Understanding the Nature of Microplastic Pollution and Identifying Environmental Impacts

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With the large-scale production of consumer plastics, comes the problem of how to deal with the disposal of long-lived single use items. Only a small percentage of plastics are recycled, leaving a significant volume accumulating in landfills or polluting our environment, where they fragment into smaller pieces, termed microplastics. Microplastics are ubiquitous and are present in every part of the environment and in the tissue of organisms, where they have physical and chemical toxic effects. To understand the extent of the problem, it is important to formulate standard methods for the collection, extraction, and identification of microplastics. In addition, laboratory-based research must be performed to understand the capacity of microplastics to effect human and environmental health. This review paper summarizes some of the key research direction in this field, in particular with respect to the research laboratories of Dr. Chelsea Rochman at the University of Toronto, one of the world's leading authorities on microplastic analysis and understanding of environmental and health impacts and risks.

Introduction

Starting in the mid-twentieth century, plastics began to replace other materials, since they were easy and inexpensive to manufacture, while still being strong and durable. With the advent of plastics, came the idea of "throwaway living": the idea that consumers could save time through single-use items including tableware and flatware, beverage and food containers, and diapers.[1] With the increase in manufacturing of disposable consumer products, the production of plastics has ballooned since 1950, with an estimated 8,300 million metric tons produced as of 2015. Of that amount, only 30% of manufactured plastics are still in use, while approximately 60% have been discarded and 10% have been incinerated.[2] The same characteristics that make plastics excellent materials for a wide variety of consumer uses, also make managing their disposal difficult. Lifetimes of plastics can range from tens of years to hundreds of years depending on the nature of the material.[3] Depending on the implementation of waste management standards, plastic waste may be landfilled, incinerated, recycled, or dumped into the environment. Evidence of plastic pollution has been well documented from the Great Pacific Garbage Patch[4] to "pristine" beaches littered with plastic trash.[5]

Plastic litter comprises a wide variety of materials and sizes, including microplastics, particles less than 5 mm in size.[6] Sources of microplastics can be both primary and secondary. Primary sources include pellets from plastic processing plants, microbeads from cosmetic and personal care products, and industrial abrasives. Secondary sources of microplastics arise from the fragmentation and degradation of larger plastics. Examples include microfibers released from textiles and tire wear particles (see Figure 1).[7] Microplastics encompass a wide variety of characteristics. Their morphologies include fibers, films, fragments, pellets, foams, and spheres.[8] Microplastics also incorporate a wide variety of polymers including polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and rubber. Microplastics are also not composed of polymers alone, but may also contain pigments or dyes and additives such as titanium dioxide and calcium carbonate. The transport of microplastics to the environment can occur through a variety of mechanisms, including through air and water, for example from laundering effluent and exhaust.[9] Sinks of microplastics in the environment include sediment, freshwater bodies (lakes, rivers), and saltwater bodies (surface water, arctic ice).[10] They can also be transported between organisms, for example from prey to predator, via trophic transfer.[11] Microplastics have been found in nearly every level of the food chain from invertebrates[12] all the way up to the largest mam-

