen in expression level of *PEN3*. The transcript expression of *CTL4* encoding C-type lectin 4 was solely significantly down-regulated in group Lo-GBH compared to negative control, but not when aged PE microplastics was combined. No statistically significant alterations in the *CTL4* expression level were recognized between single GBH exposures and combination exposures at the same GBH concentration. The expressions of other immune-related genes including *hsp70*, *Casp2*, *Toll1*, *IMD*, and *STAT* were not significantly affected by any of treatment groups compared to negative control (Fig. 6B).




Hepatopancreas
B
HSP70 mRNA
CASP2 mRNA
Toll1 mRNA
IMD mRNA
STAT mRNA
ALF3 mRNA
mRNA expression, 2^-ΔΔCt
NC
MP
MP+Lo-GBH
Lo-GBH
MP+Hi-GBH
Hi-GBH

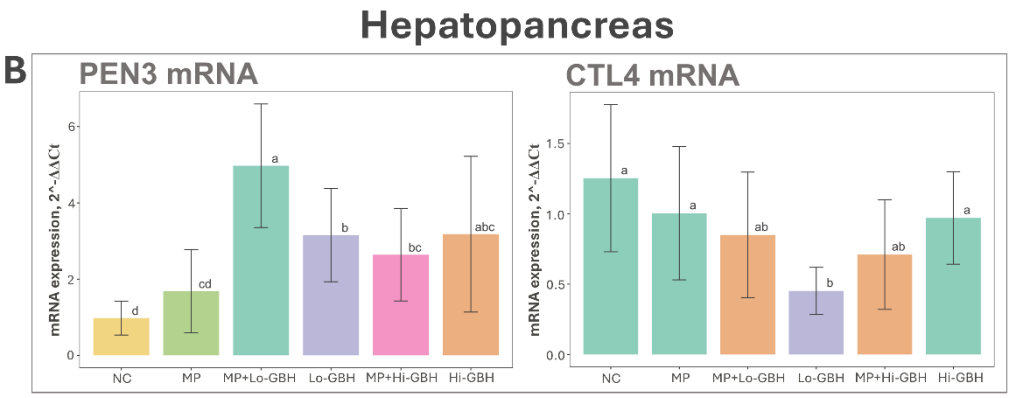


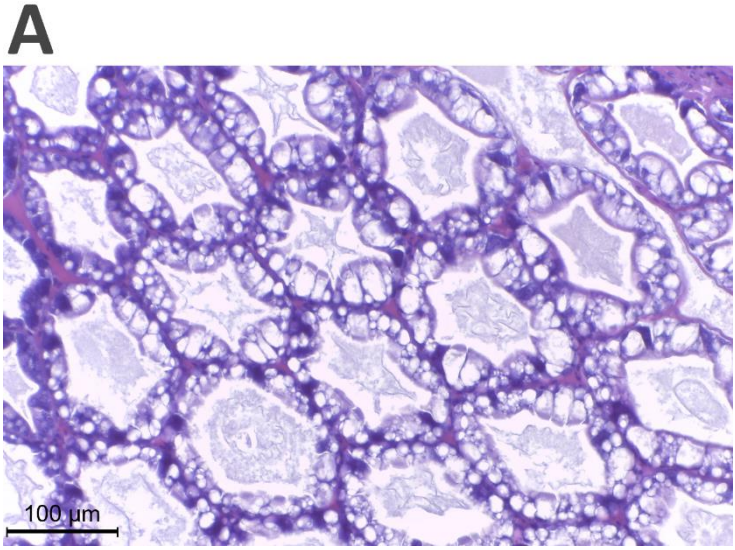
Fig. 6. Immune responses: total hemocyte count (THC) (A), phenoloxidase (PO) activity (A), immune-related gene expression (B) in different organs: hemolymph (A) and hepatopancreas (B). Data are expressed as mean ± SD (THC and PO activity: n = 10, gene expression: n = 9). Different letters denote significant difference ($P < 0.05$, Tukey multiple comparison test (A, B: *CASP2* and *STAT*) and Wilcoxon rank sum test (B: *HSP70*, *Toll1*, *IMD*, *ALF3*, *PEN3*, and *CTL4*)).

3.4. Influence of PE Microplastics and GBH in Single or Combination on Histology

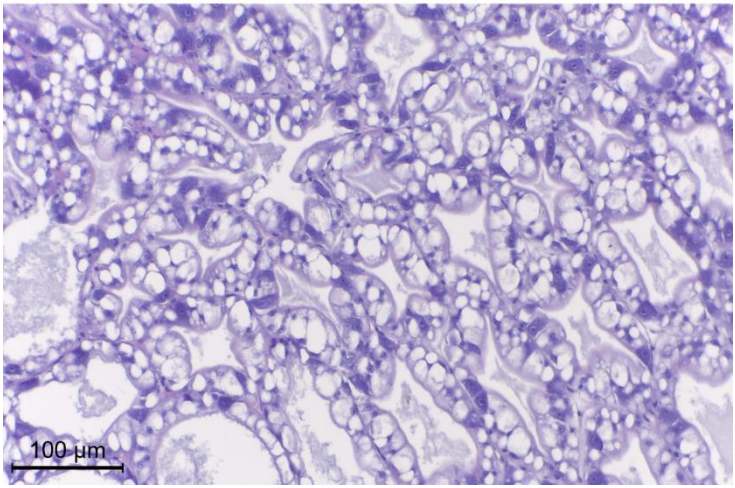
Histological observation of the hepatopancreatic tissues (HP) of shrimp treated with different combinations in feed pallets revealed the population of Blister-like or Blastozellen cells (B-cells) and Resorptive cells (R-cells) was obviously detected in the tissue section of the group treated with MP. Intact organization in cytoarchitecture was similar presented in the negative control. Nonetheless, there were a number of B-cell population

appearing in the HP tubules and it could be notably found the infiltration of hemocytes in the intertubular space of HP in group Lo-GBH compared to negative control. The HP of group Hi-GBH presented a dominant R-cells distribution along and cross-section of the tubules compared with that of the negative control. While in the histology of hepatopancreas taken from shrimp in combination groups MP+Lo-GBH and MP+Hi-GBH, numerous populations of ambiguous cells with vacuoles hardly characterized between R- and B-cells have been obviously found when compared to groups NC and MP (Fig. 7).

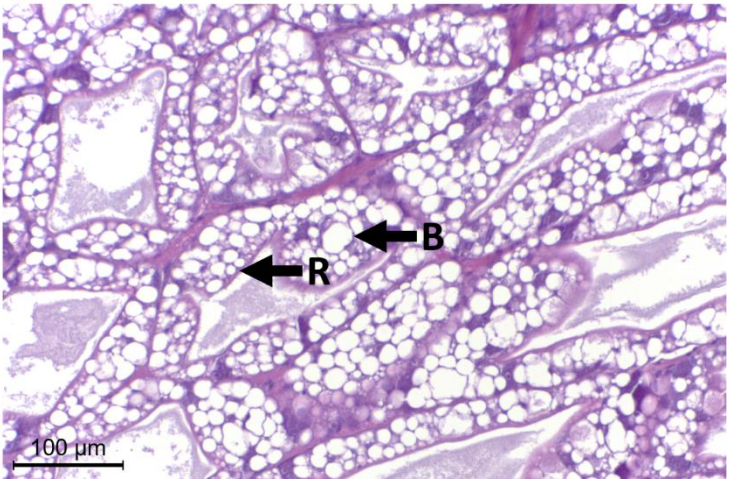
However, the histological examination was investigated in 3 shrimp from each treatment group. With this small sample size, this might not be sufficient to represent the impacts on experimental shrimp in its entirety, but merely to document the existence of possible adverse effects on physical hepatopancreas of shrimp exposed by ingestion to feed contaminated with PE microplastics and GBH, either alone or in combination.

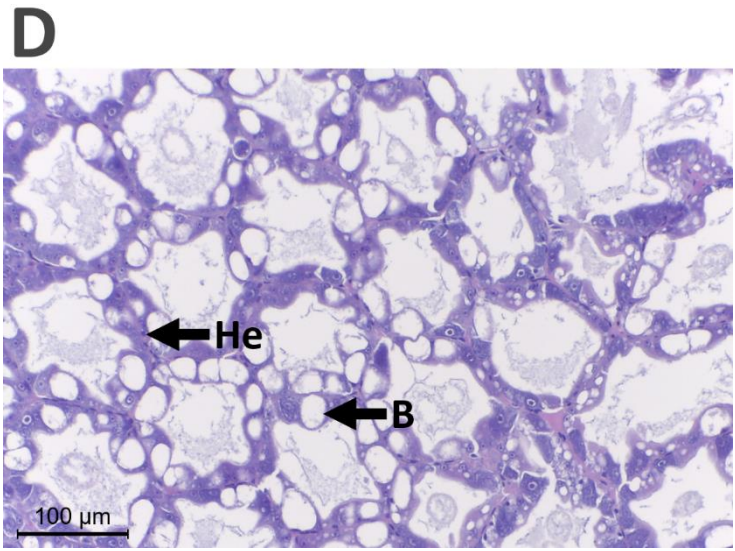


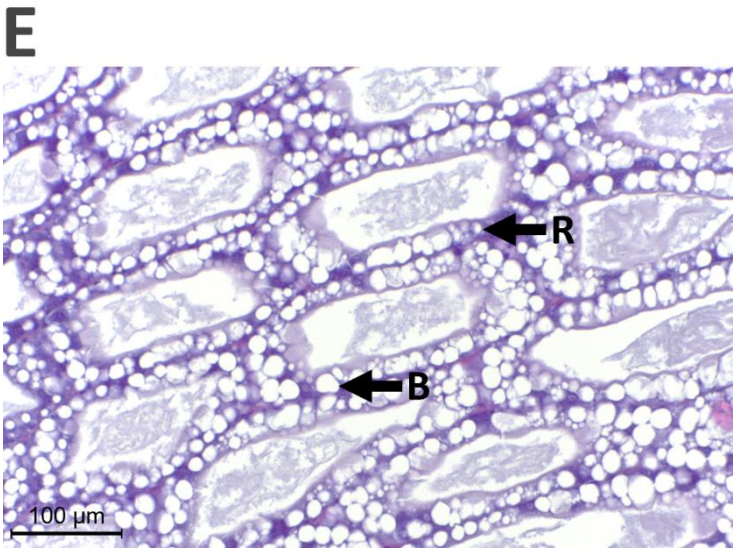
B



C




D
He
B
100 µm


E
R
B
100 µm

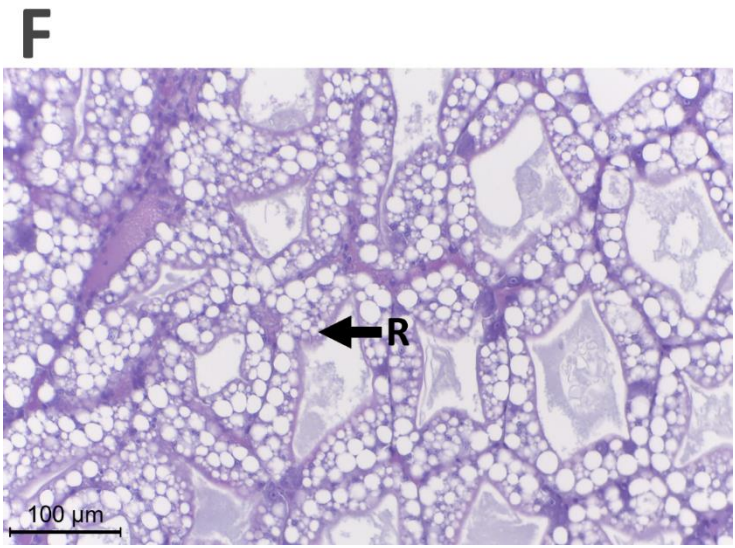


Fig. 7. Effects of PE microplastics and GBH in single or combination on histology of the hepatopancreas, H&E stain, scale bar = 100 µm. He: hemocytes, B: Blister-like or Blastozellen cells, R: Resorptive cells. A: NC, B: MP, C: MP+Lo-GBH, D: Lo-GBH, E: MP+Hi-GBH, F: Hi-GBH.

4. DISCUSSION

Several studies of glyphosate toxicity on AChE activity in crustaceans displayed glyphosate as a concentration-dependent AChE inhibitor (P. Mensah, Muller, et al., 2012a; Pala, 2019). However, this study provides evidence that the 96-hour dietary exposures of individual aged PE microplastic, GBH, and their combinations to *L. vannamei* did not cause significant changes in AChE activity when compared to the negative control. This might be because the magnitude and severity of the adverse effects depend on the concentration and bioavailability of glyphosate and adjuvants in target tissues, and the environmentally relevant GBH concentrations used in this study might not be sufficient to trigger the significant alteration of AChE activity in the exposed shrimp. In addition to glyphosate inhibitory effect on the AChE activity, it is possible that lower concentration of AChE in muscle tissue may cause decreased sensitivity of muscle samples for the purposes of detecting changes of the AChE activity in organisms exposed to GBH for a short period of time. For example, Glusczak et al. (2006) reported that exposing the piava freshwater fish (*Leporinus obtusidens*) to GBH for 96 hours significantly decreased the AChE activity in the brain, but not in the muscle. Nevertheless, the significant decreases in AChE concentration of shrimp in groups Lo-GBH and Hi-GBH suggest the potential for disruption of a regulatory mechanism involved with AChE of GBH at environmental concentrations in feed cannot not be ruled out. The bioavailability and concentration of GBH accessing the site of action could interrelate to affect not only AChE activity but also its concentration. The zebrafish (*Danio rerio*) exposed to glyphosate also showed the alteration of AChE gene expression, yet the AChE activity was not affected in the entire period of the acute

toxicity test (Lopes, Caldas, Primel, & da Rosa, 2017). On the other hand, the similar, but less pronounced inhibition of AChE concentrations of groups MP+Lo-GBH and MP+Hi-GBH compared to groups Lo-GBH and Hi-GBH, respectively implied that aged PE microplastics may reduce the bioavailability of GBH in the shrimp muscle. This scenario of microplastics reducing the transfer and bioavailability of mediated micro-pollutants is in accordance with some studies. Mohamed Nor and Koelmans (2019) reported that PE microplastics could sorb and reduce the desorption of PCBs in contaminated feed leading to the low transfer of PCBs across the simulated gut. Similarly, Dolar et al. (2021) suggested that polyester fiber and crumb rubber with mixed synthetic materials tend to reduce the bioavailability of co-exposed chlorpyrifos in woodlice (*Porcellio scaber*) based on the increases of hemocyte viability and AChE activity compared to the those of *P. scaber* exposed to chlorpyrifos alone. An explanation for this result may be that new functional groups, hydroxyl and carbonyl groups, emerged and biofilms colonized on the PE microplastics surface during the biological ageing process may increase microplastics surface hydrophilicity (Binda et al., 2023), thereby inducing the binding between these aged PE microplastics and hydrophilic GBH. In addition to the alteration of the interaction between aged PE microplastics and GBH, the interaction between feed and PE microplastics attached to GBH under the gastrointestinal conditions of *L. vannamei* could also play a major role in low desorption and uptake of GBH on the aged microplastics into intestinal lumen and target tissues. The aged PE microplastics attached to GBH might be released from the feed upon digestion and the aged PE microplastics may still sorb and trap GBH, leading to attenuated transfer of GBH into the *L. vannamei* and subsequently reduced inhibitory effect to AChE concentration in groups MP+Lo-GBH and MP+Hi-GBH.

