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How does Natural Organic Matter (NOM) affect Micro- and Nano-Plastic Pollution in the Environment? - The biophysical Mechanisms leading to the formation of 'Marine Plastic Snow'

Peter H. Santschi[1], Wei-Chun Chin[2], Antonietta Quigg[3], Chen Xu[1], Manoj Kamalanathan[3], Peng Lin[1]
[1] Department of Marine and Coastal Environmental Sciences, Texas A&M University at Galveston, TX 77553, USA
[2] Department of Bioengineering, School of Engineering, University of California at Merced, Merced, CA 95343, USA
[3] Department of Marine Biology, Texas A&M University at Galveston, TX 77553, USA
santschi@tamug.edu

Abstract
Plastics are widely produced and used with an annual global plastic production ~360 million tons per year in 2018. Plastic pollution is one of most critical environmental problems to be addressed, with the ocean as the ultimate depository for most of the waste. Intense mass media coverage around 'The Great Pacific Garbage Patch' has highlighted the severity, persistence and expansive nature of plastic pollution in the marine environment. Substantial accumulation of plastic litter through a variety of pathways such as air-water exchange, treatment plant effluents, urban runoff and river discharge, is a major scientific and public environmental concern. Marine plastic waste, through chemical mechanisms or physical forces, can be broken down (fragmented) into micro- and nano-size particles in seawater. Detailed survey (distribution) data and toxicity assessments for microplastics and nanoplastics have been documented. However, the impact of these microscopic plastic particles on fundamental aquatic processes has been understudied. We concentrate herein on the biophysical mechanisms governing the interactions between these materials and natural organic matter such as EPS in aggregating these materials and either leading to it entering food webs or being transported to the seafloor, and/or ultimately both these phenomena. Specifically, the source and composition of EPS and how they can influence the aggregation with micro/nano plastics is discussed, including the interactions between microbes, enzymes, and ROS in EPS formation and hence the aggregation into marine plastic snow.

Key Word and Phrases
Natural Organic Matter, Microplastics, Nanoplastics, Environment, Pollution, Marine Plastic Snow.

1.1 Marine Plastic Pollution

Plastics are widely produced and used synthetic polymeric materials. The global plastic production in 2018 was estimated to be ~360 million tons [1]. Plastic waste release and mismanagement have resulted in substantial accumulation of plastic litter in aquatic environments through a variety of pathways such as air-water exchange, treatment plant effluents, urban runoff and river discharge [2]-[4]. Fresh and aged plastics and debri that enter natural systems are broken down by mechanical fragmentation, chemical (oxidative and thermal) degradation, and biodegradation [3], [5]-[7] into smaller fragments, i.e., micro- and nano-plastics, the latter of which have more complex ecological and toxic impacts on the environment. Micro- and nano-plastics are defined as plastic debris with sizes <5mm and < 100 nm in diameter, respectively [8]. They spread rapidly, easily transferring to the ocean interior through food webs and aggregation processes. Many studies have shown that plastic fibers, beads and fragments not only exist in polluted environments including lakes, rivers, wastewater effluent, beaches, and coastal oceans but are also present in remote locations such as deep-sea sediments, polar seas, and frozen ice cores [9]-[14].

Plastics in the marine environment are also reservoirs for antibiotic and metal resistance genes [15]. A wide range of organisms including zooplankton, bivalves, mussels, shrimp, oysters, copepods, and lugworms have been reported to ingest smaller sized plastics [16]-[19], including the associated microbial cells and EPS, and the plastics are expected to be transferred via trophic food chains [20].

Haye et al. [21] showed for the first time that filter-feeding organisms effectively filter suspended particles, but also negatively charged colloidal-sized nanoplastics (40 nm) at ambient (~1 mg/L) concentrations, possibly as marine plastic snow (MPS). Laboratory studies indicate that ingestion could cause harmful toxicological and/or physical effects, both directly and indirectly [22]. These plastics have also been implicated as a carrier route for transboundary transport of harmful algal species, microbes and persistent organic pollutants (POPs) due to their unique properties (e.g., high surface area, porosity, buoyancy) [23], [24]. Nano-plastics are of great interest because of their high surface area to volume ratio, which makes their properties fundamentally different from those of the same polymer type in bulk form [25]. However, the behavior, transport, and distribution of nano-plastics in aquatic systems is much less studied compared to micro-plastics as most separation methods and analytical techniques are not directly applicable to nanoscale particles, even though a number of such methods have now recently been published [26], [27].