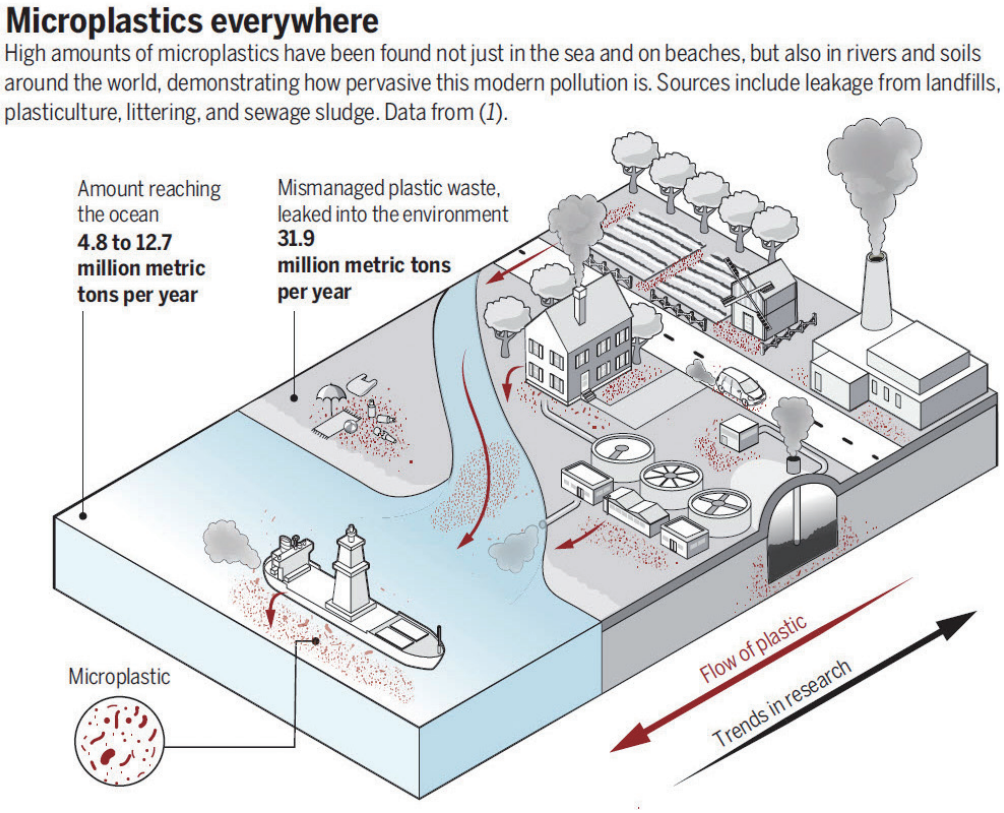

Figure 1 Reprinted with permission from Science Magazine (doi: 10.1126/science.aar7734).

mals on earth.[13]

There is ample evidence in the literature that microplastics are harmful. Toxicity can take on two forms; physical and chemical. Physical toxicity arises from the accumulation of microplastics in organisms and can have a variety of effects on health, including reduced respiratory function, hepatic stress, and the formation of granulomas through immune response.[14] As microplastics continue to break down into smaller and smaller fragments down to the nanoscale, translocation from the gut can occur leading to harmful effects in other tissues including the heart, lungs, gallbladder, and liver.[15] Chemical toxicity can arise from either additives in the plastics themselves or through the accumulation of toxins like persistent organic pollutants (POPs) or metals on the surface of microplastic particles.[14]

Critical to understanding the source of microplastics and potential toxicity is first understanding the presence and characteristics of microplastics in our environment. This includes elucidating the distribution of polymer types, presence of additives, particle morphology, and size distribution. In order to develop a clear picture, methods for collection, extraction, and identification of microplastics must be developed and standardized (or at least harmonized for data synthesis).

Collection Methods

The most common method for the collection of microplastics from marine environments is the neuston net or Manta Trawl. Using this technique, a large volume of surface water can be sampled by towing the net via a boat. Designed for collecting plankton, the net's mesh size is generally in the range of 333-335 μm, so the size of microplastics collected is restricted to those in the larger size range. However, microfibers, which are thought to be one of the most prevalent microplastic morphologies, can slip easily through a net, in addition to any other particulate with ellipsoidal shapes (thin fragments or folded/rolled films). Finally, the material of the net itself may contribute to contamination in collected samples.[16] Another method for collection from marine environments is the grab method. In this method, a 1 L (or larger volume) sample of water is collected, typically in a glass or metal sample container, to avoid contamination. Although the total volume is less than a net, a grab sample can collect plastics down to the sub-micron scale. In addition, because of the simplicity of collection, researchers of any skill level can easily collect samples, including citizen scientists. Samples may also be collected from a variety of environments including shallow tidal pools and wastewater outflow sites.[17]

Sediment samples require alternate collection methods - these include collection from coastal beaches to the deep

sea. For sediment samples collected from the seabed, specialized equipment is required. These can include grab samplers which scoop a sample from the top layer of the sea floor (Van Veen, Ekman) and core samplers, which collect columns of sediment, retaining information on the numbers of microplastics in sediment as a function of depth. As in the case of nets, contamination from plastic core samplers is also a concern. Metal is an alternate choice, however the opacity of metal precludes the ability of the researcher to actively monitor the volume of sediment collected.[18]

Other common matrices include biota, which consists of sampling animals from the environment to bring back to the lab for processing. In addition, air samples are also becoming more common and methods for collection continue to be developed to capture both wet and dry deposition.