Currently, the role played by acetylcholine in antioxidant modulation system in crustaceans is still unclear. However, Hong et al. (2018) measured AChE, SOD, and CAT activities in hemolymph of freshwater shrimp (*Macrobrachium nipponense*) exposed to GBH, and they proposed that AChE activity tends to not directly affect the activities of SOD and CAT. The alteration of SOD and CAT activities were likely results from oxidative stress and cellular damage per se. In the present study, a set of oxidative stress indicators, MDA content and expression levels of *cytMnSOD* and *CAT*, was measured to evaluate the potential oxidative damage induced by single or combined exposures to aged PE microplastics and GBH. The induction of oxidative stress occurs as a result of physiological imbalance between excessive reactive oxygen species (ROS) production and insufficient antioxidant defenses (B. P. Yu, 1994). The excessive ROS could cause damage to lipids and further lead to the overproduction of MDA. Moreover, the insufficient or ineffective antioxidative capacity of defenses including but not limited to SOD and CAT against these excessive ROS could also enhance the toxic consequences of oxidative stress, like the overwhelming MDA (Gaweł, Wardas, Niedworok, & Wardas, 2004). We found an enhancement of MDA content in the hepatopancreas across all treatment groups. Yet, the insignificant increase in MDA content in groups MP, Lo-GBH, and MP+Hi-GBH may be associated with the alterations of *cytMnSOD* and *CAT* expression levels of each respective group. SOD works in harmony with CAT to produce a broad spectrum of effects against ROS. SOD contributes superoxide radical dismutation by converting the superoxide anions to hydrogen peroxide and oxygen (Ying Wang, Branicky, Noë, & Hekimi, 2018). Thereafter, CAT contributes hydrogen peroxide detoxification by transforming it to water (Ying Wang et al., 2018). Even though the *cytMnSOD* and *CAT* expression levels of group MP were not significantly

different compared to negative control, the inverse relationship between their expression levels could still indicate the harmonization of SOD and CAT work against cellular oxidative stress, resulting in its insignificantly increased MDA contents and oxidative cellular damage. This provides support for observed histological result of no differences in organization and morphology of hepatopancreatic cells between groups MP and NC, suggesting that ingestion of feed contaminated with aged PE microplastics at environmentally relevant concentration for 96 hours might not induce significant oxidative stress in *L. vannamei*. Regarding the dietary exposure, there is only little information on negative effects of microplastics exposure to Pacific white shrimp (*L. vannamei*) (Hariharan, Purvaja, Anandavelu, Robin, & Ramesh, 2022; Niemcharoen, Haetrakul, Palić, & Chansue, 2022), as compared with direct contact exposure to contaminated water that is most commonly used in toxicological studies (Duan et al., 2021; Han et al., 2022; Z. Wang, Fan, Wang, Xie, et al., 2021; Z. Wang, Fan, Wang, Zhou, et al., 2021; Xing et al., 2023; Zhou et al., 2023). A study of oxidative stress in *L. vannamei* orally exposed to aged PE microplastics alone with environmentally relevant concentrations for a chronical period of 25 days indicated a significant increased levels of MDA and alterations of antioxidant enzyme activities, i.e. glutathione-S-transferases, reduced-glutathione, and CAT – which might be a result from starvation effect (Hariharan et al., 2022). In addition, 28-day oral exposure to high density PE microplastics mixed in feed pellet in *L. vannamei*, probably related to adverse effects on the nutritional uptake, energy reserves, and hepatopancreatic physiology, and subsequently causing statistically significant changes of antioxidant-related gene expressions including SOD, glutathione peroxidase, and lysozyme was reported by Niemcharoen et al. (2022). However, the adverse effects of microplastics exposure on oxidative stress are influenced by several factors

such as exposure time and dose, microplastics size and type, and test species (Bobori et al., 2022; Espinosa, Esteban, & Cuesta, 2019; Hariharan et al., 2022; Z. Wang, Fan, Wang, Zhou, et al., 2021; Zhou et al., 2023). The obvious difference in exposure duration of our study as acute toxicity test compared with chronic toxicity test in the mentioned studies might explain the insignificant changes of MDA content and expressions of *cytMnSOD* and *CAT* genes found in group MP here. Consistent with the results of the present study, a previous study (Korez, Gutow, & Saborowski, 2020) also showed that there was no significant difference in antioxidant defense responses in the hepatopancreas of brown shrimp (*Crangon crangon*) during acute exposure of 48 hours to synthetic microplastics. Furthermore, similar results were reported in the study about brine shrimp (*Artemia salina*) when exposed to environmental concentration of polystyrene microplastics (1 mg/L) for 48 hours and 14 days that only 14-day polystyrene microplastics exposure could induce sharp increase in ROS production (Suman et al., 2020).

Considering the oxidative stress induced by GBH alone and in combination with aged PE microplastics, we found groups Hi-GBH and MP+Lo-GBH to show the significantly increased MDA content, while groups Lo-GBH and MP+Hi-GBH also elicited the increase in MDA content, but not statistically significant, when compared to the negative control, indicating that individual and combined GBH dietary exposure could induce lipid damage. However, the post-hoc comparisons indicated that there were no significant differences of MDA content between groups exposed to single GBH and combined GBH with aged PE microplastics at the same concentrations. This suggests that the presence of aged PE microplastics did not have a significant effect on MDA induction of GBH in *L. vannamei*. Corresponding with this, histological examination revealed no evidence of any significant differences of hepatopancreatic cell damages between single

and combination groups. We found an observed trend toward increases in the presence of B- and R-cells, as a result of dietary exposures to both single and combined GBH instead, suggesting that B- and R-cells might probably be attributed to GBH detoxification, thereby maintaining shrimp homeostasis. These data were congruent with a previous study of Sreeram and Menon (2005), demonstrating that the pronounced increase in B-cell number following exposure to sublethal concentrations of petroleum hydrocarbons in hepatopancreas of Kadal shrimp (*Metapenaeus dobsoni*) was more likely be related to detoxification mechanism in a cellular level. Additionally, the increase in altered R-cells of ghost shrimp *(Palaemonetes argentinus*) exposed to pesticides suggests R-cells might be directly involved in detoxification (Petriella, 2007; Vogt & Quinitio, 1994), although R-cells were thought to be indirectly related to detoxification by providing active energy for this mechanism in response to environmental stress in *Macrobrachium borellii* Nobili, 1896 and *P. argentinus* Nobili, 1901 (Collins, 2010). Nonetheless, with the fact that roles of B- and R-cells in detoxifying organic xenobiotics, i.e., GBH, is still unclear (Vogt, 2019), further research is still needed to investigate and clarify the role of hepatopancreatic cells in depth. In addition to MDA content, expression of *cytMnSOD* and *CAT* genes were determined to understand the potential oxidative stress of dietary GBH exposures. There was no clear antioxidant response trend for the dietary exposures of *L. vannamei* to single and combined GBH but the significantly increased expression of *cytMnSOD* in group Hi-GBH when compared to negative control. The increased *cytMnSOD* expression following exposure to Hi-GBH concentration may have resulted from compensatory mechanisms in hepatopancreas to sustain the homeostasis of cells under high bioavailable GBH condition. Concerning the combination effects in our study, hepatopancreas transcript profiling revealed significant

up-regulation of *cytMnSOD* in group Hi-GBH compared to MP+Hi-GBH; however, albeit not significant, the inverse regulation trend of *CAT* between these 2 groups was observed. This may reflect harmony of feedback mechanisms governing a compensatory transcriptional response of antioxidant enzymes to a potential oxidative stress in *L. vannamei* exposed to. The result of significantly lower *cytMnSOD* expression in group MP+Hi-GBH compared to the one in group Hi-GBH contrasts with previous study in anuran tadpoles (*Scinax squalirostris*) that has found 60 mg/L virgin PE microplastic may increase toxicological effects of 6.25 mg/L GBH, resulting in an enhancement of glutathione S-transferase activity, one of antioxidants, compared to single 6.25 mg/L GBH exposure (Lajmanovich et al., 2022). Although the concentration used in the combination of virgin PE microplastic and GBH was environmental relevant and exposure duration was short term (48 hours) similar to our study, it is important to note that there are still several important differences in our study as compared to Lajmanovich et al. (2022) including but not limited to ageing process of microplastics, exposure routes, test species, and analyzed oxidative stress biomarkers, which could be factors affecting the differences in alteration of combined toxicity. Binda et al. (2021) suggested that biofouling in biological ageing process of PE microplastics might increase the affinity of microplastics with polar chemical, like GBH, however, we cannot discard the other fact that the presence of microbes not only could interact with microplastic but also with GBH, and vice versa. On the one hand, glyphosate could be utilized and degraded by different microbes, generating the primary degradation product, namely aminomethylphosphonic acid (AMPA), which is considered to be more toxic and persistent than glyphosate (Sun, Li, & Jaisi, 2019) and subsequently also degraded by microbes (Castrejón-Godínez et al., 2021; Hove-Jensen, Zechel, & Jochimsen, 2014). On the other hand, glyphosate at

specific concentrations could inhibit the growth of microbes (Pöppe, Bote, Merle, Makarova, & Roesler, 2019) or destroy them under a specific set of conditions (Barnett, Bandy, & Gibson, 2022) due to its interference in shikimate pathway involved with aromatic amino acid biosynthesis in microbes (Van Bruggen et al., 2021). Another possible microbial source in aged PE microplastics–GBH–microbe interactions besides from the biologically ageing process is gut microbiota of the test species. Previous studies have demonstrated that the exposure of glyphosate or GBH at specific concentrations to Chinese mitten crab (*Eriocheir sinensis*) (X. Yang et al., 2019), green see turtle (*Chelonia mydas*) (Kittle, McDermid, Muehlstein, & Balazs, 2018), and zebrafish (*D. rerio*) (Ding et al., 2021) could alter the composition of the gut microbiota, which might be associated with oxidative stress (J. Yang et al., 2023) and be capable of transforming glyphosate within the gastrointestinal tract (Barnett et al., 2022). These might affect the toxic concentrations of GBH released from the aged PE microplastics, interacting with the gut microbes, diffusing across the gut barrier, and directly acting on oxidative stress in target tissues, and explain why both *cytMnSOD* and *CAT* expression levels in shrimp exposed to either single or combined GBH did not significantly alter in general and showed the ability to minimize the elevated of MDA content in hepatopancreas.

Hemocytes are key cells for anti-oxidative system and cell-mediated immune responses including phagocytosis, encapsulation, nodulation, and clotting in invertebrate (Kulkarni et al., 2021). Additionally, it is commonly assumed that hemocyte alterations could affect PO activity because hemocytes are one of the main sources producing prophenoloxidase (proPO) stimulated into PO and then released to hemolymph to catalyze the oxidation of phenols to quinones in the melanization, one of important processes in invertebrate humoral immunity (Sugumaran, 2002). In the present study,

there were no statistically significant differences between PO activities between treatment groups consistent with the corresponding changes in THC. The THC and PO activity results of group MP were in accordance with a study from Dolar et al. (2021) who demonstrated insignificant alterations of THC and PO-like activity in crustacean *P. scaber* following 1.5% crumb rubber exposure. Moreover, Revel et al. (2020) reported no significant differences of PO activity in the coelomic fluid of estuarine worm (*Hediste diversicolor*) during 10-day exposure to merely polyethylene and polypropylene microplastics combination at environmentally relevant concentrations (10 and 50 mg/kg sediment). However, it is interesting to note that although not statistically significant, decreases in PO activity and THC of group Hi-GBH as compared to group MP+Hi-GBH were observed, possibly indicating lesser GBH toxicity and greater compensatory mechanisms of response following exposure to GBH with the presence of the microplastics. Like in some extent of previous studies, immunotoxicity modification by co-exposure of microplastics have been observed in other invertebrate organisms such as blood clam (*Tegillarca granosa*) (Shi et al., 2020; Tang et al., 2020), mussels *Mytilus coruscus* and *M. galloprovincialis* (Gu et al., 2020; Pittura et al., 2018), and scallop (*Chlamys farreri*) (Xia et al., 2020).

In addition to hemocytes, hepatopancreas is another important organ of shrimp, which plays an integrated pivotal role in an innate immune response, metabolism, and detoxification (Y. Li et al., 2022; D. Yu, Zhai, He, & Jia, 2022). Death via activation of caspases, enzymes that execute cytotoxic mechanism in innate immunity, could occur in cells during exposure to microplastics (Hou et al., 2021; X. Wang, Zhang, Sun, Wang, & Gong, 2022) or GBH (Benachour & Séralini, 2009; Hao et al., 2019). Here, analysis of transcript encoding caspase-2 enzyme in hepatopancreas showed

that, there was no significant difference in expression of *Casp2* between groups MP and NC, which follows the insignificant alteration of MDA content and expression levels of *cytMnSOD* and *CAT* in the hepatopancreas. Previous studies (Fei et al., 2020; H. Guo, Li, Wang, Wang, & Shen, 2017; Redza-Dutordoir & Averill-Bates, 2016) have shown that the imbalance between ROS and antioxidant system could cause oxidative stress resulting in the increase of cellular lipid peroxidation level which lead to disruption of membrane fluidity and integrity, activation of caspases, and eventually cell death. However, with the insignificantly different results we observed in group MP, microplastics sizes (32 – 500 µm) used in our experiment might be one of various factors responsible for the negligible induction of cell death (Beijer et al., 2022), supported also by Tang et al. (2020), who reported the significant down-regulation of *Caspase-3* in hemocytes of *T. granosa* exposed to 500 nm polystyrene compared to the 30 µm one. Considering the cytotoxicity effect of GBH alone and in combination, no significant differences in the hepatopancreatic expression of *Casp2* in all groups exposed to GBH might reflect that even exposure to GBH at environmentally high concentration via feed, hepatopancreatic cell death was unlikely to be a major mechanism affected. Corresponding with this, the expressions of *cytMnSOD* and *CAT* in hepatopancreas were generally altered, thereby responding to elevated MDA contents induced by GBH exposures to maintain cellular redox homeostasis and prevent hepatopancreatic cells from damages and death. Additionally, this effect was consistent with no apparent changes of the expression of transcript encoding HSP70, a protein produced by cells to maintain homeostasis, mitigate cell stress, and stimulate innate immunity (He et al., 2010; Valentim-Neto, Moser, Fraga, & Marques, 2014), across all treatment groups, supporting the suggestion that altered antioxidants may be sufficient to modulate oxidative stress resulting in no disturbance in

expression of *hsp70*. Another possibility of no significant alteration of *hsp70* expression in hepatopancreas might be that heat shock proteins (HSPs) comprising several families based on molecular weight and protein homology such as *hsp60*, *hsp70*, and *hsp90* (Nover & Scharf, 1997) are triggered by a different cascade of events and altered in different patterns from other chemical pollutants. Magni et al. (2019) presented some evidences that zebra mussel (*Dreissena polymorpha*) exposed to combination of 1- and 10-µm virgin polystyrene microbead at 2×10^6 microplastic pieces/L for 6 days exhibited an induction of oxidative stress, whereas the inactivation of HSPs in gills was observed, inferring that HSPs might be disrupted in different mechanisms by the exposed polystyrene and considered as housekeeping proteins instead of the ones against injury associated with adverse stresses. No significant difference in expression level of *hsp70*, while significantly increased lipid peroxidation observed was previously reported for chronic exposure to GBH-contaminated feed in broiler hens (Fréville et al., 2022). Similarly, the HSP70 protein expressions detected in soil nematode (*Caenorhabditis elegans*) were not significantly altered after exposure to glyphosate at concentrations of 1/1000, 1/100 and 1/10 of LC50, implying that alteration of HSPs expression might not be observed in GBH exposure at some specific concentrations (Yunbiao Wang, Ezemaduka, Li, Chen, & Song, 2017).

