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## Review

# 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

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## ABSTRACT

Microplastic pollution in marine waters around the globe is increasing exponentially. This is the first comprehensive review which focuses on microplastics as a source and vector for metals, antibiotics, toxic chemicals, pathogenic bacteria (*Vibrio cholerae*), and Harmful Algal Bloom (HAB)-forming dinoflagellates across the continents through ballast water. Microplastics in ballast waters serve as 'hotspots' for the development and spread of multiple drug-resistant human pathogens through co-selection mechanisms. Microplastic inoculation at distant countries through ballast water may pose a serious threat to human health due to higher incidences of bacterial disease outbreaks and HABs. The 2017 ballast water management convention lacks a provision for on-board treatment of microplastic-contaminated ballast water. We conclude that there is a pressing need to include microplastics in the ballast water management convention as a hazardous material. Efficient on-board ballast water treatment strategies and effective limits for microplastics in ballast waters need to be developed.

## 1. Introduction

Plastic is one of the most indiscriminately used polymers in the present world. Right from its creation in the 1870s, it has become an integral part of human life, mainly due to its advantageous properties relating to elasticity, lightness, versatility and durability. This has led to a massive increase in annual plastic production over the decades, from 0.5 million tons in the 1940s to 550 million tons in 2018 (Plastics Europe, 2017–2018). The widespread use of plastic, in addition to its high durability, is a disadvantage in terms of its persistence in the environment. This is further compounded by the low extent of recycling of plastics. In 2013, only 14% (by mass) of plastic packaging materials was recycled whereas a whopping 72% was either dumped in landfills or released into the marine environment (World Economic Forum, 2016).

Ultimately, majority of the plastics get transported from the primary site of synthesis (land) to secondary sites like freshwater and marine ecosystems (Yokota et al., 2017), where they affect marine organisms via entanglement, ingestion, etc. Entanglement in plastic has been reported for a wide variety of organisms including mammals and cetaceans (Laist, 1997; Eriksson and Burton, 2003), and can involve either

drifting plastic debris or fragments of discarded fishing nets, the latter termed 'ghost fishing' (Cole et al., 2011). Once entangled, organisms suffer from reduced mobility and feeding, and can consequently lead to drowning, suffocation and strangulation (Fischer et al., 2015; Li et al., 2016). Ingestion of plastic debris, wrongly identified as food, has been extensively reported in diverse marine organisms ranging from birds (Mallory, 2008) to turtles (Mascarenhas et al., 2004). Rios and Moore (2007) documented the ingestion of plastics in approximately 44% of marine bird species. Plastics, when ingested, can not only result in irritation and injuries to the digestive tracts of organisms, but can also result in a false sensation of satiation, impacting the fitness and reproduction of marine organisms (GESAMP, 2016).

Following fragmentation and natural physical and chemical weathering processes involving photo-oxidation and thermal degradation, most marine plastic debris consequently gets converted to microplastics (Wright et al., 2013), which, due to their small size, can impact a wide range of marine biota via shading effects, especially in microalgae (Schwab et al., 2011); ingestion, either directly or by filter-feeding (Cole et al., 2011; Thompson et al., 2009); enhancing interactions with microplastic-associated bacteria, viruses, and plankton

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(Oberbeckmann et al., 2015b; Shen et al., 2019); leaching of contaminants during degradation of microplastics (Lithner et al., 2009; Teuten et al., 2007); and release of contaminants (for e.g., toxic metals, antibiotics, endocrine disrupting chemicals, persistent organic pollutants 'POPs') adsorbed on to the surface of microplastics (Rios et al., 2007).

### 1.1. The severity and extent of plastic pollution

Marine and terrestrial environments worldwide are extensively polluted with plastics. It was estimated that global plastic pollution in the year 2016 surpassed 300 million metric tons/year (Law, 2017). On an average, every year, eight million metric tons of plastics are dumped into oceans (Imran et al., 2019). Approximately five trillion tons of plastic debris is estimated to be floating in the oceans around the globe (Barboza et al., 2018). The future projections are equally alarming. The United Nations Environment Programme (UNEP) recently estimated that there will be more plastics in ocean (by weight) than fish by the end of the year 2050 (Rocha-Santos, 2018).

The varied sources of plastic pollution in marine waters includes soft drink bottles, medical waste, wrappers, industrial waste, fishing nets, tourist activities, toys, polyethene bags, through rivers, electronic waste dumping, mineral water bottles, etc. Plastics are chemically diverse, with polyethylene terephthalate (PET), polyethylene (PE), polyurethane (PUR), polystyrene (PS) and polypropylene (PP), all contributing to marine pollution (Revel et al., 2018). Due to the uncontrolled use and dumping of plastics, a plastic garbage patch, bigger than the state of Texas, has been observed in the Pacific Ocean (Virsek et al., 2017; Mendoza et al., 2018). Studies on this 'Great Plastic Garbage Patch' by multi-vessel and aircraft surveys have revealed that at least 79 thousand tons of total oceanic plastic are floating in an area of 1.6 million km<sup>2</sup>. Moreover, this patch is rapidly accumulating plastics and expanding at an alarming rate (Lebreton et al., 2018). The severity of plastic pollution is highlighted by reports of plastic in previously pristine marine waters like Antarctic, Arctic as well as the deepest point on earth (Mariana Trench) (Mendoza et al., 2018).

### 1.2. Microplastics in marine waters around the globe

Plastics are very stable and resistant to microbial degradation. They persist in nature for years, but gradually undergo weathering processes, photo-oxidation and microbial breakdown resulting in the formation of smaller sized plastics called 'microplastics' (Rummel et al., 2017; Alimi et al., 2018). Microplastics are plastic pieces which range in size from 100 nm to 0.5 cm; below 100 nm, they are called nanoplastics (Rocha-Santos, 2018). Microplastics are divided into two types called primary and secondary microplastics. Primary microplastics are directly manufactured as < 5 mm in size and include microfibers used in textiles (Cesa et al., 2017), microbeads used in facial cleansers, toothpaste and cosmetics (Zitko and Hanlon, 1991), industrial scrubbers used for abrasive blast cleaning (Browne et al., 2007), and capsules for drug delivery (Patel et al., 2009). Secondary microplastics are formed as a result of the slow breakdown of larger plastic items over time (Smith et al., 2018).