Extraction Methods

During the collection of microplastic samples, particulate of other types may also be present including minerals, plant matter, biota, and other organic matter. There are a number of methods used to isolate microplastics of interest from other particulate including density separation and chemical digestion. Density separation is used to separate denser particulate (e.g., minerals, silica) from the more buoyant microplastics. In density separation, the mixed sample matrix is immersed in a prepared solution with high density. Those particles with lower density than the solution, including microplastics, float to the surface, while heavier particles sink to the bottom. The solution is allowed to rest for an extended period so that the denser particles have time to settle before carefully extracting the top portion of the sample containing 'floating' particles. This process may then be repeated, through the addition of fresh solution to the settled portion of the sample to ensure that all microplastics are collected. Different solutions with varying density can be prepared including sodium chloride, zinc chloride, calcium chloride, and sodium iodide.[16]

A unique extraction procedure taking advantage of the hydrophobic nature of microplastics was developed and reported by the Rochman lab at the University of Toronto. In this procedure, magnetic iron nanoparticles are functionalized with hydrophobic hydrocarbon tails. These hydrophobic groups preferentially bind to microplastics, which can then be extracted using a neodymium magnet by swirling the magnet in the sample jar and then rinsing it into a clean reservoir. Recovery of microplastics from spiked samples is demonstrated on a variety of size ranges from less than 20 µm to greater than 1 mm. It was concluded that magnetic extraction is beneficial as a secondary extraction technique after density separation or for samples that are relatively clean, as in drinking water. [19] Chemical digestion may be used to remove organic material while leaving microplastic particles behind. These methods include wet peroxide oxidation, alkaline digestion, and acidic digestion. When employing chemical digestion, it is important to ensure that the biological tissue and plant matter are removed without effecting the microplastics being collected. Acidic digestion has been shown to break down certain polymers, including nylon, polyamide, and rubber.[20] It has also been demonstrated in a publication by Munno *et al* that high temperatures generated during digestion (> 60°C) can result in the loss of some microplastics, particularly microbeads used in personal care products.[21] For biological samples, including collection of microplastics from gastrointestinal (GI) tracts, an alternative extraction procedure was developed by the California Department of Public Health, in collaboration with the Rochman lab, to avoid damage to the microplastics under study and to ensure that no biological tissue remains adhered to the surface of the extracted microplastics. In this procedure, the GI tract is dissected, isolated, and sealed in a vial. The vial is then immersed in a water tank and subjected to bursts of ultrasonic waves, termed pulsed ultrasonic extraction (PUE). The sample is then poured through a 1 mm stainless steel sieve and then filtered using a 10 µm core polycarbonate filter. Compared to samples prepared using traditional KOH digestion, samples extracted using PUE showed much cleaner surfaces and resulted in better spectral matches to reference databases.[22] For biota, biota can be extracted whole, or dissected to isolate the GI tract or target organs.[23]

Once an environmental sample has been collected and extracted, it may be sorted into various size fractions. Sieve stacks are used to separate particles into different size fractions down to approximately 300 µm.[24] For smaller particles, vacuum filtration with progressively smaller pore size membranes may be used. Large microplastics can easily clog filter membranes or obscure smaller particles if size fractioning is not employed. For particles greater than approximately 300 µm, samples may be manipulated manually using fine-tipped forceps, while smaller particles are more difficult to manipulate and can be analyzed directly from the filter membrane.[16] The use of size fractioning provides an additional benefit of collecting particles of similar size, which makes manual sorting easier.

Due to the varied nature of techniques and differing laboratory conditions, it is important to follow standard QA/QC techniques to account for any contamination that may be introduced throughout the collection and extraction

process. To limit the amount of contamination, it is best to minimize both the number of people handling samples and the amount of time the sample is exposed to air. General QA/QC lab practices include maintaining clean work surfaces, avoiding synthetic clothing, covering samples whenever possible, and installing air filters in the laboratory. In addition, proper QC/QA procedures include the generation of blank samples both in the field at collection and in the laboratory during extraction, which are treated with the exact same procedure used for measured samples. The results of particles found in the blank measurements may then be subtracted from the sample measurements or reported for each study.[8]