To explore the toxicological effects of aged PE microplastics and GBH, either alone or in combination, on humoral immunity, we investigated the expression of a number of transcripts encoding humoral immune-related molecules in the hepatopancreas. We found no significant differences across all expression of investigated immune-related genes between groups MP and NC, indicating that dietary exposure to aged PE microplastics at an environmentally relevant concentration might not significantly affect

humoral innate immunity of *L. vannamei*. A previous study (C. Zhang, Pan, Wang, Xu, & Zou, 2022) also observed no significantly altered expression of immune-related genes, interleukin-8 (*IL-8)* and nuclear factor NF-kappa-B p65 (*NF-κB p65*), in intestines of hybrid snakehead larvae (*Channa maculata* × *Channa argus*) exposed to virgin fluorescent polystyrene microplastics with 0.5 µm and 5 µm in mean diameters at concentrations of 0.2 mg/L, 2 mg/L and 20 mg/L for 48 hours. Additionally, in another study (L. a. Li et al., 2021), pristine, green, fluorescent polystyrene microplastics with 20-µm diameter caused no significant changes in the expression of *IL-8* at microplastic concentrations of 0.115 µg/L and 11.5 µg/L and interferon (*IFN*) at the one of 0.115 µg/L in juvenile yellow catfish (*Pelteobagrus fulvidraco*). To some extent, these results were consistent with our findings. However, some significant alterations of transcripts encoding humoral immune-related molecules in response to single or combined GBH exposures were observed in this study. The C-type lectin 4 (*CTL4*) and anti-lipopolysaccharide factor 3 (*ALF3*) genes, encoding probably effector molecule (H. Li et al., 2015) and antimicrobial peptide (AMP) (Yin et al., 2023) in *L. vannamei*, were significantly down-regulated in groups Lo-GBH and Hi-GBH, respectively, whereas the Penaeidin 3 (*PEN3*) gene, encoding AMP (Muñoz, Vandenbulcke, Saulnier, & Bachère, 2002), was significantly up-regulated in *L. vannamei* exposed to GBH alone or in combination, suggesting that bioavailable GBH may be uptaken across the intestinal membrane, distribute to other tissues, and affect humoral immunity in hepatopancreas of *L. vannamei*. Different from *ALF3* and *CTL4* which mostly could be detected in hepatopancreas and hemocytes (H. Li et al., 2015; Yin et al., 2023), *PEN3* is mainly expressed in hemocytes, but minimally in hepatopancreas of shrimp (Muñoz et al., 2002). As we investigated the expressions of *ALF3*, *CTL4*, and *PEN3* in hepatopancreas, it is possible that the significant upregulation of

PEN3 in hepatopancreas across GBH exposure groups might be the result of two probable causes: (1) hemocyte infiltration and accumulation in this organ by its unavoidable contamination. Corresponding to the histological examination that presented more existing hemocytes in the group of GBH-treated shrimp. However, hemocyte infiltration was not observed in some samples of shrimp that were treated with GBH. It could be the effect of sampling for histological interpretation. (2) It might be a direct response of the hepatopancreatic cells to GBH exposure. However, it remains unclear on the putative sources of expression of this gene that could be further investigated. Interestingly, statistically significant differences in expressions of *ALF3* between groups MP+Hi-GBH and Hi-GBH as well as of *PEN3* between groups MP+Lo-GBH and Lo-GBH were revealed, implying that aged PE microplastics might modify immunotoxicity of GBH. However, no similar altered expression pattern of *ALF3*, *CTL4*, and *PEN3* between exposure to GBH alone and in combination was recognized. Moreover, the expression patterns of the other immune-related molecules profiles (*Toll1*, *IMD*, and *STAT*) appeared to exhibit no significant alterations and generally not follow similar expression patterns to either *ALF3*, *CTL4*, or *PEN3*. The non-uniform pattern of differential expressions between each of the genes of interest is therefore more likely due to the crosstalk of different signaling pathways in a coordinated manner to fine-tune an effective humoral immune response. For instance, the expression pattern of *ALF3* inversely compared to those of *CTL4*, and *PEN3* across GBH treatment groups in our study was in accordance with a study from Liu et al. (2016) who demonstrated that there was significantly increased expression of *ALF*, while the expression of *PEN3* were significantly decreased in hemocytes of *L. vannamei* following *Vibrio anguillarum* infection. Furthermore, ribonucleic acid interference (RNAi) silencing of *Toll*, which encodes an receptor in Toll pathway, resulted in a

pronounced increase of transcripts involved in IMD pathway in *L. vannamei*, and vice versa (Liu et al., 2016). C. Li et al. (2015) and Guanzon and Maningas (2018) also showed that Toll3 in Toll pathway links with the regulation of another pathway, IRF Vago-JAK/STAT pathway, suggesting that humoral immune-mediated pathways may be dependent and implicate with a variety of signal-transduction pathways in *L. vannamei*.

The crustacean humoral innate immune response, mainly mediated by Toll, immune deficiency (IMD), and JAK/STAT pathways, is triggered by binding of pattern recognition receptors (PRRs) with pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), or cytokine-like molecules which elicit the expression of gene-encoded defense molecules, such as receptors, transcriptional factors, antimicrobial peptides or effectors, to protect the shrimp from an exogenous or endogenous threat (C. Li, Wang, & He, 2019; P. Yan et al., 2022). Nonetheless, with insignificant alteration of *Casp2* and *hsp70* expressions as well as no observed histological alteration linked to cellular death in the hepatopancreas, the significant alterations in the expression of *ALF3*, *CTL4*, and *PEN3* in GBH treatment groups might be considered improbable to be modulated via the trigger of DAMPs released from injured cells. More likely explanations might be microbial dysbiosis or disruptions of neuroendocrine-immune (NEI) regulation due to mechanism of action and toxicity of GBH per se. Given glyphosate being a derivative of glycine and similar to glutamate structure, which could act as neurotransmitters, exposure to glyphosate might cause interference in glycine-mediated signaling and glutamate modulation (Myers et al., 2016). A recent study (Fuxuan Wang, Li, Xiang, & Li, 2019) also elucidated that the neurotransmitters glycine and glutamate might be involved in the NEI regulation network in *L. vannamei*. Apart from being an analog of glycine, the mechanism of action of glyphosate

in GBH is to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimic acid pathway of undesired plants (Forlani, Mangiagalli, Nielsen, & Suardi, 1999; Kataoka, Ryu, Sakiyama, & Makita, 1996), which also presents and is inhibited in microbiota leading to microbial dysbiosis in the affected host (Maddalon, Galbiati, Colosio, Mandić-Rajčević, & Corsini, 2021). A recent study on Chinese mitten crab (*E. sinensis*) investigated diversity of microbial communities in intestines of the crab exposed to glyphosate solution at half of the LC50 concentration for 96 hours depicting that glyphosate might perturb gut microbial diversity involving with the immune regulation and negatively affect survival rate of *E. sinensis* (X. Yang et al., 2019). Another previous study on gut and whole body of honey bees (*Apis mellifera*) exposed glyphosate solutions also represented the decrease in abundance of gut microbiota, which might result in the down-regulation of AMP-related genes, i.e., apidaecin, defensin, and hymenoptaecin (Motta, Powell, & Moran, 2022), similarly to findings observed in Mediterranean mussel (*M. galloprovincialis*) that glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), might be involved in changes in the transcription of immune-related genes by disturbance of host microbiota communities (Iori et al., 2020). Hence, we believe that alterations in some immune-related gene expressions in shrimp exposed to GBH, either alone or in combination, may be the consequences from microbial dysbiosis or dysregulation of the NEI system rather than DAMPs released from damaged cells that trigger immune pathways.

5. CONCLUSIONS

This study explored the toxicity of aged PE microplastics and GBH, either alone or in combination, orally exposed to shrimp, *L. vannamei* and provided considerations for the potential of aged PE microplastics to modify the toxicity of GBH. We found evidence that 96 hours of exposure to environmental concentration of aged PE microplastics through feed slightly affected oxidant/antioxidant balance, immune processes, and hepatopancreas histology of shrimp. GBH alone can alter AChE, some parameters of antioxidant status and immunity, and proportion of hepatopancreatic cells. The combined microplastics are likely to attenuate the toxicity of GBH in some endpoints, however, the mechanism and pattern of such modification are still unclear. Therefore, this study indicates the need for further research into the long-term toxicology test and mechanism to broadly capture the risk related to microplastics and GBH on shrimp (*L. vannamei*).

6. SUMMARY

Plastic litter and glyphosate-based herbicides (GBHs) are increasingly becoming pervasive in aquatic environments, characterized by circulatory patterns between different compartments and continual loading with new debris. Although microplastics and GBHs unavoidably contaminate aquatic environments, their combination effect on aquatic organisms has been scarcely investigated. We present the short-term study on individual and combined effects of aged polyethylene (PE) microplastics and glyphosate-based herbicide (GBH) exposures at environmentally relevant concentrations on oxidative damage (malondialdehyde), antioxidant responses (superoxide dismutase and catalase), immune parameters (total hemocyte count, phenoloxidase activity, and immune-related genes), and hepatopancreas histology in shrimp (*Litopenaeus vannamei*). In addition, the acetylcholinesterase (AChE) was evaluated as a biomarker of GBH exposure. After 96 hours of exposure, individual PE microplastics exposure slightly influenced oxidative status, immune parameters, and histology. The decrease on AChE concentration and alterations of oxidative status, immunity, and cell population in hepatopancreas were observed in single GBH-exposed shrimp. The combinations of aged PE microplastics and GBH induced some changes that differed from individual GBH exposures. For instance, some alterations implied that the presence of aged PE microplastics may alleviate GBH toxicity to *L. vannamei* (AChE concentration and some expressions of immune-related genes). In conclusion, our results suggest that single exposure of aged PE microplastics at environmental concentration may slightly affect the health of shrimp (*L. vannamei*) and aged PE microplastics are likely to modify the toxicity of other co-exposed chemicals. Further

research on the long-term toxicology test of and mechanism between microplastics and chemicals combination is needed.

Keywords: Aged microplastics; Glyphosate; Combined toxicity; Immune response; Oxidative stress

7. ZUSAMMENFASSUNG

Plastikmüll und glyphosathaltige Herbizide (GBHs) werden zunehmend in Gewässern verbreitet, die durch zirkulatorische Muster zwischen verschiedenen Bereichen und kontinuierlicher Belastung mit neuen Ablagerungen gekennzeichnet sind. Obwohl Mikroplastik und GBHs unausweichlich verschiedenste Gewässer kontaminieren, wurde ihre kombinierte Wirkung auf im Wasser beherbergte Organismen bisher kaum untersucht. Wir präsentieren diesbezüglich eine Kurzzeitstudie zu den individuellen und kombinierten Wirkungen von gealtertem Polyethylen (PE)-Mikroplastik und glyphosathaltigem Herbizid (GBH) bei umweltrelevanten Konzentrationen auf die Parameter oxidative Schäden (Malondialdehyd), antioxidative Reaktionen (Superoxiddismutase und Katalase), Immunparameter (gesamte Hämocytenzahl, Phenoloxidase-Aktivität und immunrelevante Gene) sowie die histologische Betrachtung des Hepatopankreas bei Garnelen (*Litopenaeus vannamei*). Darüber hinaus wurde die Konzentration des Enzyms Acetylcholinesterase (AChE) als Biomarker für die GBH-Exposition bewertet. Nach bereits 96 Stunden unter Belastung mit den oben genannten Stoffen, beeinflusste die individuelle Belastung von PE-Mikroplastik leicht den oxidativen Status, die Immunparameter und die Histologie. Ein Rückgang der AChE-Konzentration und Veränderungen des oxidativen Status, der Immunaktivität und der Zellpopulation im Hepatopankreas wurden bei einzelnen unter GBHBelastung stehenden Garnelen beobachtet. Die Kombination von gealtertem PE-Mikroplastik und GBH induzierte einige Veränderungen, die sich von der Auswirkung unter Einzelbelastung nur mit GBH unterschieden. Einige Änderungen deuteten beispielsweise darauf hin, dass das

Vorhandensein von gealtertem PEMikroplastik die Toxizität von GBH für *L. vannamei* (AChE-Konzentration und einige andere Immunantworten) mildern könnte. Zusammenfassend deuten unsere Ergebnisse darauf hin, dass die einzelne Aussetzung von gealtertem PE-Mikroplastik bei umweltrelevanten Konzentrationen die Gesundheit von Garnelen (*L. vannamei*) leicht beeinträchtigen kann und, dass gealtertes PE-Mikroplastik die Toxizität anderer zusätzlicher beeinträchtigender Chemikalien vermutlich beeinflussen kann. Weitere Forschungen zur Langzeittoxizität und den Mechanismen zwischen Mikroplastik und der Kombination von anderen Chemikalien ist erforderlich.

Schlüsselbegriffe: Gealtertes Mikroplastik; Glyphosat; kombinierte Toxizität; Immunantwort; oxidativer Stress

8. REFERENCES

A. Ranjan & C.E. Boyd. (2018). Appraising pond liners for shrimp culture. Retrieved from https://www.aquaculturealliance.org/advocate/appraising-pond-liners-shrimp-culture/

Abby-Kalio NJ. (1990). *Feeding and predation in penaeid shrimps of the bony and New Calabar Estuaries of the Niger Delta*. 8th Fisheries Society of Nigeria, Akure.

Acosta-Coley, I., Duran-Izquierdo, M., Rodriguez-Cavallo, E., Mercado-Camargo, J., Mendez-Cuadro, D., & Olivero-Verbel, J. (2019). Quantification of microplastics along the Caribbean Coastline of Colombia: Pollution profile and biological effects on Caenorhabditis elegans. *Mar. Pollut. Bull., 146*, 574-583. doi:https://doi.org/10.1016/j.marpolbul.2019.06.084

Agboola, O. D., & Benson, N. U. (2021). Physisorption and chemisorption mechanisms influencing micro (nano) plastics-organic chemical contaminants interactions: A review. *Front. Environ. Sci., 9*. doi:10.3389/fenvs.2021.678574

Ahrendt, C., Perez-Venegas, D. J., Urbina, M., Gonzalez, C., Echeveste, P., Aldana, M., . . . Galbán-Malagón, C. (2020). Microplastic ingestion cause intestinal lesions in the intertidal fish *Girella*

laevifrons. *Mar. Pollut. Bull., 151*, 110795. doi:https://doi.org/10.1016/j.marpolbul.2019.110795

Akarsu, C., Kumbur, H., Gökdağ, K., Kıdeyş, A. E., & Sanchez-Vidal, A. (2020). Microplastics composition and load from three wastewater treatment plants discharging into Mersin Bay, north eastern Mediterranean Sea. *Mar. Pollut. Bull., 150*, 110776. doi:https://doi.org/10.1016/j.marpolbul.2019.110776

Aktar, M. W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip. Toxicol., 2*(1), 1-12. doi:10.2478/v10102-009-0001-7

Alonso, L. L., Demetrio, P. M., Agustina Etchegoyen, M., & Marino, D. J. (2018). Glyphosate and atrazine in rainfall and soils in agroproductive areas of the pampas region in Argentina. *Sci. Total Environ., 645*, 89-96. doi:https://doi.org/10.1016/j.scitotenv.2018.07.134

Amelia, T. S. M., Khalik, W. M. A. W. M., Ong, M. C., Shao, Y. T., Pan, H.-J., & Bhubalan, K. (2021). Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. *Prog. Earth Planet. Sci., 8*(1), 12. doi:10.1186/s40645-020-00405-4

Anderson, P. J., Warrack, S., Langen, V., Challis, J. K., Hanson, M. L., & Rennie, M. D. (2017). Microplastic contamination in Lake

Winnipeg, Canada. *Environ. Pollut., 225*, 223-231. doi:https://doi.org/10.1016/j.envpol.2017.02.072

Antunes, J. C., Frias, J. G. L., Micaelo, A. C., & Sobral, P. (2013). Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants. *Estuar. Coast. Shelf Sci., 130*, 62-69. doi:https://doi.org/10.1016/j.ecss.2013.06.016

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environ. Int., 102*, 165-176. doi:https://doi.org/10.1016/j.envint.2017.02.013

Babalola, O. O., Truter, J. C., & van Wyk, J. H. (2019). Mortality, teratogenicity and growth inhibition of three glyphosate formulations using Frog Embryo Teratogenesis Assay-Xenopus. *J. Appl. Toxicol., 39*(9), 1257-1266. doi:https://doi.org/10.1002/jat.3811

Bakir, A., Rowland, S. J., & Thompson, R. C. (2014a). Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. *Environ. Pollut., 185*, 16-23. doi:https://doi.org/10.1016/j.envpol.2013.10.007

Bakir, A., Rowland, S. J., & Thompson, R. C. (2014b). Transport of persistent organic pollutants by microplastics in estuarine conditions. *Estuar. Coast. Shelf Sci., 140*, 14-21. doi:https://doi.org/10.1016/j.ecss.2014.01.004

Barboza, L. G. A., Vieira, L. R., Branco, V., Carvalho, C., & Guilhermino, L. (2018). Microplastics increase mercury bioconcentration in gills and bioaccumulation in the liver, and cause oxidative stress and damage in *Dicentrarchus labrax* juveniles. *Sci. Rep., 8*(1), 15655. doi:10.1038/s41598-018-34125-z

Barboza, L. G. A., Vieira, L. R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., & Guilhermino, L. (2018). Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). *Aquat. Toxicol., 195*, 49-57. doi:10.1016/j.aquatox.2017.12.008

Barnes, D., Galgani, F., Thompson, R., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. Lond., B, Biol. Sci., 364*, 1985-1998. doi:10.1098/rstb.2008.0205

Barnett, J. A., Bandy, M. L., & Gibson, D. L. (2022). Is the use of glyphosate in modern agriculture resulting in increased neuropsychiatric conditions through modulation of the gut-brain-microbiome axis? *Front Nutr, 9*, 827384. doi:10.3389/fnut.2022.827384

Battaglin, W. A., Meyer, M. T., Kuivila, K. M., & Dietze, J. E. (2014). Glyphosate and its degradation product AMPA Occur frequently and widely in U.S. soils, surface water, groundwater, and

precipitation. *J. Am. Water Resour. Assoc., 50*(2), 275-290. doi:https://doi.org/10.1111/jawr.12159

Baucom, R. S. (2019). Evolutionary and ecological insights from herbicide-resistant weeds: what have we learned about plant adaptation, and what is left to uncover? *New Phytol., 223*(1), 68-82. doi:https://doi.org/10.1111/nph.15723

Beijer, N. R., Dehaut, A., Carlier, M. P., Wolter, H., Versteegen, R. M., Pennings, J. L., . . . Timmermans, B. G. (2022). Relationship between particle properties and immunotoxicological effects of environmentally-sourced microplastics. *Front. Water, 4*, 866732.