Microplastics are widespread in the marine environment, and dispersed to distant locations through ocean currents. It was estimated, in 2014, that 15–51 trillion microplastic particles, weighing approximately 93–236 thousand metric tons, were present in the global oceans (van Sebille et al., 2015). The Great Pacific Garbage Patch is dominated by microplastics, which constitute approximately 94% of the estimated 1.8 (1.1–3.6) trillion floating pieces (Lebreton et al., 2018).

Microplastic pollution has also been reported in the beaches and water column around India (Jayasiri et al., 2013; Veerasingham et al., 2016a, 2016b), and also based on microplastic-contaminated sea salt, with approximately  $103 \pm 39$  to  $56 \pm 49$  particles of microplastics kg<sup>-1</sup> of salt (Seth and Shrivastav, 2018). In the Arctic, a unique and

fragile ecosystem, microplastic pollution has been observed in sub-surface waters and in two mid-trophic level Arctic fish (*Triglops nybelini* and *Boreogadus saida*) collected off Northeast Greenland (Morgana et al., 2018). Analysis of nine sediment samples from HAUSGARTEN observatory situated in the Arctic at 2340–5570 m depth, indicated widespread occurrence of microplastics (42–6595 microplastics kg<sup>-1</sup>) in the sediment; approximately 80% of the microplastics were of size  $\leq 25 \mu\text{m}$  (Bergmann et al., 2017). Pelagic microplastics in the Southern Ocean have also been quantified; total particle counts estimated at two stations near Antarctica were of the magnitude of 100,000 pieces km<sup>-2</sup> (Isobe et al., 2017). This proves that even formerly pristine waters of the Southern Ocean are not spared from microplastic pollution. Therefore, it is now well known that all marine environments from beaches to the deep seafloor/sediments and all oceans, seas and coastal waters/sediments around the globe have been polluted with microplastics (van Sebille et al., 2015; Bergmann et al., 2017; Boucher and Friot, 2017; Isobe et al., 2017; Morgana et al., 2018; Rocha-Santos, 2018; Seth and Shrivastav, 2018; Imran et al., 2019). Therefore, microplastic pollution has become a great environmental concern.

### 1.3. Scope of the review

To the best of our knowledge, this is the first comprehensive review detailing the fate of microplastics and their associated 'plastisphere' communities in ballast water and their consequences for human health and the environment. This review highlights the role of microplastics in ballast water, as a vector for the transport of harmful chemicals, bacterial pathogens and Harmful Algal Bloom (HAB)-forming dinoflagellates across continents and their possible effect on human health. The co-selection of metal-driven, multiple antibiotic resistance in bacterial pathogens associated with microplastics is also debated. We also discuss the existing ballast water management strategies with regard to microplastics in ballast waters.

## 2. The role of microplastics in the marine environment

### 2.1. Microplastics as an emerging source and vector for harmful chemicals, antibiotics and heavy metals

During natural weathering processes, microplastics undergo changes in size, colour, morphology, crystallinity, density, etc. (Guo and Wang, 2019). Consequently, they release harmful chemicals viz. phthalates, polybrominated diphenyl ethers, Bisphenol A, Pb, Sb and Zn, into the marine environment. Microplastics, having a lipophilic nature, also have the ability to adsorb pollutants viz. pharmaceuticals, Poly Chlorinated Biphenyls (PCBs), Persistent Organic Pollutants (POPs), Polycyclic Aromatic Hydrocarbons (PAHs), and heavy metals (Ni, Ti, Pb, Zn, Cd, Cu) on their surfaces (Andrady, 2011; O'Donovan et al., 2018). They act as both a source and vector for deleterious pollutants in marine and fresh waters (Brennecke et al., 2016; Koelmans et al., 2016; Wang et al., 2016; Guo et al., 2018; Hahladakis et al., 2018; Li et al., 2018; Revel et al., 2018). Considering the tremendous increase in the use of microplastics in medicine as vectors for drug delivery (Cole et al., 2011) and their application in personal care products, it is evident that microplastics (in this case, primary microplastics) can directly reach marine waters as part of medical waste and human feces. Chemicals which are known to cause cancers in humans (perfluorooctane sulfonic acid and benzo[a]pyrene), have been reported adsorbed on the surface of such low-density polyethylene microplastics in marine waters (O'Donovan et al., 2018). These microplastic particles, in addition to the secondary microplastics that enter the marine environment through photo-oxidative, thermal and microbial degradation, act as a vector in the marine environment for heavy metals (copper and zinc) and toxic chemicals including POPs (Brennecke et al., 2016; Pittura et al., 2018).

In view of the use of microplastics as vectors for drug delivery in the medical field (Patel et al., 2009; Cole et al., 2011), the adsorption of

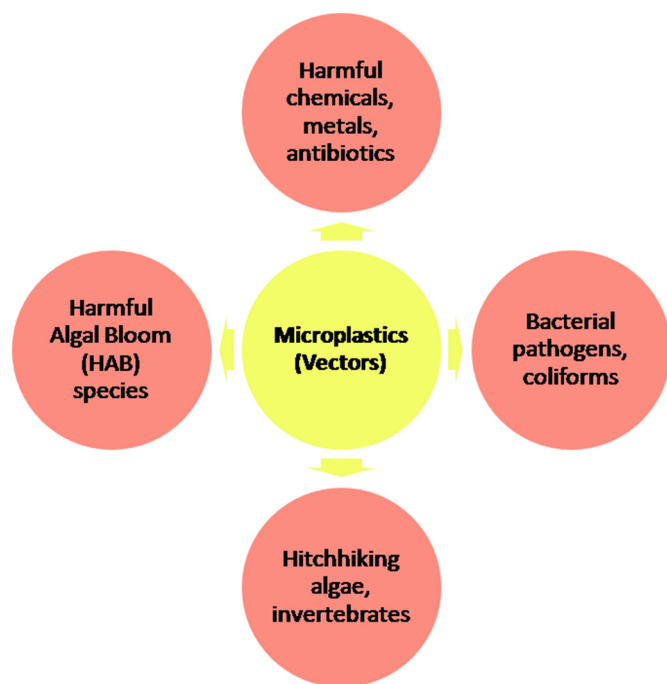


Fig. 1. The role of microplastics as vectors for a range of chemicals and biota.

antibiotics onto microplastics in the marine environment is an intriguing aspect. Li et al. (2018) have reported the adsorption of antibiotics - sulfadiazine, tetracycline ciprofloxacin, trimethoprim and amoxicillin on microplastics, resulting in dispersion of these antibiotics over long ranges and consequently, entry into the food chain. All these scientific investigations and reports provide irrefutable evidence that microplastics in the marine environment are an emerging source and vector for harmful chemicals, antibiotics and heavy metals (Fig. 1).