1.2 Relevance of Aggregates to Micro- and Nano-Plastics to Marine Plastic Snow Formation

In 1953, the first careful collection of the matrix of microorganisms embedded in suspended natural organic matter (NOM) that today we call marine snow was performed [28]. Subsequent studies reported that marine snow consists of phytoplankton, small zooplankton, fecal material, and other particles in a matrix of organic material (see recent reviews [29], [30]). Silver, Shanks and Trent [31] quantified the abundance of marine snow and its contribution to the total community *in situ,* showing that marine snow particles are "metabolic hotspots", with concentrations of microorganisms 3-4 orders of magnitude greater than those in the surrounding seawater. Rapidly sinking marine snow is important in the marine carbon cycle as it is responsible for vertical (re)distribution and demineralization of carbon, the biogeochemical cycling of elements (e.g., [32], [33]), and contaminants e.g., oil and plastics [34], [35]. Recent research has shown that plastic particles entering aquatic systems can be rapidly colonized by microbial biofilms [36]. Micro/nano-plastics have been found in marine NOM and organic aggregates such as microgels, exopolymeric substances (EPS), transparent exopolymeric particles (TEP), and aggregates [37]-[40]. Other interactions with naturally occurring colloids (i.e., biopolymers derived from phytoplankton, and colloidal-sized inorganic materials) may also cause plastic aggregation and affect other types of settling (scavenging) processes, which requires further study to obtain new insights into the environmental fate and ecological risk of plastic particles [41], [42]. De Haan, Sanchez-Vidal and Canals [43] reported from field studies that an average of 40% in abundance of the microplastics was incorporated with NOM in the marine environment. They point to evidence that marine aggregates facilitate ingestion of microplastics and nanoplastics by suspension-feeding bivalves, as well as zooplankton and other marine species. Microplastics are also found at the highest trophic level, e.g., in all the digestive tracts of birds of prey (hawks) that were investigated [20]. It was suggested that marine snows and fecal pellets have the potential to remove the majority of micro-, and nano-plastic from the surface ocean and thus is considered as a globally significant vector and sink for micro- and nano-plastic [44]. Relatively little research has been devoted to understanding the interactions between micro- and nano-plastics and marine snow, or to the mechanisms that explain the formation of MPS, and the factors that lead to its sinking. A schematic of the possible interactions leading to the formation of marine plastic snow and subsequent sedimentation is shown in Figure 1.

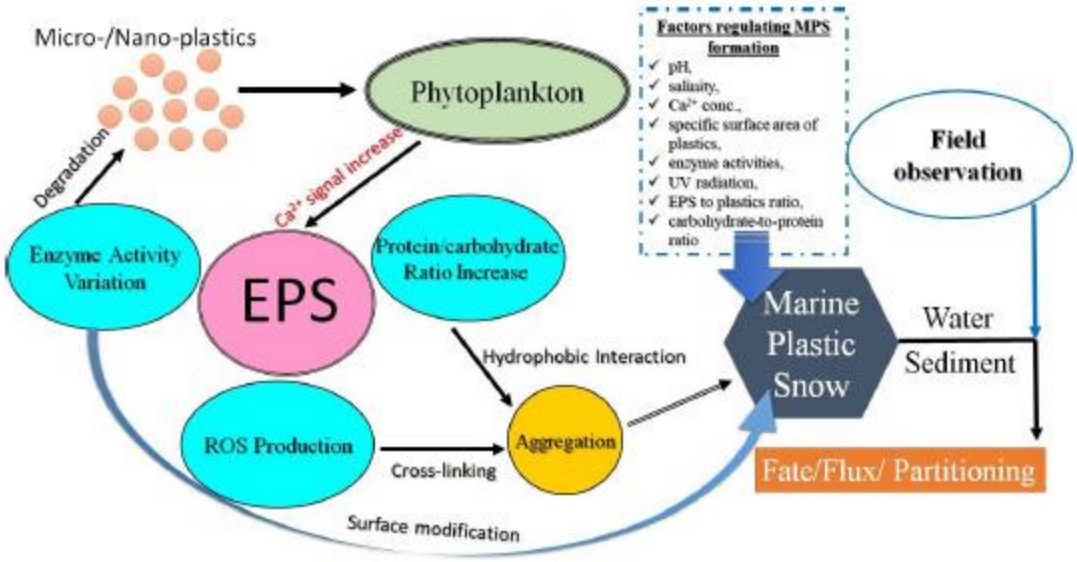

Fig. 1 Schematic of possible pathways and processes leading to the formation of 'marine plastic snow' (MPS).