Bell, T. A., & Lightner, D. V. (1988). *A handbook of normal penaeid shrimp histology*. Baton Rouge La: World Aquaculture Society.

Benachour, N., & Séralini, G.-E. (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chemical Research in Toxicology, 22*(1), 97-105. doi:10.1021/tx800218n

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environ. Sci. Eur., 28*(1), 3. doi:10.1186/s12302-016-0070-0

Bhagat, J., Nishimura, N., & Shimada, Y. (2021). Toxicological interactions of microplastics/nanoplastics and environmental contaminants:

Current knowledge and future perspectives. *J. Hazard. Mater., 405,* 123913. doi:https://doi.org/10.1016/j.jhazmat.2020.123913

Binda, G., Spanu, D., Monticelli, D., Pozzi, A., Bellasi, A., Bettinetti, R., . . . Nizzetto, L. (2021). Unfolding the interaction between microplastics and (trace) elements in water: A critical review. *Water Research, 204*, 117637. doi:https://doi.org/10.1016/j.watres.2021.117637

Binda, G., Zanetti, G., Bellasi, A., Spanu, D., Boldrocchi, G., Bettinetti, R., . . . Nizzetto, L. (2023). Physicochemical and biological ageing processes of (micro)plastics in the environment: a multi-tiered study on polyethylene. *Environmental Science and Pollution Research, 30*(3), 6298-6312. doi:10.1007/s11356-022-22599-4

Bobori, D. C., Feidantsis, K., Dimitriadi, A., Datsi, N., Ripis, P., Kalogiannis, S., . . . Kaloyianni, M. (2022). Dose-dependent cytotoxicity of polypropylene microplastics (PP-MPs) in two freshwater fishes. *International Journal of Molecular Sciences, 23*(22), 13878. Retrieved from https://www.mdpi.com/1422-0067/23/22/13878

Bonansea, R. I., Filippi, I., Wunderlin, D. A., Marino, D. J. G., & Amé, M. V. (2017). The fate of glyphosate and AMPA in a freshwater endorheic basin: An ecotoxicological risk assessment. *Toxics, 6*(1). doi:10.3390/toxics6010003

Bonnet, J. L., Bonnemoy, F., Dusser, M., & Bohatier, J. (2007). Assessment of the potential toxicity of herbicides and their degradation products to nontarget cells using two microorganisms, the bacteria *Vibrio fischeri* and the ciliate *Tetrahymena pyriformis*. *Environ. Toxicol., 22*(1), 78-91. doi:10.1002/tox.20237

Borrirukwisitsak, S., Keenan, H., & Gauchotte-Lindsay, C. (2012). Effects of salinity, pH and temperature on the octanol-water partition coefficient of bisphenol A. *Int. J. Environ. Sci. Dev., 3*, 460-464. doi:10.7763/IJESD.2012.V3.267

Botten, N., Wood, L. J., & Werner, J. R. (2021). Glyphosate remains in forest plant tissues for a decade or more. *For. Ecol. Manag., 493*, 119259. doi:https://doi.org/10.1016/j.foreco.2021.119259

Brennecke, D., Duarte, B., Paiva, F., Caçador, I., & Canning-Clode, J. (2016). Microplastics as vector for heavy metal contamination from the marine environment. *Estuar. Coast. Shelf Sci., 178*, 189-195. doi:https://doi.org/10.1016/j.ecss.2015.12.003

Briggs, M., Funge-Smith, S., Subasinghe, R., & Phillips, M. (2004). Introductions and movement of *Penaeus vannamei* and *Penaeus stylirostris* in Asia and the Pacific. *RAP publication, 10*(2004), 92.

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M., & Thompson, R. C. (2008). Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ. Sci. Technol., 42*(13), 5026-5031. doi:10.1021/es800249a

Browne, M. A., Galloway, T., & Thompson, R. (2007). Microplastic – An emerging contaminant of potential concern? *Integr. Environ. Assess. Manag., 3*, 559-561. doi:10.1897/IEAM_2007-048

Browne, Mark A., Niven, Stewart J., Galloway, Tamara S., Rowland, Steve J., & Thompson, Richard C. (2013). Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. *Curr. Biol., 23*(23), 2388-2392. doi:https://doi.org/10.1016/j.cub.2013.10.012

Burge, E. J., Madigan, D. J., Burnett, L. E., & Burnett, K. G. (2007). Lysozyme gene expression by hemocytes of Pacific white shrimp, *Litopenaeus vannamei*, after injection with *Vibrio*. *Fish & Shellfish Immunology, 22*(4), 327-339. doi:https://doi.org/10.1016/j.fsi.2006.06.004

Castrejón-Godínez, M. L., Tovar-Sánchez, E., Valencia-Cuevas, L., Rosas-Ramírez, M. E., Rodríguez, A., & Mussali-Galante, P. (2021). Glyphosate pollution treatment and microbial degradation alternatives, a review. *Microorganisms, 9*(11). doi:10.3390/microorganisms9112322

Castro Berman, M., Marino, D. J. G., Quiroga, M. V., & Zagarese, H. (2018). Occurrence and levels of glyphosate and AMPA in shallow lakes from the Pampean and Patagonian regions of Argentina. *Chemosphere, 200*, 513-522. doi:10.1016/j.chemosphere.2018.02.103

Cervellione, F., McGurk, C., Silva, P., Owen, M., & Van Den Broeck, W. (2017). Optimization of fixation methods for image analysis of the hepatopancreas in whiteleg shrimp, *Penaeus vannamei* (Boone). *Journal of fish diseases, 40*(4), 517-527.

Chemicals, D. (2005). OECD Guideline for testing of chemicals. *The Organisation for Economic Co-operation and Development: Paris, France*, 1-13.

Chen, C.-S., Le, C. B., Chiu, M.-H., & Chin, W. (2018). The impact of nanoplastics on marine dissolved organic matter assembly. *Sci. Total Environ., 634*, 316-320.

Chen, J., Rao, C., Yuan, R., Sun, D., Guo, S., Li, L., . . . Cao, X. (2021). Long-term exposure to polyethylene microplastics and glyphosate interferes with the behavior, intestinal microbial homeostasis, and metabolites of the common carp (*Cyprinus carpio* L.). *Sci. Total Environ.*, 152681. doi:https://doi.org/10.1016/j.scitotenv.2021.152681

Chen, Y.-Y., Chen, J.-C., Lin, Y.-C., Putra, D. F., Kitikiew, S., Li, C.-C., . . . Yeh, S.-T. (2014). Shrimp that have received carrageenan via immersion and diet exhibit immunocompetence in phagocytosis despite a post-plateau in immune parameters. *Fish & Shellfish Immunology, 36*(2), 352-366. doi:https://doi.org/10.1016/j.fsi.2013.12.004

Chua, E. M., Shimeta, J., Nugegoda, D., Morrison, P. D., & Clarke, B. O. (2014). Assimilation of polybrominated diphenyl ethers from microplastics by the marine amphipod, *Allorchestes Compressa*. *Environ. Sci. Technol., 48*(14), 8127-8134. doi:10.1021/es405717z

Claessens, M., Meester, S. D., Landuyt, L. V., Clerck, K. D., & Janssen, C. R. (2011). Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Mar. Pollut. Bull., 62*(10), 2199-2204. doi:https://doi.org/10.1016/j.marpolbul.2011.06.030

Collins, P. (2010). Environmental stress upon hepatopancreatic cells of freshwater prawns (Decapoda: Caridea) from the floodplain of Paraná River. *Natural Science*(2), 748-759. doi:10.4236/ns.2010.27094

Comai, L., Sen, L. C., & Stalker, D. M. (1983). An altered aroA gene product confers resistance to the herbicide glyphosate. *Science, 221*(4608), 370-371. doi:10.1126/science.221.4608.370

Contardo-Jara, V., Klingelmann, E., & Wiegand, C. (2009). Bioaccumulation of glyphosate and its formulation Roundup Ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes. *Environ. Pollut., 157*(1), 57-63. doi:https://doi.org/10.1016/j.envpol.2008.07.027

Corteel, M., Lima, J., Wille, M., Alday-Sanz, V., Pensaert, M., & Sorgeloos, P. (2009). Molt stage and cuticle damage influence White Spot Syndrome Virus immersion infection in penaeid

shrimp. *Vet. Microbiol., 137*, 209-216. doi:10.1016/j.vetmic.2009.01.018

Courtene-Jones, W., Quinn, B., Gary, S. F., Mogg, A. O. M., & Narayanaswamy, B. E. (2017). Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. *Environ. Pollut., 231*, 271-280. doi:https://doi.org/10.1016/j.envpol.2017.08.026

Cozar, A., Sanz-Martin, M., Marti, E., Gonzalez-Gordillo, J. I., Ubeda, B., Galvez, J. A., . . . Duarte, C. M. (2015). Plastic accumulation in the Mediterranean sea. *PLOS ONE, 10*(4), e0121762. doi:10.1371/journal.pone.0121762

Curren, E., Leaw, C. P., Lim, P. T., & Leong, S. C. Y. (2020). Evidence of marine microplastics in commercially harvested seafood. *Front. Bioeng. Biotechnol., 8*(1390). doi:10.3389/fbioe.2020.562760

Daam, M. A., Moutinho, M. F., Espíndola, E. L. G., & Schiesari, L. (2019). Lethal toxicity of the herbicides acetochlor, ametryn, glyphosate and metribuzin to tropical frog larvae. *Ecotoxicology, 28*(6), 707-715. doi:10.1007/s10646-019-02067-5

De Falco, F., Di Pace, E., Cocca, M., & Avella, M. (2019). The contribution of washing processes of synthetic clothes to microplastic pollution. *Sci. Rep., 9*(1), 6633. doi:10.1038/s41598-019-43023-x

de Melo, M. S., dos Santos, T. P. G., Jaramillo, M., Nezzi, L., Rauh Muller, Y. M., & Nazari, E. M. (2019). Histopathological and ultrastructural indices for the assessment of glyphosate-based herbicide cytotoxicity in decapod crustacean hepatopancreas. *Aquat. Toxicol., 210*, 207-214. doi:https://doi.org/10.1016/j.aquatox.2019.03.007

de Melo, M. S., Nazari, E. M., Müller, Y. M. R., & Gismondi, E. (2020). Modulation of antioxidant gene expressions by Roundup® exposure in the decapod *Macrobrachium potiuna*. *Ecotoxicol. Environ. Saf., 190*, 110086. doi:https://doi.org/10.1016/j.ecoenv.2019.110086

Desforges, J.-P. W., Galbraith, M., Dangerfield, N., & Ross, P. S. (2014). Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Mar. Pollut. Bull., 79*(1), 94-99. doi:https://doi.org/10.1016/j.marpolbul.2013.12.035

Ding, W., Shangguan, Y., Zhu, Y., Sultan, Y., Feng, Y., Zhang, B., . . . Li, X. (2021). Negative impacts of microcystin-LR and glyphosate on zebrafish intestine: Linked with gut microbiota and microRNAs? *Environmental Pollution, 286*, 117685. doi:https://doi.org/10.1016/j.envpol.2021.117685

Dolar, A., Selonen, S., van Gestel, C. A. M., Perc, V., Drobne, D., & Jemec Kokalj, A. (2021). Microplastics, chlorpyrifos and their mixtures modulate immune processes in the terrestrial crustacean *Porcellio*

scaber. *Science of The Total Environment, 772*, 144900. doi:https://doi.org/10.1016/j.scitotenv.2020.144900

Dost, F. N. (2003). Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options: Part IV, Risk to workers using glyphosate formulations. Retrieved from http://www.llbc.leg.bc.ca/public/pubdocs/bcdocs/363001/6_dost_glyphosate.pdf

Duan, Y., Xiong, D., Wang, Y., Zhang, Z., Li, H., Dong, H., & Zhang, J. (2021). Toxicological effects of microplastics in *Litopenaeus vannamei* as indicated by an integrated microbiome, proteomic and metabolomic approach. *Science of The Total Environment, 761*, 143311. doi:https://doi.org/10.1016/j.scitotenv.2020.143311

Duke, S. O., Lydon, J., Koskinen, W. C., Moorman, T. B., Chaney, R. L., & Hammerschmidt, R. (2012). Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *J. Agric. Food. Chem., 60*(42), 10375-10397. doi:10.1021/jf302436u

Duke, S. O., & Powles, S. B. (2008). Glyphosate: a once-in-a-century herbicide. *Pest Manag. Sci., 64*(4), 319-325. doi:10.1002/ps.1518

EC. (1998). Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Off. J. Eur. Comm., 330(32)*, 0032–0054.

Eerkes-Medrano, D., Thompson, R. C., & Aldridge, D. C. (2015). Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res., 75*, 63-82. doi:https://doi.org/10.1016/j.watres.2015.02.012

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*(2), 88-95. doi:https://doi.org/10.1016/0006-2952(61)90145-9

Espinosa, C., Esteban, M. Á., & Cuesta, A. (2019). Dietary administration of PVC and PE microplastics produces histological damage, oxidative stress and immunoregulation in European sea bass (*Dicentrarchus labrax* L.). *Fish & Shellfish Immunology, 95*, 574-583. doi:https://doi.org/10.1016/j.fsi.2019.10.072

Fantón, N., Bacchetta, C., Rossi, A., & Gutierrez, M. F. (2020). Effects of a glyphosate-based herbicide on the development and biochemical biomarkers of the freshwater copepod *Notodiaptomus carteri* (Lowndes, 1934). *Ecotoxicol. Environ. Saf., 196*, 110501. doi:https://doi.org/10.1016/j.ecoenv.2020.110501

FAO. (2005). *Pesticide residues in food, FAO plant production and protection paper 183*. Retrieved from https://www.fao.org/3/a0209e/a0209e00.htm

FAO. (2017). Aquaculture feed and fertilizer resources information system. In: Food and Agriculture Organization Rome.

Fatima, M., Ahmad, I., Sayeed, I., Athar, M., & Raisuddin, S. (2000). Pollutant-induced over-activation of phagocytes is concomitantly associated with peroxidative damage in fish tissues. *Aquatic Toxicology, 49*(4), 243-250. doi:https://doi.org/10.1016/S0166-445X(99)00086-7

Fei, F., Gao, X., Wang, X., Liu, Y., Bin, H., & Liu, B. (2020). Effect of spectral composition on growth, oxidative stress responses, and apoptosis-related gene expression of the shrimp, Penaeus vannamei. *Aquaculture Reports, 16*, 100267. doi:https://doi.org/10.1016/j.aqrep.2019.100267

Fisner, M., Majer, A., Taniguchi, S., Bícego, M., Turra, A., & Gorman, D. (2017). Colour spectrum and resin-type determine the concentration and composition of polycyclic aromatic hydrocarbons (PAHs) in plastic pellets. *Mar. Pollut. Bull., 122*(1), 323-330. doi:https://doi.org/10.1016/j.marpolbul.2017.06.072

Fletcher, R. (2021). Global shrimp production sees significant growth in 2021. Retrieved from https://thefishsite.com/articles/global-shrimp-production-sees-significant-growth-in-2021-gorjan-nikolik-rabobank

Forlani, G., Mangiagalli, A., Nielsen, E., & Suardi, C. M. (1999). Degradation of the phosphonate herbicide glyphosate in soil:

evidence for a possible involvement of unculturable microorganisms. *Soil Biology and Biochemistry, 31*(7), 991-997. doi:https://doi.org/10.1016/S0038-0717(99)00010-3

Fréville, M., Estienne, A., Ramé, C., Lefort, G., Chahnamian, M., Staub, C., . . . Hondelatte, A. (2022). Chronic dietary exposure to a glyphosate-based herbicide results in total or partial reversibility of plasma oxidative stress, cecal microbiota abundance and short-chain fatty acid composition in broiler hens. *Frontiers in Physiology*, 1908.