## 2.2. Ecological effects: effect of microplastics on marine biota

In 2015, it was estimated that 693 species of marine organisms had been affected by marine plastic pollution whereas 267 species had marine plastic debris in their bodies (Gall and Thompson, 2015). The effects were mainly through entanglement, causing reduced mobility and feeding (Eriksson and Burton, 2003; Fischer et al., 2015; Li et al., 2016) and ingestion (Mallory, 2008; Mascarenhas et al., 2004; Rios and Moore, 2007). The 2016 UN report cited > 800 animal species to be contaminated with plastic through ingestion or entanglement (UNEP 2016). In comparison to these consequences associated with visible plastic debris, microplastics have far more debilitating effects on marine organisms, including impairment, reduced fitness and mortality (Fig. 2). They often get bioaccumulated through the food web, ultimately entering the food chain (Seltenrich, 2015). Yokota et al. (2017) and Prata et al. (2019) have recently compiled the available reports on the effect of microplastics on microalgae in freshwater and marine environments. Some authors have reported attachment of microalgae to microplastics, associated with growth inhibition of microalgae (Casado et al., 2013; Besseling et al., 2014; Bergami et al., 2017; Lyakurwa, 2017), often attributed to shading and agglomeration (Schwab et al., 2011). Morphological changes include unclear pyrenoid, plasma detached from the cell wall, deformed thylakoids and cell wall thickening (Mao et al., 2018). Other effects include internalisation of microplastics during cell division (Chae et al., 2018) or by mixotrophic organisms (Long et al., 2017), reduction in photosynthesis, growth rate and expression of some chloroplast genes (*rbcl*) (Bhattacharya et al., 2010; Besseling et al., 2014; Lagarde et al., 2016; Sjollem et al., 2016). Microplastics interfere with photosynthesis by affecting the electron donor

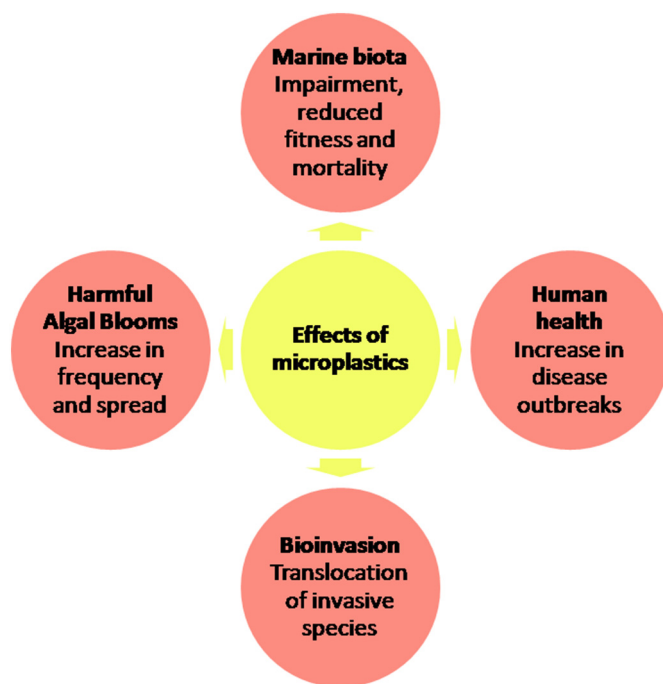


Fig. 2. The effects of microplastics on various aspects of human health and the environment.

site, the reaction center of photosystem II (responsible for energy conversion), and the electron transport chain, resulting in production of Reactive Oxygen Species (ROS), responsible for oxidative stress (Bhattacharya et al., 2010; Mao et al., 2018). The adsorption of microplastics onto the cell surface possibly contributes to the decrease in chlorophyll content, due to the shading effect and the reduced area available for exchange of substances across the cell (Bhattacharya et al., 2010).

Microplastics adversely affect the feeding behaviour, movement and reproductive success of zooplankton, mussels, oysters, crabs and fish (Carbery et al., 2018). Since microplastics are very small in size (100 nm – 5 mm), they float in marine waters and can easily enter marine organisms during feeding or through gills (Watt et al., 2016; Carbery et al., 2018; Smith et al., 2018). Zooplankton are highly susceptible to microplastics (Foley et al., 2018); the effects may span generations (Martins and Guilhermino, 2018). They may accidentally ingest microplastics, mistaking them for food (Rochman et al., 2013). A meta-analysis of fish and zooplankton populations by Foley et al. (2018) highlighted a negative impact of microplastics on their growth and reproduction. Prolonged exposure of copepods to high concentrations of microplastics leads to a reduction in energy levels, feeding, egg production and finally, death (Lee et al., 2013; Kaposi et al., 2014; Cole et al., 2016). Microplastics travel through the food web and ultimately reach seals and other higher organisms (Nelms et al., 2018; Revel et al., 2018).

Microplastics can affect organisms directly or through harmful chemicals released from their surface. Therefore, there is trophic transfer of not only microplastics but chemical contaminants released from microplastics or adsorbed on surface of microplastics in marine food webs (Carbery et al., 2018). In fact, chemicals and heavy metal load on microplastic surfaces may be enriched up to  $10^6$ -fold compared to those in the surrounding seawater (Mato et al., 2001). Due to this, marine organisms in close proximity to microplastics are subjected to very high concentrations of diverse pollutants. Microplastics have been reported to increase the toxicity of the antibiotics – doxycycline and procainamide, to the marine microalga, *Tetraselmis chuii* (Prata et al., 2018). Behavioural responses and reduction in swimming velocity of juveniles of *Dicentrarchus labrax* (European seabass) was noticed as a

consequence of mercury and microplastic co-contamination in marine waters (Barboza et al., 2018). O'Donovan et al. (2018) recently reported oxidative stress in the bivalve – *Scrobicularia plana* in response to perfluorooctane sulfonic acid and benzo[a]pyrene, adsorbed on the surface of polyethylene microplastics.