1.3 A Major Component of MPS: Exopolymeric Substances (EPS)

Marine microbes are capable of producing high molecular weight exudates ([29]). EPS (also called extracellular substances, exopolysaccharides) describes a heterogeneous group of materials released from cells existing in a size continuum from dissolved to colloidal phases, and includes gels, which function as particles. EPS may be attached to cell coatings, biofilms or colony matrices, or free floating. These polymeric substances have varying functional roles (e.g., protecting microbes, aiding their attachment), chemical and physical properties [45]-[49] and appearance. The production of EPS by planktonic microbes can influence the fate of pollutants (e.g., oil, dispersants, nanomaterials) in the ocean through emulsification, degradation, dispersion, aggregation, and/or sedimentation [29], [50]. In turn, microbial community structure and function, including the production and characteristics of EPS, is influenced by the concentration and chemical composition of the pollutant materials. Research has shown that EPS is the key component of NOM that mediates the formation of MPS [51]. As such, EPS may serve an important role in determining the fate and transport of micro- and nano-plastics, but relatively little is known about EPS production and the biophysical mechanisms in relation to interactions with plastics (both physically and chemically), especially at molecular and chemical levels.

1.4 The Key Aspects of Marine Plastic Snow (MPS)

1.4.1 Protein/Carbohydrate (P/C) Ratios and Hydrophobic Interactions

Microbial EPS are ubiquitous in marine systems and are known as the precursor of microgels and marine snow [29], [45]. EPS are released by microbes (phytoplankton, bacteria), and contain proteins, carbohydrates, and other biological macromolecules [52]. The impacts of EPS and biopolymers on the marine ecosystem are vast, serving active roles in marine particle aggregation and gravitational sinking, biofouling, microbial colonization, and pollutant mobility [37], [53]-[58]. Notably, studies have shown that the secretion of EPS from microbial cells is significantly affected by surrounding environmental conditions. For example, EPS with higher protein to carbohydrate

(P/C) ratios are induced by unfavorable growth conditions including nutrient limitation, toxins (nanoparticles, oil, and dispersant), and light exposure [59]-[62]. Shiu et al. [58] showed that the P/C ratio of microbially produced EPS increased in the presence of oil and the dispersant, Corexit. EPS with high P/C ratios are more hydrophobic and sticky, thus easily aggregating around the cells and forming a natural barrier against the hazardous agents [63], resulting in the lower effective concentration of toxins entering microbial cells. Furthermore, protein-rich EPS can potentially facilitate faster assembly rates of marine aggregates and alter their aggregation sizes [56], [64]. Thus, the P/C ratio of EPS can be used to predict stickiness, attachment and aggregation propensity [65]. The interactions between microbial growth/survival and critical characteristics of EPS (P/C ratio) in the presence of micro- and nano-plastics has received little attention. Therefore, understanding the complex biochemical and biophysical interactions between three key components (microbes, plastics, and EPS, Figure 1) during plastic exposure will help to better elucidate the fate of plastics, especially in their aggregation and scavenging processes in marine environments.

1.4.2 Activities of Microbially produced Enzymes and other Compounds within MPS

Some if not all microbes possess the ability to degrade plastics in the environment via secretion of extracellular enzymes [66]. One of the most promising lines of evidence of this phenomenon comes from [67], where the biological degradation of poly(ethylene terephthalate) (PET) by the bacterium, *Ideonella sakaiensis* occurred via a hydrolase PETase. Through this enzyme, the bacterium *I. sakaiensis* was able to utilize PET as a sole source of carbon. Several other extracellular enzymes also help in degrading PET such as lipases (EC 3.1.1.3), cutinases (EC 3.1.1.74), carboxylesterases (EC 3.1.1.1) and laccases (EC 1.10.3.2) [68]. Our previous studies show that although polysaccharides are a major carbon source in the marine environment, introduction of another carbon source such as oil can change the profile of extracellular enzymes produced to specifically target the new carbon source [69]. For example, under control conditions (treatment without petroleum contamination), the carbon acquisition enzyme β-Glucosidase (that provides carbon through breakdown of polysaccharides) was linearly correlated with the nitrogen acquisition enzyme leucine-amino-peptidase (LAP, which provides nitrogen through protein breakdown). A linear correlation was also observed between the global homolog abundance of β-Glucosidase and LAP derived from the global sampling project Tara Ocean Database [69]. Given the linear relationships measured and the potential for gene production (Tara metagenomes), this may be a general rule in marine systems. However, the correlation between β-Glucosidase and LAP was not observed in experiments in which contaminants were present [69]. These results indicate that introduction of micro- or nano-plastics as alternative carbon sources for microbes into the marine environment may trigger similar changes in the extracellular enzyme profile, potentially leading to higher expression and activities of PETase, lipase, cuinase, carboxyexterase, laccases etc.