Frias, J. P. G. L., Otero, V., & Sobral, P. (2014). Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. *Mar. Environ. Res., 95*, 89-95. doi:https://doi.org/10.1016/j.marenvres.2014.01.001

Fries, E., & Zarfl, C. (2012). Sorption of polycyclic aromatic hydrocarbons (PAHs) to low and high density polyethylene (PE). *Environ. Sci. Pollut. Res., 19*(4), 1296-1304. doi:10.1007/s11356-011-0655-5

Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M.-C., & Séralini, G.-E. (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology, 262*(3), 184-191. doi:https://doi.org/10.1016/j.tox.2009.06.006

Gaweł, S., Wardas, M., Niedworok, E., & Wardas, P. (2004). [Malondialdehyde (MDA) as a lipid peroxidation marker]. *Wiad Lek, 57*(9-10), 453-455.

Glozier, N. E., Struger, J., Cessna, A. J., Gledhill, M., Rondeau, M., Ernst, W. R., . . . Donald, D. B. (2012). Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007. *Environ. Sci. Pollut. Res. Int., 19*(3), 821-834. doi:10.1007/s11356-011-0600-7

Glusczak, L., dos Santos Miron, D., Crestani, M., Braga da Fonseca, M., Araújo Pedron, F. d., Duarte, M. F., & Vieira, V. L. P. (2006). Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). *Ecotoxicology and Environmental Safety, 65*(2), 237-241. doi:https://doi.org/10.1016/j.ecoenv.2005.07.017

Gomiero, A., Øysæd, K. B., Agustsson, T., van Hoytema, N., van Thiel, T., & Grati, F. (2019). First record of characterization, concentration and distribution of microplastics in coastal sediments of an urban fjord in south west Norway using a thermal degradation method. *Chemosphere, 227*, 705-714. doi:https://doi.org/10.1016/j.chemosphere.2019.04.096

Göpferich, A. (1996). Mechanisms of polymer degradation and erosion. *Biomaterials, 17*(2), 103-114. doi:https://doi.org/10.1016/0142-9612(96)85755-3

Gouin, T., Becker, R. A., Collot, A.-G., Davis, J. W., Howard, B., Inawaka, K., . . . Hopp, P. W. (2019). Toward the development and application of an environmental risk assessment framework for

microplastic. *Environ. Toxicol. Chem., 38*(10), 2087-2100. doi:https://doi.org/10.1002/etc.4529

Graham, E. R., & Thompson, J. T. (2009). Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. *J. Exp. Mar. Biol. Ecol., 368*(1), 22-29. doi:https://doi.org/10.1016/j.jembe.2008.09.007

Grandcoin, A., Piel, S., & Baurès, E. (2017). Aminomethylphosphonic acid (AMPA) in natural waters: Its sources, behavior and environmental fate. *Water Res., 117*, 187-197. doi:https://doi.org/10.1016/j.watres.2017.03.055

Greco, W. R., Bravo, G., & Parsons, J. C. (1995). The search for synergy: a critical review from a response surface perspective. *Pharmacol. Rev., 47*(2), 331-385. Retrieved from http://pharmrev.aspetjournals.org/cgi/content/abstract/47/2/331

Gu, H., Wei, S., Hu, M., Wei, H., Wang, X., Shang, Y., . . . Wang, Y. (2020). Microplastics aggravate the adverse effects of BDE-47 on physiological and defense performance in mussels. *Journal of Hazardous Materials, 398*, 122909. doi:https://doi.org/10.1016/j.jhazmat.2020.122909

Guanzon, D. A. V., & Maningas, M. B. B. (2018). Functional elucidation of LvToll 3 receptor from *P. vannamei* through RNA interference and its potential role in the shrimp antiviral response. *Developmental &*

Comparative Immunology, 84, 172-180. doi:https://doi.org/10.1016/j.dci.2018.01.020

Guilhermino, L., Vieira, L. R., Ribeiro, D., Tavares, A. S., Cardoso, V., Alves, A., & Almeida, J. M. (2018). Uptake and effects of the antimicrobial florfenicol, microplastics and their mixtures on freshwater exotic invasive bivalve *Corbicula fluminea*. *Sci. Total Environ., 622-623*, 1131-1142. doi:https://doi.org/10.1016/j.scitotenv.2017.12.020

Guo, H., Li, K., Wang, W., Wang, C., & Shen, Y. (2017). Effects of copper on hemocyte apoptosis, ROS production, and gene expression in White shrimp *Litopenaeus vannamei*. *Biological Trace Element Research, 179*(2), 318-326. doi:10.1007/s12011-017-0974-6

Guo, J., Davis, R., Starkey, C., & Davis, D. A. (2021). Efficacy of various coated materials to prevent nutrient leaching for Pacific white shrimp *Litopenaeus vannamei* commercial diets. *Journal of the World Aquaculture Society, 52*(1), 195-203.

Guo, X., Pang, J., Chen, S., & Jia, H. (2018). Sorption properties of tylosin on four different microplastics. *Chemosphere, 209*, 240-245. doi:https://doi.org/10.1016/j.chemosphere.2018.06.100

Guo, X., Wang, X., Zhou, X., Kong, X., Tao, S., & Xing, B. (2012). Sorption of four hydrophobic organic compounds by three chemically distinct polymers: Role of chemical and physical

composition. *Environ. Sci. Technol., 46*(13), 7252-7259. doi:10.1021/es301386z

Gustinasari, K., Sługocki, Ł., Czerniawski, R., Pandebesie, E. S., & Hermana, J. (2021). Acute toxicity and morphology alterations of glyphosate-based herbicides to *Daphnia magna* and *Cyclops vicinus*. *Toxicol. Res., 37*(2), 197-207. doi:10.1007/s43188-020-00054-1

Gutierrez, M. F., Battauz, Y., & Caisso, B. (2017). Disruption of the hatching dynamics of zooplankton egg banks due to glyphosate application. *Chemosphere, 171*, 644-653. doi:https://doi.org/10.1016/j.chemosphere.2016.12.110

Guzzetti, E., Sureda, A., Tejada, S., & Faggio, C. (2018). Microplastic in marine organism: Environmental and toxicological effects. *Environ. Toxicol. Pharmacol., 64*, 164-171. doi:https://doi.org/10.1016/j.etap.2018.10.009

Han, Y., Shi, W., Tang, Y., Zhou, W., Sun, H., Zhang, J., . . . Liu, G. (2022). Microplastics and bisphenol A hamper gonadal development of whiteleg shrimp (*Litopenaeus vannamei*) by interfering with metabolism and disrupting hormone regulation. *Science of The Total Environment, 810*, 152354. doi:https://doi.org/10.1016/j.scitotenv.2021.152354

Hao, Y., Chen, H., Xu, W., Gao, J., Yang, Y., Zhang, Y., & Tao, L. (2019). Roundup® confers cytotoxicity through DNA damage and

Mitochondria-Associated apoptosis induction. *Environmental Pollution, 252*, 917-923. doi:https://doi.org/10.1016/j.envpol.2019.05.128

Hariharan, G., Purvaja, R., Anandavelu, I., Robin, R. S., & Ramesh, R. (2022). Ingestion and toxic impacts of weathered polyethylene (wPE) microplastics and stress defensive responses in whiteleg shrimp (*Penaeus vannamei*). *Chemosphere, 300*, 134487. doi:https://doi.org/10.1016/j.chemosphere.2022.134487

He, S., Liang, X.-F., Li, R.-Q., Li, G.-G., Wang, L., & Shen, D. (2010). Molecular characterization of heat shock protein 70 genes in the liver of three warm freshwater fishes with differential tolerance to microcystin-LR. *Journal of Biochemical and Molecular Toxicology, 24*(5), 293-302. doi:https://doi.org/10.1002/jbt.20338

Helmenstine, A. M. (2020). Plastic definition and examples in chemistry. Retrieved from https://www.thoughtco.com/plastic-chemical-composition-608930

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environ. Sci. Technol., 46*(6), 3060-3075. doi:10.1021/es2031505

Holmes, L. A., Turner, A., & Thompson, R. C. (2014). Interactions between trace metals and plastic production pellets under estuarine

conditions. *Mar. Chem., 167*, 25-32. doi:https://doi.org/10.1016/j.marchem.2014.06.001

Hong, Y., Yang, X., Huang, Y., Yan, G., & Cheng, Y. (2018). Assessment of the oxidative and genotoxic effects of the glyphosate-based herbicide roundup on the freshwater shrimp, Macrobrachium nipponensis. *Chemosphere, 210*, 896-906. doi:https://doi.org/10.1016/j.chemosphere.2018.07.069

Hou, J., Lei, Z., Cui, L., Hou, Y., Yang, L., An, R., . . . Zhang, L. (2021). Polystyrene microplastics lead to pyroptosis and apoptosis of ovarian granulosa cells via NLRP3/Caspase-1 signaling pathway in rats. *Ecotoxicology and Environmental Safety, 212*, 112012. doi:https://doi.org/10.1016/j.ecoenv.2021.112012

Hove-Jensen, B., Zechel, D. L., & Jochimsen, B. (2014). Utilization of glyphosate as phosphate source: Biochemistry and genetics of bacterial carbon-phosphorus lyase. *Microbiology and Molecular Biology Reviews, 78*(1), 176-197. doi:doi:10.1128/MMBR.00040-13

Hu, J.-Q., Yang, S.-Z., Guo, L., Xu, X., Yao, T., & Xie, F. (2017). Microscopic investigation on the adsorption of lubrication oil on microplastics. *J. Mol. Liq., 227*, 351-355. doi:https://doi.org/10.1016/j.molliq.2016.12.043

Huang, J., Yang, Y., & Wang, A. (2010). Reconsideration of phenoloxidase activity determination in white shrimp *Litopenaeus vannamei*. *Fish*

& Shellfish Immunology, 28(1), 240-244. doi:https://doi.org/10.1016/j.fsi.2009.10.010

Hüffer, T., Weniger, A.-K., & Hofmann, T. (2018). Sorption of organic compounds by aged polystyrene microplastic particles. *Environ. Pollut., 236*, 218-225. doi:https://doi.org/10.1016/j.envpol.2018.01.022

Ikeogu, C. F., Ikpeze, O. O., Omobowale, T. O., & Olufemi, B. E. (2020). Effect of Ascorbic Acid on Glyphosate-Induced Residues in Muscles of Juvenile Catfish (*Clarias gariepinus*). *Asian J. Fish. Aquat. Res., 10(1)*, 40-49. doi:https://doi.org/10.9734/ajfar/2020/v10i130174

International Agency for Research on Cancer (IARC). (2017). *Some Organophosphate Insecticides and Herbicides*. Retrieved from Lyon, France: http://monographs.iarc.fr/ENG/Monographs/vol112/mono112.pdf

Iori, S., Rovere, G. D., Ezzat, L., Smits, M., Ferraresso, S. S., Babbucci, M., . . . Milan, M. (2020). The effects of glyphosate and AMPA on the mediterranean mussel *Mytilus galloprovincialis* and its microbiota. *Environ. Res., 182*, 108984. doi:https://doi.org/10.1016/j.envres.2019.108984

Iummato, M. M., Sabatini, S. E., Cacciatore, L. C., Cochón, A. C., Cataldo, D., de Molina, M. d. C. R., & Juárez, Á. B. (2018). Biochemical responses of the golden mussel *Limnoperna fortunei* under dietary

glyphosate exposure. *Ecotoxicol. Environ. Saf., 163*, 69-75. doi:https://doi.org/10.1016/j.ecoenv.2018.07.046

Janewanthanakul, S., Supungul, P., Tang, S., & Tassanakajon, A. (2020). Heat shock protein 70 from *Litopenaeus vannamei* (LvHSP70) is involved in the innate immune response against white spot syndrome virus (WSSV) infection. *Developmental & Comparative Immunology, 102*, 103476. doi:https://doi.org/10.1016/j.dci.2019.103476

Johansen, M. P., Prentice, E., Cresswell, T., & Howell, N. (2018). Initial data on adsorption of Cs and Sr to the surfaces of microplastics with biofilm. *J. Environ. Radioact., 190-191*, 130-133. doi:https://doi.org/10.1016/j.jenvrad.2018.05.001

Kane, I. A., & Clare, M. A. (2019). Dispersion, accumulation, and the ultimate fate of microplastics in deep-marine environments: A review and future directions. *Front. Earth Sci., 7*(80). doi:10.3389/feart.2019.00080

Kanissery, R., Gairhe, B., Kadyampakeni, D., Batuman, O., & Alferez, F. (2019). Glyphosate: Its environmental persistence and impact on crop health and nutrition. *Plants, 8*(11), 499. Retrieved from https://www.mdpi.com/2223-7747/8/11/499

Karapanagioti, H. K., & Klontza, I. (2008). Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). *Mar. Environ.*

Res., 65(4), 283-290. doi:https://doi.org/10.1016/j.marenvres.2007.11.005

Kataoka, H., Ryu, S., Sakiyama, N., & Makita, M. (1996). Simple and rapid determination of the herbicides glyphosate and glufosinate in river water, soil and carrot samples by gas chromatography with flame photometric detection. *Journal of Chromatography A, 726*(1), 253-258. doi:https://doi.org/10.1016/0021-9673(95)01071-8

Kim, D., Chae, Y., & An, Y.-J. (2017). Mixture toxicity of nickel and microplastics with different functional groups on *Daphnia magna*. *Environ. Sci. Technol., 51*(21), 12852-12858. doi:10.1021/acs.est.7b03732

Kittle, R. P., McDermid, K. J., Muehlstein, L., & Balazs, G. H. (2018). Effects of glyphosate herbicide on the gastrointestinal microflora of Hawaiian green turtles (*Chelonia mydas*) Linnaeus. *Marine pollution bulletin*, *127*, 170-174. doi:https://doi.org/10.1016/j.marpolbul.2017.11.030

Klein, S., Dimzon, I. K., Eubeler, J., & Knepper, T. P. (2018). Analysis, occurrence, and degradation of microplastics in the aqueous environment. In M. Wagner & S. Lambert (Eds.), *Freshwater Microplastics : Emerging Environmental Contaminants?* (pp. 51-67).

Korez, S., Gutow, L., & Saborowski, R. (2020, 23 November 2020 - 27 November 2020). *Coping with synthetic and natural*

microparticles: brown shrimp and antioxidant defence. Paper presented at the Micro2020, Fate and Impacts of Microplastics: Knowledge and Responsibilities, Lanzarote and beyond.