### 2.2.1. Role of microplastics in translocating fouling communities

In a Scanning Electron Microscopy (SEM)-based study of the epiplastic community of millimeter-sized plastics, floating in marine surface waters around Australia, Reisser et al. (2014) visualized numerous and diverse fouling organisms, including diatoms from 14 genera, coccolithophores, a dinoflagellate (*Ceratium* sp.), bryozoans, barnacles, isopods, marine worm, marine insect eggs, and cells putatively identified as cyanobacteria, bacteria and fungi. This provides evidence that microplastics provide a unique substrate for attachment and growth of fouling organisms, which can then translocate to distant sites via surface currents (Fig. 1). This has implications for the emergence of novel species of fouling organisms having superior attachment/degradative abilities with respect to plastic. For e.g., a new species of fouling Stylonematophycean red algae, *Tsunamiya transpacific* gen. nov. et sp. nov., was reported from floating plastic debris in the North Pacific (West et al., 2016). These 'hitch-hikers' are dispersed widely via prevailing ocean currents. Given the increase in microplastic pollution in aquatic environments, it can be postulated that new 'plastisphere' niches are opening up for fouling algae such as Stylonematophycean red algae. Floating microplastics also offer a potential pathway for the invasion of alien species (Derraik, 2002).

### 2.2.2. Role of microplastics in dissemination of HAB species

Several potential HAB-causing dinoflagellates (for e.g., *Coolia* and *Ostreopsis* spp.), vegetative cells and temporary cysts of *Alexandrium taylori*, and resting cysts of unidentified dinoflagellates, have been recovered from plastic debris floating along the Catalan coast (Masó et al., 2003). SEM studies and DNA sequencing have detected several groups of eukaryotic algae - diatoms, prasinophytes, rhodophytes, cryptophytes, haptophytes, dinoflagellates (including potentially harmful *Alexandrium*), chlorarachniophytes, chrysophytes, pelagophytes, and phaeophytes in plastisphere communities (Zettler et al., 2013; Reisser et al., 2014; Zhang et al., 2017). Based on this, it can be said that microplastics play a very important role in disseminating HAB species (either vegetative cells or cysts), as part of their associated microfouling communities, to new areas, where they may then form blooms (Figs. 1, 2). When transferred to humans through consumption of filter-feeding bivalves, HAB toxins can cause several poisoning syndromes, such as Amnesic Shellfish Poisoning, Diarrhetic Shellfish Poisoning, Neurotoxic Shellfish Poisoning, Paralytic Shellfish Poisoning, Ciguatera Fish Poisoning, Putative Estuary Associated Syndrome (van Dolah et al., 2001; James et al., 2010).

Cyanobacteria, responsible for HABs in freshwaters, flourish on microplastic particles (Lee, 2008). Cyanobacterial akinetes (the resting stages) can tide over unfavourable conditions by overwintering in the sediment-water interface. Attachment to sinking microplastics will hasten their settling onto the sediment, resulting in an increase in the cyanobacterial 'seed bank' (Lee, 2008). A similar scenario may hold true for dinoflagellate cysts. The temporary cyst of *Alexandrium taylori* has a sticky nature which promotes cluster formation and settling into the sediment where it contributes to the 'seed-banks' for blooms (Masó et al., 2003). Its sticky nature can also facilitate its attachment to microplastics. Whether microplastics serve as an additional reservoir of cysts of HAB species remains to be explored. This will definitely influence the frequency and severity of HABs in the future (Lee, 2008). Additionally, the ability of HAB species to grow attached to plastic, and utilize it either through primary metabolism or through consortia with other organisms, raises the possibility that several new HAB species with enhanced survival compared to competitors, may emerge in the new future, particularly in plastic-polluted environments.

### 2.2.3. The emergence of plastic-degrading microorganisms

Microplastics, not only provide a substrate for organisms to attach and proliferate, but also harbor several microplastic-degrading organisms. Several lines of evidence support this. Firstly, Reisser et al. (2014) noticed a variety of plastic surface microtextures, including pits and grooves, conforming to the shape of several bacterial, fungal and eukaryotic colonizers (details in Section 2.2.1 above), revealing that these epiplastic communities are pivotal in plastic degradation. Secondly, several plastic-degrading bacterial, fungal and algal genera have been retrieved from plastics and microplastics (reviewed in Oberbeckmann et al., 2015a). For e.g., the PAH-degrading bacterial genus – *Erythrobacter*, was recorded on microplastics under a broad range of environmental conditions (Oberbeckmann et al., 2018). Canniff and Hoang (2018), in their study on the microalga – *Raphidocelis subcapitata*, noticed that it grew better in the presence of plastic microbeads than without them, suggesting that plastic microbeads could serve as substrates for *R. subcapitata*. Thirdly, small-subunit rRNA gene surveys have reported several hydrocarbon-degrading bacteria on Plastic Marine Debris (PMD), supporting the idea of microbes being involved in the degradation of PMD (Zettler et al., 2013).

### 2.3. Microplastics as a potential vector for human pathogens in the marine environment

Microorganisms have the potential to form biofilms on microplastic surfaces in aquatic environments. They constitute the 'plastisphere', i.e., the assemblage of microorganisms inhabiting the surfaces of macro and microplastics in the marine environment (Mincer et al., 2016). Thus, microplastics are not only effective vectors for transport of harmful chemicals, resulting in propagation across the food web, but are also responsible for the dispersal of microbial communities across large distances (Fig. 1). In the North and Baltic Seas, biofilm-forming human bacterial pathogens such as *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* were reported on polystyrene, polyethylene and polypropylene microplastics (Kirstein et al., 2016), which helped to transport them to distant sites. In a separate study on the microplastic-associated microbial assemblages in the intertidal zone of the Yangtze estuary, China, high-throughput sequencing techniques revealed the presence of bacterial communities which are mainly responsible for pathogenesis in corals, human and fish (Jiang et al., 2018). In fact, Virsek et al. (2017) confirmed that microplastics in marine waters can act as a potential vector for the spread of *Aeromonas salmonicida*, a fish pathogen that also causes human infection via consumption of infected fish. Thus, pathogenic bacteria that have the potential to form biofilms on microplastics can be successfully transferred through the food chain to humans (Rummel et al., 2017).