Detection and Identification Methods

Visual examination of extracted samples using a stereo zoom microscope is arguably the most prevalent technique for identifying microplastics. Using visual examination, a suspected microplastic can be characterized by color, and morphology. In addition, visual identification can be used to discriminate natural particles from anthropogenic particles. Different modes of imaging can help to improve contrast and aid in identification including reflected/transmitted light, polarized light microscopy, and dark field microscopy. Microscopy images of the particles can be taken and used to record measurements for exact particle dimensions with the implementation of software such as ImageJ.[25] The reliability of visual examination alone to definitively identify microplastics is low: depending on the researcher, false negatives and positives may occur with varying frequency.[24] The addition of fluorescent staining can improve identification using optical microscopy. The most common stain used in the identification of microplastics is Nile Red, which binds to plastics in both exposure experiments in the lab and in environmental samples through hydrophobic interactions. Nile Red fluoresces at a variety of wavelengths and is dependent on the hydrophobicity of the microplastic particle's surface. However, certain types of plastics including polycarbonate, polyurethane, PET, and PVC display weak signals, while microplastic fibers are particularly difficult to stain.[26] In response to these difficulties, alternate stains have been tested in the Rochman lab, including those designed specifically for textiles. For both laboratory tests and environmental samples, different dyes have been identified as promising stain alternatives (see Figure 2).[27-29]

For definitive chemical identification, there are a number of techniques that may be used including pyrolysis gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy, and scanning electron microscopy (SEM) plus energy dispersive x-ray spectroscopy (SEM/EDS). The application of these techniques has been described in detail elsewhere,[24] therefore a short overview of each technique's use in microplastics research will be given here. Pyrolysis GC-MS works by thermally breaking down the sample under measurement: the masses of the daughter fragments are analyzed in the resulting pyrogram to elucidate the parent molecule. GC-MS is considered a "gold standard" in analytical labs and is a readily available piece of analytical instrumentation. Pyrolysis GC-MS provides simultaneous identification and quantification of microplastics in complex samples. Because of

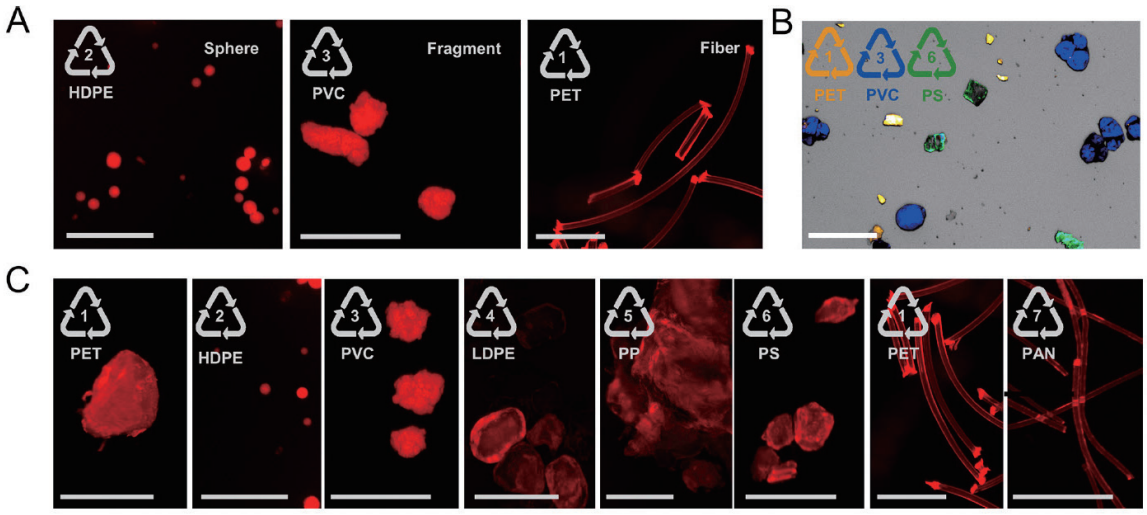

Figure 2 Examples of plastic types and morphologies dyed with different dyes. (A) Different morphologies: spheres (10-90 μm), fragments (50-300 μm), and fibers (30-60 μm/150-5000 μm) dyed with pink dye. (B) Different fluorophores: green (kentucky dye), red (pink dye), and far-red (blue dye). Pseudocolors are applied to different fluorescent channels for the purpose of differentiation. (C) Different polymer types dyed with pink dye: polyethylene terephthalate (PET) fragments (50-500 μm), high-density polyethylene (HDPE) spheres (10-90 μm), polyvinyl chloride (PVC) fragments (50-300 μm), low-density polyethylene (LDPE) fragments (100-500 μm), polypropylene (PP) fragments (500-4000 μm), polystyrene (PS) fragments (100-300 μm), polyester (PET) fiber (30-60 μm/150-5000 μm), and polyacrylonitrile (PAN) fiber (20-50 μm/300-3000 μm). Scale bars are 550 μm. Reprinted with permission from Environmental Science & Technology Letters (doi: 10.1021/acs.estlett.9b00241) Copyright (2019) American Chemical Society.