Kulkarni, A., Krishnan, S., Anand, D., Kokkattunivarthil Uthaman, S., Otta, S. K., Karunasagar, I., & Kooloth Valappil, R. (2021). Immune responses and immunoprotection in crustaceans with special reference to shrimp. *Reviews in Aquaculture, 13*(1), 431-459. doi:https://doi.org/10.1111/raq.12482

Kwon, J.-H., Chang, S., Hong, S. H., & Shim, W. J. (2017). Microplastics as a vector of hydrophobic contaminants: Importance of hydrophobic additives. *Integr. Environ. Assess. Manag., 13*(3), 494-499. doi:https://doi.org/10.1002/ieam.1906

Lajmanovich, R. C., Attademo, A. M., Lener, G., Cuzziol Boccioni, A. P., Peltzer, P. M., Martinuzzi, C. S., . . . Repetti, M. R. (2022). Glyphosate and glufosinate ammonium, herbicides commonly used on genetically modified crops, and their interaction with microplastics: Ecotoxicity in anuran tadpoles. *Sci. Total Environ., 804*, 150177. doi:https://doi.org/10.1016/j.scitotenv.2021.150177

Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., . . . Reisser, J. (2018). Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Sci. Rep., 8*(1), 4666. doi:10.1038/s41598-018-22939-w

Lebreton, L. C. M., van der Zwet, J., Damsteeg, J.-W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. *Nat. Commun.*, *8*(1), 15611. doi:10.1038/ncomms15611

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., . . . Schludermann, E. (2014). The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environ. Pollut.*, *188*, 177-181. doi:https://doi.org/10.1016/j.envpol.2014.02.006

Lei, K., Qiao, F., Liu, Q., Wei, Z., Qi, H., Cui, S., . . . An, L. (2017). Microplastics releasing from personal care and cosmetic products in China. *Mar. Pollut. Bull.*, *123*(1), 122-126. doi:https://doi.org/10.1016/j.marpolbul.2017.09.016

Levy, G. (1965). Apparent potentiating effect of a second dose of drug. *Nature*, *206*(4983), 517-518. doi:10.1038/206517a0

Li, C., Li, H., Chen, Y., Chen, Y., Wang, S., Weng, S.-P., . . . He, J. (2015). Activation of Vago by interferon regulatory factor (IRF) suggests an interferon system-like antiviral mechanism in shrimp. *Scientific Reports*, *5*(1), 15078. doi:10.1038/srep15078

Li, C., Wang, S., & He, J. (2019). The two NF-κB pathways regulating bacterial and WSSV infection of shrimp. *Frontiers in Immunology*, *10*. doi:10.3389/fimmu.2019.01785

Li, H., Chen, Y., Li, M., Wang, S., Zuo, H., Xu, X., . . . Li, C. (2015). A C-type lectin (LvCTL4) from *Litopenaeus vannamei* is a downstream molecule of the NF-κB signaling pathway and participates in antibacterial immune response. *Fish & Shellfish Immunology, 43*(1), 257-263. doi:https://doi.org/10.1016/j.fsi.2014.12.024

Li, J., Liu, H., & Paul Chen, J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res., 137*, 362-374. doi:https://doi.org/10.1016/j.watres.2017.12.056

Li, J., Zhang, K., & Zhang, H. (2018). Adsorption of antibiotics on microplastics. *Environ. Pollut., 237*, 460-467. doi:https://doi.org/10.1016/j.envpol.2018.02.050

Li, L. a., Xu, R., Jiang, L., Xu, E. G., Wang, M., Wang, J., . . . Wang, Y. (2021). Effects of microplastics on immune responses of the yellow catfish *Pelteobagrus fulvidraco* under hypoxia. *Frontiers in Physiology, 12*, 753999.

Li, M.-H., Ruan, L.-Y., Zhou, J.-W., Fu, Y.-H., Jiang, L., Zhao, H., & Wang, J.-S. (2017). Metabolic profiling of goldfish (*Carassius auratis*) after long-term glyphosate-based herbicide exposure. *Aquat. Toxicol., 188*, 159-169. doi:https://doi.org/10.1016/j.aquatox.2017.05.004

Li, Y., Zhou, F., Yang, Q., Jiang, S., Huang, J., Yang, L., . . . Jiang, S. (2022). Single-cell sequencing reveals types of hepatopancreatic

cells and haemocytes in black tiger shrimp (*Penaeus monodon*) and their molecular responses to ammonia stress. *Front Immunol, 13*, 883043. doi:10.3389/fimmu.2022.883043

Lima, A. R. A., Costa, M. F., & Barletta, M. (2014). Distribution patterns of microplastics within the plankton of a tropical estuary. *Environ. Res., 132*, 146-155. doi:https://doi.org/10.1016/j.envres.2014.03.031

Lithner, D., Damberg, J., Dave, G., & Larsson, Å. (2009). Leachates from plastic consumer products – Screening for toxicity with *Daphnia magna*. *Chemosphere, 74*(9), 1195-1200. doi:https://doi.org/10.1016/j.chemosphere.2008.11.022

Lithner, D., Larsson, Å., & Dave, G. (2011). Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Sci. Total Environ., 409*(18), 3309-3324. doi:https://doi.org/10.1016/j.scitotenv.2011.04.038

Liu, G., Zhu, Z., Yang, Y., Sun, Y., Yu, F., & Ma, J. (2019). Sorption behavior and mechanism of hydrophilic organic chemicals to virgin and aged microplastics in freshwater and seawater. *Environ. Pollut., 246*, 26-33. doi:https://doi.org/10.1016/j.envpol.2018.11.100

Liu, J., Zhang, T., Tian, L., Liu, X., Qi, Z., Ma, Y., . . . Chen, W. (2019). Aging significantly affects mobility and contaminant-mobilizing

ability of nanoplastics in saturated loamy sand. *Environ. Sci. Technol., 53*(10), 5805-5815. doi:10.1021/acs.est.9b00787

Liu, Y., Song, L., Sun, Y., Liu, T., Hou, F., & Liu, X. (2016). Comparison of immune response in Pacific white shrimp, *Litopenaeus vannamei*, after knock down of Toll and IMD gene in vivo. *Developmental & Comparative Immunology, 60*, 41-52. doi:https://doi.org/10.1016/j.dci.2016.02.004

Llorca, M., Schirinzi, G., Martínez, M., Barceló, D., & Farré, M. (2018). Adsorption of perfluoroalkyl substances on microplastics under environmental conditions. *Environ. Pollut., 235*, 680-691. doi:https://doi.org/10.1016/j.envpol.2017.12.075

Lobelle, D., & Cunliffe, M. (2011). Early microbial biofilm formation on marine plastic debris. *Marine pollution bulletin, 62*(1), 197-200. doi:https://doi.org/10.1016/j.marpolbul.2010.10.013

Lopes, F. M., Caldas, S. S., Primel, E. G., & da Rosa, C. E. (2017). Glyphosate adversely affects *Danio rerio* males: Acetylcholinesterase modulation and oxidative stress. *Zebrafish, 14*(2), 97-105.

Lusher, A. L., Burke, A., O'Connor, I., & Officer, R. (2014). Microplastic pollution in the Northeast Atlantic Ocean: Validated and opportunistic sampling. *Mar Pollut Bull, 88*(1-2), 325-333. doi:10.1016/j.marpolbul.2014.08.023

Lusher, A. L., Tirelli, V., O'Connor, I., & Officer, R. (2015). Microplastics in Arctic polar waters: The first reported values of particles in surface and sub-surface samples. *Sci. Rep., 5*(1), 14947. doi:10.1038/srep14947

Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., & Xing, B. (2020). Microplastics in aquatic environments: Toxicity to trigger ecological consequences. *Environ. Pollut., 261*, 114089. doi:https://doi.org/10.1016/j.envpol.2020.114089

Ma, J., & Li, X. (2015). Alteration in the cytokine levels and histopathological damage in common carp induced by glyphosate. *Chemosphere, 128*, 293-298. doi:https://doi.org/10.1016/j.chemosphere.2015.02.017

Maddalon, A., Galbiati, V., Colosio, C., Mandić-Rajčević, S., & Corsini, E. (2021). Glyphosate-based herbicides: Evidence of immune-endocrine alteration. *Toxicology, 459*, 152851. doi:https://doi.org/10.1016/j.tox.2021.152851

Magni, S., Della Torre, C., Garrone, G., D'Amato, A., Parenti, C. C., & Binelli, A. (2019). First evidence of protein modulation by polystyrene microplastics in a freshwater biological model. *Environmental Pollution, 250*, 407-415. doi:https://doi.org/10.1016/j.envpol.2019.04.088

Magnusson, K., & Norén, F. (2014). *Screening of microplastic particles in and down-stream a wastewater treatment plant* (Report C55).

Retrieved from https://urn.kb.se/resolve?urn=urn:nbn:se:naturvardsverket:diva-2226

Martinez, A., & Al-Ahmad, A. J. (2019). Effects of glyphosate and aminomethylphosphonic acid on an isogeneic model of the human blood-brain barrier. *Toxicol. Lett., 304*, 39-49. doi:https://doi.org/10.1016/j.toxlet.2018.12.013

Martínez, M.-A., Rodríguez, J.-L., Lopez-Torres, B., Martínez, M., Martínez-Larrañaga, M.-R., Maximiliano, J.-E., . . . Ares, I. (2020). Use of human neuroblastoma SH-SY5Y cells to evaluate glyphosate-induced effects on oxidative stress, neuronal development and cell death signaling pathways. *Environ. Int., 135*, 105414. doi:https://doi.org/10.1016/j.envint.2019.105414

Mason, S. A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., . . . Rogers, D. L. (2016). Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environ. Pollut., 218*, 1045-1054. doi:https://doi.org/10.1016/j.envpol.2016.08.056

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., & Kaminuma, T. (2001). Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environ. Sci. Technol., 35*(2), 318-324. doi:10.1021/es0010498

Matozzo, V., Munari, M., Masiero, L., Finos, L., & Marin, M. G. (2019). Ecotoxicological hazard of a mixture of glyphosate and aminomethylphosphonic acid to the mussel *Mytilus galloprovincialis* (Lamarck 1819). *Sci. Rep., 9*(1), 14302. doi:10.1038/s41598-019-50607-0

Mattsson, K., Hansson, L. A., & Cedervall, T. (2015). Nano-plastics in the aquatic environment. *Environ. Sci.: Process. Impacts, 17*(10), 1712-1721. doi:10.1039/C5EM00227C

Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., . . . Zambonino-Infante, J. (2015). Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. *Mar. Environ. Res., 112*, 78-85. doi:https://doi.org/10.1016/j.marenvres.2015.09.009

McLaughlin, M., & Lanno, R. (2014). Use of "Bioavailability" as a term in ecotoxicology. *Integr. Environ. Assess. Manag., 10*, 138-140. doi:10.1002/ieam.1497

Menon, M. K. The life history and bionomics of an Indian penaeid prawn *Metapenaeus dobsoni,* Miers. *Proc. Indo-Pacif. Fish. Counc,.* Retrieved from https://www.fao.org/apfic/meeting-reports/detail-events/en/c/420315/

Mensah, P., Muller, W., & Palmer, C. (2012a). Acetylcholinesterase activity in the freshwater shrimp *Caridina nilotica* as a biomarker of Roundup (R) herbicide pollution of freshwater systems in South

Africa. *Water Sci. Technol., 66*, 402-408. doi:10.2166/wst.2012.206

Mensah, P., Muller, W., & Palmer, C. (2012b). Using growth measures in the freshwater shrimp *Caridina nilotica* as biomarkers of Roundup (R) pollution of South African freshwater systems. *Phys. Chem. Earth, 50-52*, 262-268. doi:10.1016/j.pce.2012.08.003

Mensah, P., Palmer, C., & Muller, W. (2012). Lipid peroxidation in the freshwater shrimp *Caridina nilotica* as a biomarker of Roundup (R) herbicide pollution of freshwater systems in South Africa. *Water Sci. Technol., 65*, 1660-1666. doi:10.2166/wst.2012.060

Mensah, P. K., Muller, W. J., & Palmer, C. G. (2011). Acute toxicity of Roundup® herbicide to three life stages of the freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae). *Phys. Chem. Earth., 36*(14), 905-909. doi:https://doi.org/10.1016/j.pce.2011.07.071

Mercurio, P., Flores, F., Mueller, J. F., Carter, S., & Negri, A. P. (2014). Glyphosate persistence in seawater. *Mar. Pollut. Bull., 85*(2), 385-390. doi:10.1016/j.marpolbul.2014.01.021

Mikó, Z., Ujszegi, J., Gál, Z., & Hettyey, A. (2017). Effects of a glyphosate-based herbicide and predation threat on the behaviour of agile frog tadpoles. *Ecotoxicol. Environ. Saf., 140*, 96-102. doi:https://doi.org/10.1016/j.ecoenv.2017.02.032

Milan, M., Dalla Rovere, G., Smits, M., Ferraresso, S., Pastore, P., Marin, M. G., . . . Matozzo, V. (2018). Ecotoxicological effects of the herbicide glyphosate in non-target aquatic species: Transcriptional responses in the mussel *Mytilus galloprovincialis*. *Environ. Pollut., 237*, 442-451. doi:https://doi.org/10.1016/j.envpol.2018.02.049

Mintenig, S. M., Int-Veen, I., Löder, M. G. J., Primpke, S., & Gerdts, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. *Water Res., 108*, 365-372. doi:https://doi.org/10.1016/j.watres.2016.11.015

Mohamed Nor, N. H., & Koelmans, A. A. (2019). Transfer of PCBs from microplastics under simulated gut fluid conditions is biphasic and reversible. *Environ. Sci. Technol., 53*(4), 1874-1883. doi:10.1021/acs.est.8b05143

Moore, C. J., Moore, S. L., Leecaster, M. K., & Weisberg, S. B. (2001). A comparison of plastic and plankton in the North Pacific Central Gyre. *Mar. Pollut. Bull., 42*(12), 1297-1300. doi:https://doi.org/10.1016/S0025-326X(01)00114-X

Moore, J. (2021). Controlling aquatic weeds in farm dams in Western Australia. Retrieved from https://agric.wa.gov.au/n/1501

Motta, E. V. S., Powell, J. E., & Moran, N. A. (2022). Glyphosate induces immune dysregulation in honey bees. *Anim Microbiome, 4*(1), 16. doi:10.1186/s42523-022-00165-0

Mottier, A., Séguin, A., Devos, A., Pabic, C. L., Voiseux, C., Lebel, J. M., . . . Costil, K. (2015). Effects of subchronic exposure to glyphosate in juvenile oysters (*Crassostrea gigas*): From molecular to individual levels. *Mar. Pollut. Bull., 95*(2), 665-677. doi:https://doi.org/10.1016/j.marpolbul.2014.10.026

Muñoz, M., Vandenbulcke, F., Saulnier, D., & Bachère, E. (2002). Expression and distribution of penaeidin antimicrobial peptides are regulated by haemocyte reactions in microbial challenged shrimp. *European Journal of Biochemistry, 269*(11), 2678-2689. doi:https://doi.org/10.1046/j.1432-1033.2002.02934.x

Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environ. Sci. Technol., 50*(11), 5800-5808. doi:10.1021/acs.est.5b05416

Murray, F., & Cowie, P. R. (2011). Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Mar. Pollut. Bull., 62*(6), 1207-1217. doi:https://doi.org/10.1016/j.marpolbul.2011.03.032

Myers, J. P., Antoniou, M. N., Blumberg, B., Carroll, L., Colborn, T., Everett, L. G., . . . Benbrook, C. M. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environmental Health, 15*(1), 19. doi:10.1186/s12940-016-0117-0

Mylläri, V., Ruoko, T.-P., & Syrjälä, S. (2015). A comparison of rheology and FTIR in the study of polypropylene and polystyrene photodegradation. *J. Appl. Polym. Sci., 132*. doi:10.1002/app.42246

Nagy, K., Tessema, R. A., Budnik, L. T., & Ádám, B. (2019). Comparative cyto- and genotoxicity assessment of glyphosate and glyphosate-based herbicides in human peripheral white blood cells. *Environ. Res., 179*, 108851. doi:https://doi.org/10.1016/j.envres.2019.108851

Naik, R. K., Naik, M. M., D'Costa, P. M., & Shaikh, F. (2019). Microplastics in ballast water as an emerging source and vector for harmful chemicals, antibiotics, metals, bacterial pathogens and HAB species: A potential risk to the marine environment and human health. *Mar. Pollut. Bull., 149*, 110525. doi:https://doi.org/10.1016/j.marpolbul.2019.110525

Ng, K. L., & Obbard, J. P. (2006). Prevalence of microplastics in Singapore's coastal marine environment. *Mar. Pollut. Bull., 52*(7), 761-767. doi:https://doi.org/10.1016/j.marpolbul.2005.11.017

Niemcharoen, S., Haetrakul, T., Palić, D., & Chansue, N. (2022). Microplastic-contaminated feed interferes with antioxidant enzyme and lysozyme gene expression of Pacific White shrimp (*Litopenaeus vannamei*) leading to hepatopancreas damage and increased mortality. *Animals, 12*(23), 3308. Retrieved from https://www.mdpi.com/2076-2615/12/23/3308

Nover, L., & Scharf, K. D. (1997). Heat stress proteins and transcription factors. *Cellular and Molecular Life Sciences CMLS, 53*(1), 80-103. doi:10.1007/PL00000583

OECD. (2006). *"Section 2 - General Information Concerning the Genes and their Enzymes that Confer Tolerance to Glyphosate Herbicide", in Safety Assessment of Transgenic Organisms* (OECD Ed. Vol. 1). Paris: OECD Consensus Documents.