### 2.4. Co-selection of antibiotic-metal resistance in bacteria on surface of microplastics in marine waters

Microplastics act as vectors for the development and spread of multidrug-resistant pathogens. This was confirmed by recent research carried out at King George Island (Antarctica) that highlighted the potential of plastics as an effective vector for the dissemination of antibiotic-resistant bacteria to distant sites (Laganà et al., 2019). Microplastics are also known as vectors for diverse harmful chemicals, metals and other pollutants which are either released during the natural weathering of plastics, or which had been earlier adsorbed and concentrated on the surface of the microplastics. These conditions provide a perfect environment and selection pressure on bacterial pathogens in marine waters for co-selection (cross-resistance and co-resistance) of metal and antibiotic resistance. When one resistance mechanism is responsible for resistance to various compounds, it is called cross-resistance, whereas, in co-resistance, two or more different resistance conferring genes are present on a single mobile genetic element (transposon/plasmid/integrans) in microorganisms and confer



resistance to different toxic compounds (antibiotic, heavy metals, PHAs, PCBs) simultaneously (Baker-Austin et al., 2006; Imran et al., 2019).

In phylogenetically-diverse bacteria present on surfaces of microplastics, an increased rate of plasmid transfer as compared to free-living (planktonic) bacteria in the aquatic environment has been observed (Arias-Andres et al., 2018). Additionally, it was proven that with an increase in microplastic particles, there was a corresponding increase in the abundance of integrase 1 (*int1*) in the 'plastisphere' but not in the water surrounding the microplastics (Eckert et al., 2018). All these studies proved that microplastic pollution in both marine and freshwater environments serves as a hotspot for development and spread of antibiotic resistance between phylogenetically distinct bacterial pathogens due to selection pressure and horizontal gene transfer. This has been recognized as a rising environmental concern, especially in metal- and microplastic-contaminated environments (Baker-Austin et al., 2006; Imran et al., 2019).

### 2.5. Effects of microplastics on human health

The microplastics ingested by shellfish and fish finally reach humans through the food chain (Carbery et al., 2018; Smith et al., 2018). It has also been proven that salts made from sea water around the globe are contaminated with microplastics which may enter the human body through consumption (Barboza et al., 2018; Seth and Shrivastav, 2018). Humans can be exposed to microplastics through ingestion of contaminated fish as well. Recently, studies on cell lines of cerebral and epithelial human cells showed the potential cytotoxic effect of microplastics and nanoplastics (40 nm–10 µm) (Schirinzi et al., 2017). Very fine microplastic particles have the potential to cross cell membranes, the blood-brain barrier, the placenta and may cause oxidative stress, cell damage, inflammation and impairment of energy allocation (Vethaak and Leslie, 2016; Carbery et al., 2018).

Humans are not only exposed to microplastics but also to the various contaminants leached from/adsorbed on to their surface. The chemicals leached from microplastics during weathering and also chemicals which are adsorbed on the surface of microplastics from polluted marine waters (phthalates, heavy metals, bisphenol A, pesticides, flame retardants and PCBs, fertilizers) can act as endocrine disruptors, mutagenic and carcinogenic agents. These may be harmful to humans at very low concentrations (Rochman et al., 2015; Alimi et al., 2018; Gallo et al., 2018). These may enter the human body via consumption of contaminated fish. Wardrop et al. (2016) has reported the adsorption of polybrominated diphenyl ethers (persistent organic pollutants/POPs) onto microplastic beads from personal care products. These POPs were then assimilated by fish (*Melanotaenia fluviatilis*) following particle ingestion (Wardrop et al., 2016). Consumption of such fish by humans introduces the microplastics and their adsorbed pollutants into the human body and poses a serious health hazard. Other health effects resulting from bioaccumulation and biomagnification of microplastics and chemical contaminants in the human body include skin irritations, respiratory problems, cardiovascular diseases, digestive problems and reproductive effects (Carbery et al., 2018). In addition, microplastics also support the emergence of Multiple Drug Resistant (MDR) pathogens, which are difficult to treat with conventional antibiotics and thus, pose an upcoming threat to human health. Therefore, microplastics are termed as a cocktail of toxic contaminants (Rochman et al., 2015).

It has been experimentally ascertained that evisceration of fish does not eliminate the risk of microplastic intake by fish consumers (Karami et al., 2017). European countries which consume high amount of shellfish in their diet are estimated to ingest on an average 11,000 microplastic particles/year (microplastic size ranges 5–1000 µm) whereas, on an average, 1,800 microplastics/year per person are consumed by countries which have comparatively less shellfish in their diet (Barboza et al., 2018). This is a critical concern from the perspective of human health (Fig. 2).

### 2.6. Policies and regulatory guidelines regarding microplastics

The problem of microplastics has been addressed by several organizations working in the field of environmental protection. The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) – an advisory body to the United Nations on the prevention, reduction, and control of the degradation of the marine environment – emphasizes the need to address the relevance of plastics and microplastics as a vector for the transfer of organisms ([www.gesamp.org](http://www.gesamp.org)). Amaral-Zettler et al. (2015) has pointed out that one of the GESAMP action-oriented recommendations was the need for 'identifying the main sources and categories of plastics and microplastics entering the ocean'. However, attempts to identify the origin of the plastic/microplastics based on the plastisphere microorganisms are misleading since the 'plastisphere' tends to reflect the local surroundings, more than their potential sources of origin (Amaral-Zettler et al., 2015). Several other agencies, including US EPA and European agencies have identified the problem of marine litter, particularly plastic, and recognized that it is critical to develop sound environmental policies for microplastic risk assessment and conservation of marine ecosystems. The EU member states adopted the Marine Strategy Framework Directive (MSFD) in 2008. Zarfl et al. (2011) have pointed out that, under that directive, EU member states aim to develop activities to achieve 'good environmental status' (GES) in the European marine environment by the year 2020. Future challenges in this regard, with regard to microplastics, include determining the longevity of different types of PMD in marine systems and characterizing the microbial interactions with the persistent, bioaccumulating, and toxic substances contained on microplastics (Amaral-Zettler et al., 2015).