In addition, Galgani et al. [70], [71] showed that plastics tend to provide a stable mechanical surface for microbial growth and biofilm production, which then in turn boosts microbial metabolism by EPS embedded extracellular enzymes. The increasing microbial metabolism occurring in the aggregates and/or marine snow could have modified the chemical properties of the dissolved organic carbon and thus altering the marine biological pump. Several studies have shown that extracellular enzyme activities are higher in marine snow and biofilms as they are a critical component of external digestive system allowing metabolism of dissolved, nanoparticulate (colloidal) and solid biopolymers [72]-[74]. Therefore, it can be hypothesized that micro and nano-plastics, by acting as nuclei for biofilm and marine snow development, will boost the synthesis and activity of extracellular enzymes such as β-glucosidase, LAP, and alkaline phosphatases in addition to plastic biodegradation enzymes. The fact that antibiotic and metal resistance genes are observed in biofilms forming on plastic surfaces was explained by a combination of lateral gene transfer and release of antibiotics by selected bacteria [15]. This suggests that micro- and nano-plastics can become a vector for food chain transfer of not only various hydrophobic pollutants but also of antibiotic resistance genes.

1.4.3 Reactive Oxygen Species (ROS) and EPS

Nano/micro-plastics are known to increase production of reaction oxygen species (ROS) (e.g., superoxide, hydrogen peroxide and hydroxyl radical) from electron accumulation in cells via reduction of the electron transfer, reactivity of photosynthesis reaction center, and efficiency of the oxygen-evolving complex [75], [76]. This excess ROS can then lead to cellular injury in the form of damaged DNA, lipids and proteins. Cells tend to produce a variety of antioxidant defensive enzymes e.g. superoxide dismutase (SOD), catalase (CAT) [77] and peroxidase (POD), to decrease the ROS levels and reduce the intracellular damage [78], [79]. It has been shown that in the presence of nano/micro-plastics [80] and nanoparticles in general [81], [82], the enzymes SOD, CAT, and POD activities in microbes could be induced and elevated. The other strategy is to produce EPS to generate a buffer zone between the living cell and the ambient environment to chemically quench hazardous ROS [83]. Many cyanobacteria [84], e.g., *Nostoc commune* [85], [86], *Microcoleus vaginatus* [87], [88], produce EPS under UV-induced oxidative conditions. Sun et al. [89] showed that ROS was produced by irradiation of seawater. In addition, there is a mutual interplay between ROS and intracellular Ca^{2+} production [90], that is closely related to the release of EPS. Enhanced intracellular free Ca^{2+} levels are known to lead to the activation of protein kinase C, which is involved in many intracellular signaling pathways. The Ca^{2+}-mediated secretion process controls the release of EPS from phytoplankton cells. Chin et al. [61] provided direct evidence for Ca^{2+} signaling related to exocytosis. Other studies have also demonstrated that unfavorable environmental conditions can trigger intracellular Ca^{2+} changes leading to EPS secretion [61], [91].