Oliver, M. J. (2014). Why we need GMO crops in agriculture. *Missouri medicine, 111*(6), 492-507. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6173531/

P. Kungvankij & T.E. Chua. (1986). Shrimp culture: Pond design, operation and management. Retrieved from https://www.fao.org/3/ac210e/AC210E00.htm#TOC

Pala, A. (2019). The effect of a glyphosate-based herbicide on acetylcholinesterase (AChE) activity, oxidative stress, and antioxidant status in freshwater amphipod: *Gammarus pulex* (Crustacean). *Environmental Science and Pollution Research, 26*(36), 36869-36877. doi:10.1007/s11356-019-06804-5

Pascall, M. A., Zabik, M. E., Zabik, M. J., & Hernandez, R. J. (2005). Uptake of polychlorinated biphenyls (PCBs) from an aqueous medium by polyethylene, polyvinyl chloride, and polystyrene films. *J. Agric. Food Chem., 53*(1), 164-169. doi:10.1021/jf048978t

Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., . . . Soudant, P. (2016). Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: Toxicity and influence on fluoranthene bioaccumulation. *Environ. Pollut., 216*, 724-737. doi:https://doi.org/10.1016/j.envpol.2016.06.039

Peng, J., Wang, J., & Cai, L. (2017). Current understanding of microplastics in the environment: Occurrence, fate, risks, and what we should do. *Integr. Environ. Assess. Manag., 13*(3), 476-482. doi:https://doi.org/10.1002/ieam.1912

Petriella, L. S.-A. (2007). Functional morphology of the hepatopancreas of *Palaemonetes argentinus* (Crustacea: Decapoda): influence of environmental pollution. *Revista de Biología Tropical*.

Piccini, C., Fazi, S., Pérez, G., Batani, G., Martínez de la Escalera, G., & Sotelo-Silveira, J. R. (2020). Resistance to degradation and effect of the herbicide glyphosate on the bacterioplankton community of a large river system dominated by agricultural activities. *Mar. Freshw. Res., 71*(8), 1026-1032. doi:https://doi.org/10.1071/MF19079

Pittura, L., Avio, C. G., Giuliani, M. E., d'Errico, G., Keiter, S. H., Cormier, B., . . . Regoli, F. (2018). Microplastics as vehicles of environmental PAHs to marine organisms: Combined chemical and physical hazards to the mediterranean mussels, *Mytilus galloprovincialis*. *Frontiers in Marine Science, 5*(APR). doi:10.3389/fmars.2018.00103

Pöppe, J., Bote, K., Merle, R., Makarova, O., & Roesler, U. (2019). Minimum inhibitory concentration of glyphosate and a glyphosate-containing herbicide in *Salmonella enterica* isolates originating from different time periods, hosts, and serovars. *Eur J Microbiol Immunol (Bp), 9*(2), 35-41. doi:10.1556/1886.2019.00005

Powles, S. B. (2008). Evolved glyphosate-resistant weeds around the world: lessons to be learnt. *Pest Manag. Sci., 64*(4), 360-365. doi:10.1002/ps.1525

Prado, J. R., Segers, G., Voelker, T., Carson, D., Dobert, R., Phillips, J., . . . Martino-Catt, S. (2014). Genetically engineered crops: from idea to product. *Annu. Rev. Plant Biol., 65*, 769-790. doi:10.1146/annurev-arplant-050213-040039

Primost, J. E., Marino, D. J. G., Aparicio, V. C., Costa, J. L., & Carriquiriborde, P. (2017). Glyphosate and AMPA, "pseudo-persistent" pollutants under real-world agricultural management practices in the Mesopotamic Pampas agroecosystem, Argentina. *Environ. Pollut., 229*, 771-779. doi:10.1016/j.envpol.2017.06.006

Pruder, G. D., Duerr, E. O., Walsh, W. A., Lawrence, A. L., & Bray, W. A. (1992). The technical feasibility of pond liners for rearing Pacific white shrimp (*Penaeus vannamei*) in terms of survival, growth, water exchange rate and effluent water quality. *Aquac. Eng., 11*(3), 183-201. doi:https://doi.org/10.1016/0144-8609(92)90004-H

Qian, Z., Liu, X., Wang, L., Wang, X., Li, Y., Xiang, J., & Wang, P. (2012). Gene expression profiles of four heat shock proteins in response to different acute stresses in shrimp, *Litopenaeus vannamei*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 156*(3), 211-220. doi:https://doi.org/10.1016/j.cbpc.2012.06.001

Qu, H., Ma, R., Wang, B., Zhang, Y., Yin, L., Yu, G., . . . Wang, Y. (2018). Effects of microplastics on the uptake, distribution and biotransformation of chiral antidepressant venlafaxine in aquatic ecosystem. *J. Hazard. Mater., 359*, 104-112. doi:10.1016/j.jhazmat.2018.07.016

Quaghebeur, D., De Smet, B., De Wulf, E., & Steurbaut, W. (2004). Pesticides in rainwater in Flanders, Belgium: Results from the monitoring program 1997-2001. *J. Environ. Monit., 6*(3), 182-190. doi:10.1039/b312558k

Rampazzo Todorovic, G., Rampazzo, N., Mentler, A., Blum, W. E. H., Eder, A., & Strauss, P. (2014). Influence of soil tillage and erosion on the dispersion of glyphosate and aminomethylphosphonic acid in agricultural soils. *Int. Agrophys., 28*(1), 93-100. doi:10.2478/intag-2013-0031

Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research,*

1863(12), 2977-2992. doi:https://doi.org/10.1016/j.bbamcr.2016.09.012

Rendon-von Osten, J., & Dzul-Caamal, R. (2017). Glyphosate residues in groundwater, drinking water and urine of subsistence farmers from intensive agriculture localities: A survey in Hopelchén, Campeche, Mexico. *Int. J. Environ. Res. Public Health, 14*(6), 595. doi:10.3390/ijerph14060595

Reno, U., Doyle, S. R., Momo, F. R., Regaldo, L., & Gagneten, A. M. (2018). Effects of glyphosate formulations on the population dynamics of two freshwater cladoceran species. *Ecotoxicology, 27*(7), 784-793. doi:10.1007/s10646-017-1891-3

Revel, M., Yakovenko, N., Caley, T., Guillet, C., Châtel, A., & Mouneyrac, C. (2020). Accumulation and immunotoxicity of microplastics in the estuarine worm *Hediste diversicolor* in environmentally relevant conditions of exposure. *Environmental Science and Pollution Research, 27*(4), 3574-3583. doi:10.1007/s11356-018-3497-6

Riaño, C., Ortiz-Ruiz, M., Pinto-Sánchez, N. R., & Gómez-Ramírez, E. (2020). Effect of glyphosate (Roundup Active®) on liver of tadpoles of the colombian endemic frog *Dendropsophus molitor* (amphibia: Anura). *Chemosphere, 250,* 126287. doi:https://doi.org/10.1016/j.chemosphere.2020.126287

Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., & Seralini, G.-E. (2005). Differential effects of glyphosate and roundup on human placental cells and aromatase. *Environ. Health Perspect., 113*(6), 716-720. doi:10.1289/ehp.7728

Rocha-Santos, T., & Duarte, A. C. (2015). A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. *TrAC - Trends Anal. Chem., 65*, 47-53. doi:https://doi.org/10.1016/j.trac.2014.10.011

Rochman, C. M., Hoh, E., Hentschel, B. T., & Kaye, S. (2013). Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris. *Environ. Sci. Technol., 47*(3), 1646-1654. doi:10.1021/es303700s

Rochman, C. M., Hoh, E., Kurobe, T., & Teh, S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci. Rep., 3*(1), 3263. doi:10.1038/srep03263

Roell, K. R., Reif, D. M., & Motsinger-Reif, A. A. (2017). An introduction to terminology and methodology of chemical synergy—perspectives from across disciplines. *Front. Pharmacol., 8*(158). doi:10.3389/fphar.2017.00158

Rossi, A. S., Fantón, N., Michlig, M. P., Repetti, M. R., & Cazenave, J. (2020). Fish inhabiting rice fields: Bioaccumulation, oxidative stress and neurotoxic effects after pesticides application. *Ecol.*

Indic., 113, 106186. doi:https://doi.org/10.1016/j.ecolind.2020.106186

Seidensticker, S., Zarfl, C., Cirpka, O. A., Fellenberg, G., & Grathwohl, P. (2017). Shift in mass transfer of wastewater contaminants from microplastics in the presence of dissolved substances. *Environ. Sci. Technol., 51*(21), 12254-12263. doi:10.1021/acs.est.7b02664

Servos, M. R. (1999). Review of the aquatic toxicity, estrogenic responses and bioaccumulation of alkylphenols and alkylphenol polyethoxylates. *Water Qual. Res. J., 34*(1), 123-178. doi:10.2166/wqrj.1999.005

Shen, X.-C., Li, D.-C., Sima, X.-F., Cheng, H.-Y., & Jiang, H. (2018). The effects of environmental conditions on the enrichment of antibiotics on microplastics in simulated natural water column. *Environ. Res., 166*, 377-383. doi:https://doi.org/10.1016/j.envres.2018.06.034

Shi, W., Han, Y., Sun, S., Tang, Y., Zhou, W., Du, X., & Liu, G. (2020). Immunotoxicities of microplastics and sertraline, alone and in combination, to a bivalve species: size-dependent interaction and potential toxication mechanism. *Journal of hazardous materials, 396*, 122603. doi:https://doi.org/10.1016/j.jhazmat.2020.122603

Silva, V., Montanarella, L., Jones, A., Fernández-Ugalde, O., Mol, H. G. J., Ritsema, C. J., & Geissen, V. (2018). Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils

of the European Union. *Sci. Total Environ., 621*, 1352-1359. doi:https://doi.org/10.1016/j.scitotenv.2017.10.093

Smedes, F., & Booij, K. (2012). Guidelines for passive sampling of hydrophobic contaminants in water using silicone rubber samplers. In (pp. 20): ICES Techniques in Marine Environmental Sciences No. 52.

Song, H. L., Tan, B. P., Chi, S. Y., Liu, Y., Chowdhury, M. K., & Dong, X. H. (2017). The effects of a dietary protease-complex on performance, digestive and immune enzyme activity, and disease resistance of *Litopenaeus vannamei* fed high plant protein diets. *Aquaculture Research, 48*(5), 2550-2560.

Sørensen, L., Groven, A. S., Hovsbakken, I. A., Del Puerto, O., Krause, D. F., Sarno, A., & Booth, A. M. (2021). UV degradation of natural and synthetic microfibers causes fragmentation and release of polymer degradation products and chemical additives. *Sci. Total Environ., 755*, 143170. doi:https://doi.org/10.1016/j.scitotenv.2020.143170

Sreeram, M. P., & Menon, N. (2005). Histopathological changes in the hepatopancreas of the penaeid shrimp *Metapenaeus dobsoni* exposed to petroleum hydrocarbons. *Journal of the Marine Biological Association of India, 47*(2), 160-168.

Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., & Shi, H. (2016). Microplastics in Taihu Lake, China. *Environ. Pollut., 216*, 711-719. doi:https://doi.org/10.1016/j.envpol.2016.06.036

Suaria, G., Avio, C. G., Mineo, A., Lattin, G. L., Magaldi, M. G., Belmonte, G., . . . Aliani, S. (2016). The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. *Sci. Rep., 6*(1), 37551. doi:10.1038/srep37551

Sugumaran, M. (2002). Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. *Pigment Cell Research, 15*(1), 2-9. doi:https://doi.org/10.1034/j.1600-0749.2002.00056.x

Suman, T. Y., Jia, P.-P., Li, W.-G., Junaid, M., Xin, G.-Y., Wang, Y., & Pei, D.-S. (2020). Acute and chronic effects of polystyrene microplastics on brine shrimp: First evidence highlighting the molecular mechanism through transcriptome analysis. *Journal of Hazardous Materials, 400*, 123220. doi:https://doi.org/10.1016/j.jhazmat.2020.123220

Sun, M., Li, H., & Jaisi, D. P. (2019). Degradation of glyphosate and bioavailability of phosphorus derived from glyphosate in a soil-water system. *Water Research, 163*, 114840. doi:https://doi.org/10.1016/j.watres.2019.07.007

Takahashi, T., Sutherland, S. C., Chipman, D. W., Goddard, J. G., Ho, C., Newberger, T., . . . Munro, D. R. (2014). Climatological

distributions of pH, pCO_2, total CO_2, alkalinity, and $CaCO_3$ saturation in the global surface ocean, and temporal changes at selected locations. *Mar. Chem., 164*, 95-125. doi:https://doi.org/10.1016/j.marchem.2014.06.004

Tang, Y., Rong, J., Guan, X., Zha, S., Shi, W., Han, Y., . . . Liu, G. (2020). Immunotoxicity of microplastics and two persistent organic pollutants alone or in combination to a bivalve species. *Environmental Pollution, 258*, 113845. doi:https://doi.org/10.1016/j.envpol.2019.113845

Teuten, E. L., Rowland, S. J., Galloway, T. S., & Thompson, R. C. (2007). Potential for plastics to transport hydrophobic contaminants. *Environ. Sci. Technol., 41*(22), 7759-7764. doi:10.1021/es071737s

Then, C. (2013). *High Levels of Residues from Spraying with Glyphosate Found in Soybeans in Argentina*. Retrieved from München, Germany: http://www.testbiotech.de/en/node/926

Thiagarajan, V., Iswarya, V., P, A. J., Seenivasan, R., Chandrasekaran, N., & Mukherjee, A. (2019). Influence of differently functionalized polystyrene microplastics on the toxic effects of P25 TiO_2 NPs towards marine algae *Chlorella* sp. *Aquat. Toxicol., 207*, 208-216. doi:https://doi.org/10.1016/j.aquatox.2018.12.014

Thompson, R., Swan, S., Moore, C., & vom Saal, F. (2009). Our plastic age. *Philos. Trans. R. Soc. Lond., B, Biol. Sci., 364*, 1973-1976. doi:10.1098/rstb.2009.0054

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., . . . Russell, A. E. (2004). Lost at sea: Where is all the plastic? *Science, 304*(5672), 838. doi:10.1126/science.1094559

Thongprakaisang, S., Thiantanawat, A., Rangkadilok, N., Suriyo, T., & Satayavivad, J. (2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food Chem. Toxicol., 59*, 129-136. doi:https://doi.org/10.1016/j.fct.2013.05.057

Tiseo, I. (2021). Production of plastics worldwide from 1950 to 2019 (in million metric tons). Retrieved from https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/

Topal, A., Atamanalp, M., Uçar, A., Oruç, E., Kocaman, E. M., Sulukan, E., . . . Ceyhun, S. B. (2015). Effects of glyphosate on juvenile rainbow trout (*Oncorhynchus mykiss*): Transcriptional and enzymatic analyses of antioxidant defence system, histopathological liver damage and swimming performance. *Ecotoxicol. Environ. Saf., 111*, 206-214. doi:https://doi.org/10.1016/j.ecoenv.2014.09.027

Tourinho, P. S., Kočí, V., Loureiro, S., & van Gestel, C. A. M. (2019). Partitioning of chemical contaminants to microplastics: Sorption mechanisms, environmental distribution and effects on toxicity and bioaccumulation. *Environ. Pollut., 252*, 1246-1256. doi:https://doi.org/10.1016/j.envpol.2019.06.030

Tu, H. T., Silvestre, F., Bernard, A., Douny, C., Phuong, N. T., Tao, C. T., . . . Kestemont, P. (2008). Oxidative stress response of black tiger shrimp (*Penaeus monodon*) to enrofloxacin and to culture system. *Aquaculture, 285*(1), 244-248. doi:https://doi.org/10.1016/j.aquaculture.2008.08.032

Tu, H. T., Silvestre, F., Scippo, M.-L., Thome, J.-P., Phuong, N. T., & Kestemont, P. (2009). Acetylcholinesterase activity as a biomarker of exposure to antibiotics and pesticides in the black tiger shrimp (*Penaeus monodon*). *Ecotoxicology and Environmental Safety, 72*(5), 1463-1470. doi:https://doi.org/10.1016/j.ecoenv.2009.04.008

Turner, A., & Holmes, L. (2015). Adsorption of trace metals by microplastic pellets in fresh water. *Environ. Chem., 12*. doi:10.1071/EN14143

Tzaskos, D., Marcovicz, C., Dias, N., & Rosso, N. (2012). Development of sampling for quantification of glyphosate in natural waters. *Cienc. e Agrotecnologia, 36*, 399-405. doi:10.1590/S1413-70542012000400003

UN GHS. (2015). *Globally harmonized system of classification and labelling of chemicals (GHS)* (Sixth revised edition ed.). New York and Geneva: United Nations.