## 3. Ballast water as a vector for microplastic transfer across continents

An important feature of microplastic pollution is its dissemination to distant sites across the globe. A pivotal yet unexplored vector for the dissemination of microplastics is ballast water, associated with the commercial shipping industry. Approximately 80% of the world's cargo is transported across the oceans, with shipping routes crisscrossing the world's oceans. Consequently, over 12 billion tons of ballast water is mobilized from one port to the other (Anil et al., 2002). Considering the small size of microplastics (100 nm–0.5 mm) and the tremendous volume of shipping traffic, microplastics have a very high probability of being transferred from one continent to another along with ballast water. However, ballast water-mediated transfer of microplastics has not been given the attention it deserves.

### 3.1. Ballast water as a vector for bioinvasion

Ballast water maintains the stability and structural integrity of ships. When a ship unloads cargo, ballast water is taken in, and vice versa. Ballast water contains a plethora of organisms, including microorganisms, phytoplankton, zooplankton, etc. When these are introduced to new marine environments through ballast water, they may pose a threat to the native biota of the local marine environment. Organisms carried in ships' ballast water may not survive the voyage, or the conditions in the recipient environment. Yet, some species survive, propagate to form viable populations, and may cause detrimental effects in the environment. In this scenario, the introduced organism is called a 'bioinvasive' species. Invasive aquatic species is one of the four greatest threats to the world's oceans; the others include land-based sources of marine pollution, over-exploitation of living marine resources, and physical alteration and destruction of coastal and marine habitats (Tsolaki and Diamadopoulos, 2010).

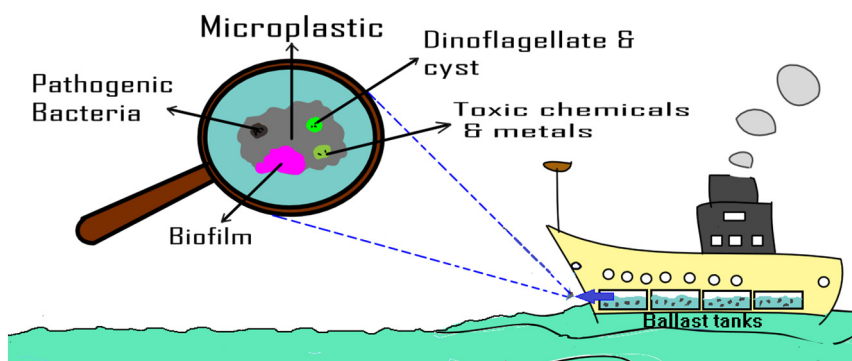


Fig. 3. Fate of microplastics in ballast water.

### 3.2. Ballast water-mediated translocation of human pathogenic *Vibrio* spp.: a global concern

Ballast water is responsible for global translocation of life-threatening, diarrheal disease (cholera)-causing bacterium - *Vibrio cholerae*. *Vibrio cholerae* reaches humans through fish/shellfish consumption or through recreational activities in polluted marine waters (Halpern and Izhaki, 2017). Out of 200 serotypes found globally, only serotype O1 and O139 (called toxigenic *V. cholerae*), are known to cause epidemics and pandemics. An outbreak of cholera, resulting in mortality in Haiti in 2010 highlighted the severity of this disease globally (Centers for Disease Control and Prevention, 2010). The biggest cholera epidemic in the world occurred in Yemen between 2016 and 18, where over 1,000,000 cholera cases were reported (Camacho et al., 2018). Earlier, cholera was confined to the South-East Asian countries, but has now spread to North American countries too. This is primarily due to translocation of *Vibrio* globally through ballast water. A cholera pandemic, linked to ballast water-related transfer of toxigenic *V. cholerae* O1, was observed in Latin American countries in 1991. In 1992, this spread to USA (Cohen et al., 2012; Khandeparker and Anil, 2017). Thus, *V. cholerae* is the most extensively studied, human pathogenic bacterium in ballast water (Khandeparker and Anil, 2017). In ballast water, *V. cholerae* is associated with plankton (zooplankton and phytoplankton) in higher numbers compared to that in the water column (Ruiz et al., 2000; Tang, 2005; Rivera et al., 2013). Therefore, *V. cholerae* is in the list of Global Ballast Water Management Programme as one of the 'Ten Most Unwanted' bacteria in ballast water (McConnell, 2002). Other pathogenic species of *Vibrio* reported to translocate through ballast water are *V. alginolyticus*, *V. carchariae*, *V. parahaemolyticus* and *V. vulnificus* (Wu et al., 2017). Therefore, considering the urgency of this matter, in 2004, the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM) was adopted by the International Maritime Organization (IMO) in order to reduce the risk for transfer of invasive species and pathogens (*V. cholerae*) through ballast water (Cohen et al., 2012).

The emergence of Asiatic cholera diseases is also influenced by the warming phase (El Niño) of the El Niño Southern Oscillation (ENSO). Martínez-Urtaza et al. (2016) confirmed through microbiological, genomics and bioinformatics tools, that El-Niño episodes caused outbreaks of devastating *Vibrio* diseases in Latin America. *Vibrio* spp., in the VBNC (Viable But Not Culturable) state are usually transferred across continents (Asia to South America) through ballast water. Due to the El-Niño episode in 1997, surface temperature increased, creating a suitable environment for resuscitation and growth of *Vibrio*, and ultimately cholera outbreaks (Martínez-Urtaza et al., 2008; Vezzulli et al., 2015; Martínez-Urtaza et al., 2016), facilitated by the intercontinental transfer of *V. cholerae* through ballast water (Moore et al., 2017).