1.4.4. Experimental Evidence for Micro- and Nano-Plastics in EPS Aggregates

Ding, et al. [92] demonstrated for the first time that EPS microgel formation in seawater was greatly accelerated by small amounts of nano-plastics. Later, Ding, et al. [93], that phytoplankton EPS microgel formation is also accelerated by nano-plastics in seawater, but to a lesser extent. In both cases, it was demonstrated that hydrophobic interactions dominated, which cannot be affected by EDTA additions, in contrast to hydrophilic interactions. Chen, et al. [39] and Shiu, et al. [37], showed that phytoplankton EPS microgel formation is accelerated by nano-plastics in seawater likely due to a higher protein content of the EPS produced. Evidence for the importance of protein/carbohydrate ratios of EPS aggregates was reviewed by Santschi, et al. [64]. In Shiu, et al. [37], it was demonstrated that EPS microgel formation from dissolved organic matter was greatly accelerated by nano-plastics in different river and lake waters as well as seawater. Patches of algal cells with 1 μm polystyrene micro-particles encased in an EPS matrix have been observed [37], suggesting that plastics are incoporated into the phytoplankton EPS matrix when forming marine plastic snow aggregates. The ‘stickiness’ of EPS increased with the P/C ratio of EPS was also demonstrated with a magnetic tweezer technique [65].

1.5. How do buoyant MPS Aggregates Sink to the Bottom?

When buoyant MPS aggregates on encounter biologically produced or allochthonous mineral particles, they sorb to these mineral particles by electrostatic and/or hydrophobic interactions imparting greater density to the aggregate resulting in its removal from the water column. Mineral components for sufficient sedimentation of aggregates to occur are more abundant in near-shore than offshore areas [94]. These are also the areas of the ocean where plastic pollution is most pressing. However, existing data from field studies are mostly correlative rather than predictive based on mechanistic insights. Thus, studies of individual processes such as outlined here are needed.

2.0 Future Perspectives in Studying MPS—A Significant Vector for Removal of Micro-, and Nano-Plastics from Ocean Water.

During the formation of MPS, ROS are overproduced by microbial cells. This can trigger cellular Ca^{2+} increases leading to protein-rich EPS release from microbes and thus, ultimately resulting in stickier aggregates. The overproduction of ROS should be more evident for nano-plastics than micro-plastics due to their higher specific surface area. It will also be more evident in surface waters than in sediments, due to UV radiation at the surface ocean as another external trigger for ROS production. If this is the biophysical mechanism, then ROS will be produced in proportion to plastic particle surface area, under identical conditions, and the abundance and composition of EPS will change at different ROS levels [89], [95]. Secondly, the amount of antioxidant enzymes e.g. superoxide dismutase, catalase, and peroxidase, is expected to change in the presence of micro- and nano-plastics [78], [79], [96], [97]. In addition, it is expected that ROS can trigger cellular Ca^{2+} uptake leading to further EPS release [90], [98]. However, the actual relationship between intra-cellular (e.g., ROS) and extra-cellular factors that trigger EPS production needs to be further investigated.

The extent to which environmental factors determine the formation of MPS and its subsequent sedimentation flux, is not clear. In the marine environment, pH and redox conditions, salinity, Ca^{2+} concentrations, specific surface area of micro-/nano-plastics, enzyme activities, UV radiation, the ratio of EPS to micro- and nano-plastics and carbohydrate-to-protein ratio (Fig. 1), will be the key variables [45], [64], [99]. A combination of microfluidic, batch and roller table experiments could be useful in testing the influence of these variables to the production of EPS (quantities and qualities), the partitioning of micro-, and nano-plastics between water and aggregates, and the formation of MPS. In addition, active enzymatic and chemical/physical modifications of the plastic surface will continue for aged plastics in the ocean [94], [100]-[104]. Thus, how results from fresh plastic surfaces used in experimental systems compare to aged plastics in the natural environment might need further investigations.

3. Conclusions

Interactions between micro- and nano-plastics and natural organic matter such as EPS in aggregating to MPS lead to the plastic particles either being transported to the sea floor or entering food webs. Sources and composition of EPS and how they can influence the aggregation with micro/nano plastics are complex, and include various interactions between microbes, enzymes, and ROS that lead to EPS aggregation, enmeshing other particulate matter such as minerals, micro- and nano-plastics, and leading to the formation of MPS. These various aggregation phenomena occur in a dynamic system of microbial growth and decay, irradiated by sunlight that fuels not only photosynthesis, but also to ROS mediated chemical crosslinking of proteins, all concurring concurrently. While various factors are discussed in this review, the effects of various environmental factors that either trigger, enhance or change EPS production, e.g., pH and redox conditions, salinity, Ca^{2+} concentrations, specific surface area of micro-/nano-plastics, enzyme activities, UV radiation, the ratio of EPS to micro- and nano-plastics and carbohydrate-to-protein, require further studies.

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