Uren Webster, T. M., Laing, L. V., Florance, H., & Santos, E. M. (2014). Effects of glyphosate and its formulation, Roundup, on

reproduction in zebrafish (*Danio rerio*). *Environ. Sci. Technol., 48*(2), 1271-1279. doi:10.1021/es404258h

US Environmental Protection Agency. (2016). *Ecological effects test guidelines OCSPP 850.1045: Penaeid acute toxicity test*. Washington, DC.

Utley, H. G., Bernheim, F., & Hochstein, P. (1967). Effect of sulfhydryl reagents on peroxidation in microsomes. *Archives of biochemistry and biophysics, 118*(1), 29-32.

Valentim-Neto, P. A., Moser, J. R., Fraga, A. P., & Marques, M. R. (2014). Hsp70 expression in shrimp *Litopenaeus vannamei* in response to IHHNV and WSSV infection. *Virusdisease, 25*(4), 437-440. doi:10.1007/s13337-014-0236-6

Van Bruggen, A., Finckh, M., He, M., Ritsema, C., Harkes, P., Knuth, D., & Geissen, V. (2021). Indirect effects of the herbicide glyphosate on plant, animal and human health through its effects on microbial communities. *Frontiers in Environmental Science*, 464.

Van Cauwenberghe, L., Vanreusel, A., Mees, J., & Janssen, C. R. (2013). Microplastic pollution in deep-sea sediments. *Environ. Pollut., 182*, 495-499. doi:https://doi.org/10.1016/j.envpol.2013.08.013

van Emmerik, T., & Schwarz, A. (2020). Plastic debris in rivers. *WIREs Water, 7*(1), e1398. doi:https://doi.org/10.1002/wat2.1398

Van Stempvoort, D. R., Spoelstra, J., Senger, N. D., Brown, S. J., Post, R., & Struger, J. (2016). Glyphosate residues in rural groundwater, Nottawasaga River Watershed, Ontario, Canada. *Pest Manag. Sci.*, *72*(10), 1862-1872. doi:10.1002/ps.4218

Van Thuong, K., Van Tuan, V., Li, W., Sorgeloos, P., Bossier, P., & Nauwynck, H. (2016). Effects of acute change in salinity and moulting on the infection of white leg shrimp (*Penaeus vannamei*) with white spot syndrome virus upon immersion challenge. *J. Fish Dis.*, *39*(12), 1403-1412. doi:https://doi.org/10.1111/jfd.12471

Velzeboer, I., Kwadijk, C. J. A. F., & Koelmans, A. A. (2014). Strong sorption of PCBs to nanoplastics, microplastics, carbon nanotubes, and fullerenes. *Environ. Sci. Technol.*, *48*(9), 4869-4876. doi:10.1021/es405721v

Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., & Da Ros, L. (2013). Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. *Estuar. Coast. Shelf Sci.*, *130*, 54-61. doi:https://doi.org/10.1016/j.ecss.2013.03.022

Vogt, G. (2019). Functional cytology of the hepatopancreas of decapod crustaceans. *Journal of Morphology*, *280*(9), 1405-1444. doi:https://doi.org/10.1002/jmor.21040

Vogt, G., & Quinitio, E. T. (1994). Accumulation and excretion of metal granules in the prawn, *Penaeus monodon*, exposed to water-borne

copper, lead, iron and calcium. *Aquatic Toxicology, 28*(3), 223-241. doi:https://doi.org/10.1016/0166-445X(94)90035-3

Wang, F., Li, S., Xiang, J., & Li, F. (2019). Transcriptome analysis reveals the activation of neuroendocrine-immune system in shrimp hemocytes at the early stage of WSSV infection. *BMC Genomics, 20*(1), 247. doi:10.1186/s12864-019-5614-4

Wang, F., Shih, K. M., & Li, X. Y. (2015). The partition behavior of perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (FOSA) on microplastics. *Chemosphere, 119*, 841-847. doi:https://doi.org/10.1016/j.chemosphere.2014.08.047

Wang, J., Liu, X., Liu, G., Zhang, Z., Wu, H., Cui, B., . . . Zhang, W. (2019). Size effect of polystyrene microplastics on sorption of phenanthrene and nitrobenzene. *Ecotoxicol. Environ. Saf., 173*, 331-338. doi:https://doi.org/10.1016/j.ecoenv.2019.02.037

Wang, W., Ge, J., & Yu, X. (2020). Bioavailability and toxicity of microplastics to fish species: A review. *Ecotoxicol. Environ. Saf., 189*, 109913. doi:https://doi.org/10.1016/j.ecoenv.2019.109913

Wang, X., Chang, L., Zhao, T., Liu, L., Zhang, M., Li, C., . . . Zhu, W. (2019). Metabolic switch in energy metabolism mediates the sublethal effects induced by glyphosate-based herbicide on tadpoles of a farmland frog *Microhyla fissipes*. *Ecotoxicol. Environ. Saf., 186*, 109794. doi:https://doi.org/10.1016/j.ecoenv.2019.109794

Wang, X., Zhang, X., Sun, K., Wang, S., & Gong, D. (2022). Polystyrene microplastics induce apoptosis and necroptosis in swine testis cells via ROS/MAPK/HIF1α pathway. *Environmental Toxicology, 37*(10), 2483-2492. doi:https://doi.org/10.1002/tox.23611

Wang, Y., Branicky, R., Noë, A., & Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *Journal of Cell Biology, 217*(6), 1915-1928. doi:10.1083/jcb.201708007

Wang, Y., Ezemaduka, A. N., Li, Z., Chen, Z., & Song, C. (2017). Joint toxicity of arsenic, copper and glyphosate on behavior, reproduction and heat shock protein response in *Caenorhabditis elegans*. *Bulletin of Environmental Contamination and Toxicology, 98*(4), 465-471. doi:10.1007/s00128-017-2042-5

Wang, Z., Fan, L., Wang, J., Xie, S., Zhang, C., Zhou, J., . . . Zou, J. (2021). Insight into the immune and microbial response of the white-leg shrimp *Litopenaeus vannamei* to microplastics. *Marine Environmental Research, 169*, 105377. doi:https://doi.org/10.1016/j.marenvres.2021.105377

Wang, Z., Fan, L., Wang, J., Zhou, J., Ye, Q., Zhang, L., . . . Zou, J. (2021). Impacts of microplastics on three different juvenile shrimps: Investigating the organism response distinction. *Environmental Research, 198*, 110466. doi:https://doi.org/10.1016/j.envres.2020.110466

Wardrop, P., Shimeta, J., Nugegoda, D., Morrison, P. D., Miranda, A., Tang, M., & Clarke, B. O. (2016). Chemical pollutants sorbed to ingested microbeads from personal care products accumulate in fish. *Environ. Sci. Technol., 50*(7), 4037-4044. doi:10.1021/acs.est.5b06280

Welden, N. A. C., & Cowie, P. R. (2016). Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. *Environ. Pollut., 218*, 895-900. doi:https://doi.org/10.1016/j.envpol.2016.08.020

Wilson, B. W., & Henderson, J. D. (2007). Determination of cholinesterase in blood and tissue. *Current protocols in toxicology, 34*(1), 12.13. 11-12.13. 16.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environ. Pollut., 178*, 483-492. doi:https://doi.org/10.1016/j.envpol.2013.02.031

Wu, B., Taylor, C. M., Knappe, D. R. U., Nanny, M. A., & Barlaz, M. A. (2001). Factors controlling alkylbenzene sorption to municipal solid waste. *Environ. Sci. Technol., 35*(22), 4569-4576. doi:10.1021/es010893a

Xia, B., Zhang, J., Zhao, X., Feng, J., Teng, Y., Chen, B., . . . Qu, K. (2020). Polystyrene microplastics increase uptake, elimination and cytotoxicity of decabromodiphenyl ether (BDE-209) in the marine

scallop *Chlamys farreri*. *Environmental Pollution, 258*, 113657. doi:https://doi.org/10.1016/j.envpol.2019.113657

Xing, Y., Zhu, X., Duan, Y., Huang, J., Nan, Y., & Zhang, J. (2023). Toxic effects of nitrite and microplastics stress on histology, oxidative stress, and metabolic function in the gills of Pacific white shrimp, *Litopenaeus vannamei*. *Marine pollution bulletin, 187*, 114531. doi:https://doi.org/10.1016/j.marpolbul.2022.114531

Xu, B., Liu, F., Brookes, P., & xu, J. (2018). Microplastics play a minor role in tetracycline sorption in the presence of dissolved organic matter. *Environ. Pollut., 240*. doi:10.1016/j.envpol.2018.04.113

Yan, M., Wang, W., Huang, X., Wang, X., & Wang, Y. (2020). Interactive effects of dietary cholesterol and phospholipids on the growth performance, expression of immune-related genes and resistance against Vibrio alginolyticus in white shrimp (Litopenaeus vannamei). *Fish & Shellfish Immunology, 97*, 100-107. doi:https://doi.org/10.1016/j.fsi.2019.11.048

Yan, P., Lin, C., He, M., Zhang, Z., Zhao, Q., & Li, E. (2022). Immune regulation mediated by JAK/STAT signaling pathway in hemocytes of Pacific white shrimps, *Litopenaeus vannamei* stimulated by lipopolysaccharide. *Fish & Shellfish Immunology, 130*, 141-154. doi:https://doi.org/10.1016/j.fsi.2022.07.048

Yang, J., Zhou, W.-W., Shi, D.-D., Pan, F.-F., Sun, W.-W., Yang, P.-L., & Li, X.-M. (2023). The interaction between oxidative stress

biomarkers and gut microbiota in the antioxidant effects of extracts from *Sonchus brachyotus* DC. in oxazolone-induced intestinal oxidative stress in adult zebrafish. *Antioxidants, 12*(1), 192. Retrieved from https://www.mdpi.com/2076-3921/12/1/192

Yang, L., Luo, M., He, J., Zuo, H., Weng, S., He, J., & Xu, X. (2019). A JAK-STAT pathway target gene encoding a single WAP domain (SWD)-containing protein from *Litopenaeus vannamei*. *Fish & Shellfish Immunology, 89*, 555-563. doi:https://doi.org/10.1016/j.fsi.2019.04.046

Yang, X., Song, Y., Zhang, C., Pang, Y., Song, X., Wu, M., & Cheng, Y. (2019). Effects of the glyphosate-based herbicide roundup on the survival, immune response, digestive activities and gut microbiota of the Chinese mitten crab, *Eriocheir sinensis*. *Aquatic Toxicology, 214*, 105243. doi:https://doi.org/10.1016/j.aquatox.2019.105243

Yang, X., Wang, F., Bento, C. P. M., Xue, S., Gai, L., van Dam, R., . . . Geissen, V. (2015). Short-term transport of glyphosate with erosion in Chinese loess soil — A flume experiment. *Sci. Total Environ., 512-513*, 406-414. doi:https://doi.org/10.1016/j.scitotenv.2015.01.071

Yin, C., Shen, X., Wang, Y., Hu, J., Bao, Z., & Wang, M. (2023). Comparative study of five anti-lipopolysaccharide factor genes in *Litopenaeus vannamei*. *Developmental & Comparative Immunology, 139*, 104557. doi:https://doi.org/10.1016/j.dci.2022.104557

Yu, B. P. (1994). Cellular defenses against damage from reactive oxygen species. *Physiological Reviews, 74*(1), 139-162. doi:10.1152/physrev.1994.74.1.139

Yu, D., Zhai, Y., He, P., & Jia, R. (2022). Comprehensive transcriptomic and metabolomic analysis of the *Litopenaeus vannamei* hepatopancreas after WSSV challenge. *Front Immunol, 13*, 826794. doi:10.3389/fimmu.2022.826794

Yu, F., Yang, C., Zhu, Z., Bai, X., & Ma, J. (2019). Adsorption behavior of organic pollutants and metals on micro/nanoplastics in the aquatic environment. *Sci. Total Environ., 694*, 133643. doi:https://doi.org/10.1016/j.scitotenv.2019.133643

Yu, Q., Xie, J., Huang, M., Chen, C., Qian, D., Qin, J. G., . . . Li, E. (2020). Growth and health responses to a long-term pH stress in Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Reports, 16*, 100280. doi:https://doi.org/10.1016/j.aqrep.2020.100280

Zhan, Z., Wang, J., Peng, J., Xie, Q., Huang, Y., & Gao, Y. (2016). Sorption of 3,3′,4,4′-tetrachlorobiphenyl by microplastics: A case study of polypropylene. *Mar. Pollut. Bull., 110*(1), 559-563. doi:https://doi.org/10.1016/j.marpolbul.2016.05.036

Zhang, C., Pan, Z., Wang, S., Xu, G., & Zou, J. (2022). Size and concentration effects of microplastics on digestion and immunity of hybrid snakehead in developmental stages. *Aquaculture*

Reports, 22, 100974. doi:https://doi.org/10.1016/j.aqrep.2021.100974

Zhang, H., Fei, Y., Wang, H., Chen, Y., Huang, S., Yu, B., . . . Luo, Y. (2020). Interaction of microplastics and organic pollutants: Quantification, environmental fates, and ecological consequences. In D. He & Y. Luo (Eds.), *Microplastics in Terrestrial Environments: Emerging Contaminants and Major Challenges* (pp. 161-184). Cham: Springer International Publishing.

Zhang, H., Wang, J., Zhou, B., Zhou, Y., Dai, Z., Zhou, Q., . . . Luo, Y. (2018). Enhanced adsorption of oxytetracycline to weathered microplastic polystyrene: Kinetics, isotherms and influencing factors. *Environ. Pollut., 243*, 1550-1557. doi:https://doi.org/10.1016/j.envpol.2018.09.122

Zhang, K., Hamidian, A. H., Tubić, A., Zhang, Y., Fang, J. K. H., Wu, C., & Lam, P. K. S. (2021). Understanding plastic degradation and microplastic formation in the environment: A review. *Environ. Pollut., 274*, 116554. doi:https://doi.org/10.1016/j.envpol.2021.116554

Zhang, L., Pan, L., Xu, L., & Si, L. (2018). Effects of ammonia-N exposure on the concentrations of neurotransmitters, hemocyte intracellular signaling pathways and immune responses in white shrimp *Litopenaeus vannamei*. *Fish & Shellfish Immunology, 75*, 48-57. doi:https://doi.org/10.1016/j.fsi.2018.01.046

Zhang, Q., Qu, Q., Lu, T., Ke, M., Zhu, Y., Zhang, M., . . . Qian, H. (2018). The combined toxicity effect of nanoplastics and glyphosate on *Microcystis aeruginosa* growth. *Environ. Pollut., 243*, 1106-1112. doi:https://doi.org/10.1016/j.envpol.2018.09.073

Zhou, N., Wang, Z., Yang, L., Zhou, W., Qin, Z., & Zhang, H. (2023). Size-dependent toxicological effects of polystyrene microplastics in the shrimp *Litopenaeus vannamei* using a histomorphology, microbiome, and metabolic approach. *Environmental Pollution, 316*, 120635. doi:https://doi.org/10.1016/j.envpol.2022.120635

Zhu, Z.-l., Wang, S.-c., Zhao, F.-f., Wang, S.-g., Liu, F.-f., & Liu, G.-z. (2019). Joint toxicity of microplastics with triclosan to marine microalgae *Skeletonema costatum*. *Environ. Pollut., 246*, 509-517. doi:https://doi.org/10.1016/j.envpol.2018.12.044

Ziajahromi, S., Kumar, A., Neale, P., & Leusch, F. (2019). Effects of polyethylene microplastics on the acute toxicity of a synthetic pyrethroid to midge larvae (*Chironomus tepperi*) in synthetic and river water. *Sci. Total Environ., 671*. doi:10.1016/j.scitotenv.2019.03.425

Zocchi, M., & Sommaruga, R. (2019). Microplastics modify the toxicity of glyphosate on *Daphnia magna*. *Sci. Total Environ., 697*, 134194. doi:https://doi.org/10.1016/j.scitotenv.2019.134194

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