### 3.3. Microplastics in ballast water – unexplored aspects

The presence of microplastics in ballast water is a relatively unexplored aspect that deserves attention. An extensive literature survey about this aspect revealed only two brief reports, one about the occurrence of microplastics in ballast water by Matiddi et al. (2017), and the other report, by Kirchner (2017), about the necessity of including consideration for microplastic occurrence and effective regulations to curb the same, in the Ballast Water Conventions. Matiddi et al. (2017) provided evidence, for the first time, for the presence of microplastics in ballast water of commercial tanks. Microplastic abundance was assessed in the ballast water of nine cargo vessels, arriving at the port of Bari, Italy from July to October 2015. This pioneering study was carried out under the IPA Adriatic project BALMAS (Ballast Water Management System for Adriatic Sea Protection). Microplastic concentrations in ballast water ranged from 100 to 1410 items  $m^{-3}$  (average  $651 \pm 160$  items  $m^{-3}$ ); this is much higher than the corresponding values observed in samples collected at sea in other parts of the Mediterranean ( $0.116\text{--}0.15$  items  $m^{-3}$  (in other words,  $< 1$  item  $m^{-3}$ )). Synthetic filaments were predominant followed by thin plastic layers and fragments. Plastic virgin pellets, in the form of spheres, were below detection levels. Blue was the main colour observed, followed by black and red items (Matiddi et al., 2017). These observations, though limited, highlight the potential of ballast water as a 'hotspot' for the occurrence and concentration of microplastics. This study also brings to light the significant role of ballast water in transporting microplastics and its associated entourage of harmful chemicals, pathogens and plastisphere organisms (including invasive and HAB species) across the globe.

### 3.4. Ballast water as a vector for transfer of microplastics and its associated perils across continents

Microplastics in ballast water have numerous impacts (Fig. 3). Firstly, they may act as efficient vectors for the inter-continental transfer of bacterial pathogens. Potentially pathogenic *Vibrio* sp. (Kirstein et al., 2016) and fecal indicator bacteria (Keswani et al., 2016) have been reported from plastic debris. *Vibrio cholerae*, already known to be transferred through ballast water, may form biofilms on the readily available microplastic surfaces, that will then serve as an emerging potential vector for the spread of bacterial diseases in marine environments. Given the unique conditions of the microplastic-ballast water interface, the chances of pathogens enhancing their virulence as well as resilience, in the presence of the cocktail of harmful chemicals, metals and contaminants present there, are very high. Secondly, transfer of microplastic-laden ballast water ensures the transport of the plastisphere microbiota across continents. This may facilitate the dispersal of invasive fouling species to distant sites. Thirdly, ballast water is already recognized as a vector for HAB species (Wu et al., 2017). Microplastics in ballast water may be a crucial, yet unrecognized

vector, responsible for the spread and frequency of HABs in the last few decades, concurrent with the increase of commercial shipping (Fig. 3).

### 3.5. Fate of microplastics and their associated 'plastisphere' communities in ballast water

Microplastics are already recognized as hotspots for co-selection of metal-driven antibiotic resistance in the marine environment, since they adsorb a wide variety of pollutants (metals, metalloids, antibiotics, persistent organic pollutants) onto their surfaces (Imran et al., 2019). They are also a potential vector for development and spread of multi-drug-resistant human and fish pathogens, which have been reported to form biofilms on microplastic particles (Kirstein et al., 2016; Virsek et al., 2017; Imran et al., 2019). Drake et al. (2005) have reported that biofilm-forming microorganisms on the internal surfaces of ballast tanks are present at higher densities as compared to planktonic forms in ballast water (Drake et al., 2005). This may be true for microplastics as well.

Bacteria associated with microplastics show higher frequency of horizontal gene transfer between phylogenetically distinct bacteria as compared to free-living bacteria (Arias-Andres et al., 2018; Eckert et al., 2018). Thus, microplastics provide a perfect 'hotspot' for metal-driven co-selection (cross-resistance and co-resistance) of antibiotic resistance in human pathogenic bacteria associated with microplastics (Imran et al., 2019). Additionally, the 'plastisphere' microorganisms are subjected to a confined set of conditions in the ballast water tank – absence of light, lowered oxygen concentrations, oxidation potential and pH, leaching of metal ions from corrosion-resistant paints, which are different from that of the pelagic environment outside the tank (Anil et al., 2002). In view of these conditions in ballast water tanks as detailed above, and the high abundance of microplastics, there is a high probability of the 'plastisphere' community developing resistance to metals, antibiotics, persistent organic pollutants, etc. by co-selection mechanisms and horizontal gene transfer, during the voyage duration. These conditions could result in the evolution of multidrug resistant (MDR) human pathogens, for e.g., *V. cholerae*, which is known to enhance its virulence through mobile genetic elements (Munn, 2011). It is plausible that these MDR pathogens may transfer from one continent to other through ballast water by using microplastics as a vector and may pose a serious risk to human health.

Cysts of HAB species may also survive in ballast tanks attached to microplastics, and proliferate in the recipient environment, depending on the suitability of the environmental conditions. Transport on microplastics in ballast water may thus increase the resilience of microorganisms (pathogens, HAB species, etc.) and enhance their chances of survival in the recipient environment.

### 3.6. The management of microplastics in ballast water – pressing need for guidelines and regulatory frameworks

Microplastics in ballast waters have high potential for translocation of biofilm-forming human pathogenic *Vibrio* spp. and HABs on microplastics. These microplastics (along with chemicals and bacterial human pathogens) may enter the human body during recreational activities or through the food chain and pose a serious threat to humans. Also, the release of chemicals adsorbed on microplastics may be detrimental to the marine biota in the recipient environment and may have several ecological repercussions. Thus, it is imperative to have effective ballast water treatment protocols that reduce to a significant degree, the microplastic concentrations in ballast water prior to discharge. However, given the urgency of this issue, it is pertinent to note that there are no existing guidelines by IMO for the management of microplastics in ballast water. Several policies exist for the management of plastics and microplastics in the marine environment. However, their occurrence in ballast water, though posing a serious problem, has not warranted attention so far. This is a matter of grave concern. In fact,

Kirchner (2017), the only other paper available on microplastics in ballast water, besides Matiddi et al. (2017), stresses the need to include regulations for microplastics in ballast water, within the framework of the Ballast Water Convention guidelines.

### 3.7. Ballast water on-board treatment according to the International Convention for the Control and Management of Ships' Ballast Water and Sediments

According to the International Convention for the Control and Management of Ships' Ballast Water and Sediments, in force since 2017, there are two categories of ballast water management protocols: (1) ballast water exchange at sea, and (2) ballast water treatment on-board.

#### 3.7.1. Ballast water exchange at sea

This method, also called re-ballasting, is recommended by the International Maritime Organization (IMO). It is the most effective measure to reduce the transfer of harmful aquatic organisms, and can be performed in either of two ways: sequential or reballasting and flow-through or continuous flushing. The first approach consists of completely emptying ballast tanks (individually or in sequence) and refilling them with open ocean water. The second approach (flow-through) involves partially emptying and refilling the tanks.

There are ballast water management standards for these methods (the D-1 and D-2 standards). D-1 pertains to ballast water exchange, while D-2 specifies the maximum amount of viable organisms that can be discharged, including specified indicator microbes harmful to human health (toxigenic *Vibrio cholerae*, *Escherichia coli*, intestinal enterococci). The D-1 standard requires ships to exchange their ballast water in open seas, such that at least 95% of water by volume is exchanged far away from the coast away from coastal areas. Ideally, this means at least 200 nautical miles from land and in water at least 200 m deep. This is based on the rationale that oceanic organisms will find coastal conditions unsuitable for growth and vice versa. However, it must be noted that microplastics are ubiquitous, and thus, will find their way into ballast water tanks, irrespective of where the ballast water is taken in. The D-2 standard states that the ballast water discharged by ships should meet the following criteria: (1) < 10 viable organisms per cubic metre for organisms in the size range  $\geq 50 \mu\text{m}$  in minimum dimension; (2) < 10 viable organisms per millilitre for organisms 10–50  $\mu\text{m}$  in minimum dimension; (3) < 1 colony-forming unit (cfu) per 100 millilitres of toxigenic *Vibrio cholerae*; (4) < 250 cfu per 100 ml of *Escherichia coli*; and (5) < 100 cfu per 100 ml of intestinal enterococci (IMO website). So, though harmful bacterial pathogens and larger organisms have been taken into account in the guidelines, it should be noted that there are no corresponding standards set for microplastics. Thus, microplastics along with their plastisphere organisms, can gain entry into ballast tanks.

Ballast water exchange is not 100% effective in removing organisms from ballast water. In a study of four container ships that took on ballast in Mexico and discharged it after 21 days in Hong Kong, Dickman and Zhang (1999) noticed few viable diatoms and dinoflagellates after the 21 day journey. Five ships that carried out ballast water exchange in the open ocean reduced diatom and dinoflagellate populations by 48%. Another study, by Zhang and Dickman (1999), of the seasonal factors affecting transport of harmful phytoplankton on 34 ships, reported that newer ships were more effective in removing phytoplankton species compared to older ships. Besides this issue of incomplete removal of harmful organisms, ballast water exchange procedures are also fraught with ship-safety limitations (Tsolaki and Diamadopoulos, 2010).

#### 3.7.2. Ballast water treatment

There are several technologies employing mechanical, physical and chemical methods to treat ballast water (reviewed in Vorkapić et al., 2016). Mechanical methods include filtration and cyclonic separation; physical methods are cavitation, ultrasound, electrolysis, heat treatment, deoxygenation and UV radiation whereas chemical methods



include chlorination, ozonation and peroxyacetic acid treatment. On-board ballast water treatment systems are reported to be effective, but not 100% efficient, in reducing the risk of spreading invasive species across continents (Vorkapić et al., 2016). The most common ballast water treatment systems are two-stage electrochlorination for high-capacity systems and UVR systems for low-capacity systems, both combined with mechanical filtration method (filtration or cyclonic separation for the necessary initial treatment) for the removal of organisms and particles bigger than 20 µm (Vorkapić et al., 2016).

Considering the < 10 µm fraction and particularly heterotrophic bacteria, Hess-Erga et al. (2019) have recently reported that the presence of particles (biotic and abiotic) in ballast water interferes with the disinfection process, particularly in UV treatment. Thus, it is highly probable that microplastics not only serve as vectors and hotspots of microbial activity but also serve to protect the bacteria associated with them from ballast water disinfection processes. Biofilm-forming plastisphere microorganisms are more resilient compared to planktonic microorganisms (Drake et al., 2005) and thus, cannot be easily dislodged from microplastic particles. This is particularly true for cysts adsorbed on the surface of microplastics and covered with sediment. All these aspects need to be considered within the framework of the existing ballast water strategies. Currently, self-cleaning filters are used in mechanical filtration (Vorkapić et al., 2016). However, whether these treatment systems are effective in reducing microplastic concentrations to acceptable levels needs to be established. So far, to our knowledge, reducing microplastic concentration in ballast water has not been incorporated as a criterion into any of the ballast water treatment guidelines so far. Thus, there are no standard limits for the occurrence of microplastics in ballast water. These aspects need to be urgently looked into. Based on the International Convention for the Control and Management of Ships' Ballast Water and Sediments, in force since 2017, ships need to install on-board systems for treatment of ballast water, prior to discharge (Ng et al., 2018). There is a pressing need to incorporate microplastics as a relevant parameter, so that effective treatment technologies and the corresponding changes in ship design can be carried out.

#### 4. Conclusions and suggestions

This review is the first one showcasing microplastics in ballast waters, and their role as vectors for transport of harmful chemicals, metals, associated bacterial pathogens, invasive species, HAB-forming dinoflagellates, etc. across continents. Microplastics in ballast waters serve as hotspots for horizontal gene transfer and co-selection of metal-driven, multiple antibiotic resistance in bacterial pathogens associated with microplastics. This is an emerging global health risk, especially for inhabitants of coastal areas. Thus, in this context, we strongly advocate the amendment of the Ballast Water Management Convention by the International Maritime Organization to notify microplastics as a hazardous material at the earliest.

#### CRediT authorship contribution statement

**Ravidas Krishna Naik:** Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Milind Mohan Naik:** Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Priya Mallika D'Costa:** Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Fauzia Shaikh:** data curation, formal analysis, visualization, writing the original draft.

#### Declaration of competing interest

None.

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