the quantitative nature of this technique (in mass, not number of particles), there is risk of matrix effects from remaining organic matter and materials or chemicals from extraction techniques, so extra care must be taken to accommodate for potential contaminants.[24]

Raman spectroscopy and FT-IR spectroscopy are both techniques that probe the vibrational bonds in a molecule. FT-IR uses broadband infrared light to illuminate a sample; when the light is resonant with a vibrational band in the molecule under study, a decrease in the intensity of the infrared light is observed. In Raman spectroscopy, a monochromatic laser source illuminates the sample; most of the light is elastically scattered (Rayleigh scattering), while a small portion of light is inelastically scattered to lower or higher frequency (Stokes and anti-Stokes scattering). The difference in energy between the inelastically scattered photon and the laser corresponds to a vibrational band in the molecule under study. These two vibrational spectroscopy techniques are complementary and provide different structural information on the particle under study. For example, Raman active vibrational modes can provide information on the backbone of a polymer, while infrared active vibrational modes provide information on side chains. In addition, Raman spectroscopy can provide information on additives and pigments or dyes in microplastics, which can help in tracking the source of microplastics. While micro-FT-IR spectroscopy can measure a minimum particle size of approximately 10 µm, Raman spectroscopy can measure particles down to < 1 µm in size.[24] For microplastics, this is critically important because it is generally agreed that, as particle sizes become smaller, the abundance of microplastics increases. Smaller particle sizes also have bigger ramifications when it comes to toxicology, which will be discussed in more detail below.[24,30,31]

SEM/EDS combines scanning electron microscopy and energy dispersive x-ray spectroscopy to provide high resolution imaging at the nanoscale with elemental characterization of heavier elements. SEM focuses an electron beam onto the sample under study and measures the resulting scattered electrons. EDS works in combination with SEM and measures the resulting x-ray radiation from the sample. SEM/EDS provides a means to quickly distinguish plastics from minerals, which in marine environments, are primarily Si (sand) and Ca (shell fragments).[22,24] Each technique described above has advantages, disadvantages, and varying associated costs and measurement times. In the characterization of microplastics, it is important to note that multiple techniques may be required for complete characterization.[24] It is also important to standardize methods across different laboratories to ensure consistency in reporting. This is one of the main goals of the microplastics study plan organized by the Southern California Coastal Water Research Project (SCCWRP) described in a separate article.

As a single microplastics sample can contain hundreds or thousands of particles, a critical part of the process of standardization is automation of sample measurements, in order to reduce the time required for sample analysis. One such method of automation relies on the use of the optical image of a sample, for example microplastic particulate on a filter membrane, to distinguish particles from the background substrate. The optical image provides the spatial contrast needed to identify the particulate, and then the use of a motorized stage together with Raman or FT-IR spectroscopy allows the user to collect spectra at each isolated particle. Using this technique, large areas can be covered without collecting spectra from areas that are not of interest, for example from the filter membrane itself.[32]

While automated routines like those described above are sufficient for analysis of larger microplastics across an entire filter membrane (for example a 47 mm diameter filter), for analysis of particulate in the lower size range, < 20 µm, measurements across an entire filter becomes prohibitively expensive in both time and data size. As part of standardizing the analysis of microplastics, it is important to formulate sampling and sub-sampling schemes that are representative of the sample under study. There are a number of different ways to define sub-sampling, for example, by percentage of filter area covered in a measurement, or by the percentage of total particles measured. In a paper published by Anger *et al*, it was proposed that sub-sampling by percentage of total particles is most appropriate for two reasons; one, particles may not be evenly distributed across the filter and two, different filter diameters may be used across different laboratories. The proposed working method was to first estimate the total number of particles on a filter using the optical image, and then chemically identify a chosen subset of particles.[33]

Once spectroscopic measurements are complete, it is necessary to determine the number of unique components present. Understanding the complete picture of chemical signatures can provide indication of the source of microplastics and potentially provide information on possible contamination as well (see Toxicology section).[8] Multivariate analysis techniques including principal component analysis (PCA), multivariate curve resolution (MCR), and cluster analysis techniques can be used to determine the number of unique spectral signatures in a sample set.[34] Once a model has been built, spectral matching algorithms may be used to identify the exact species present in a microplastics sample. The development of spectral

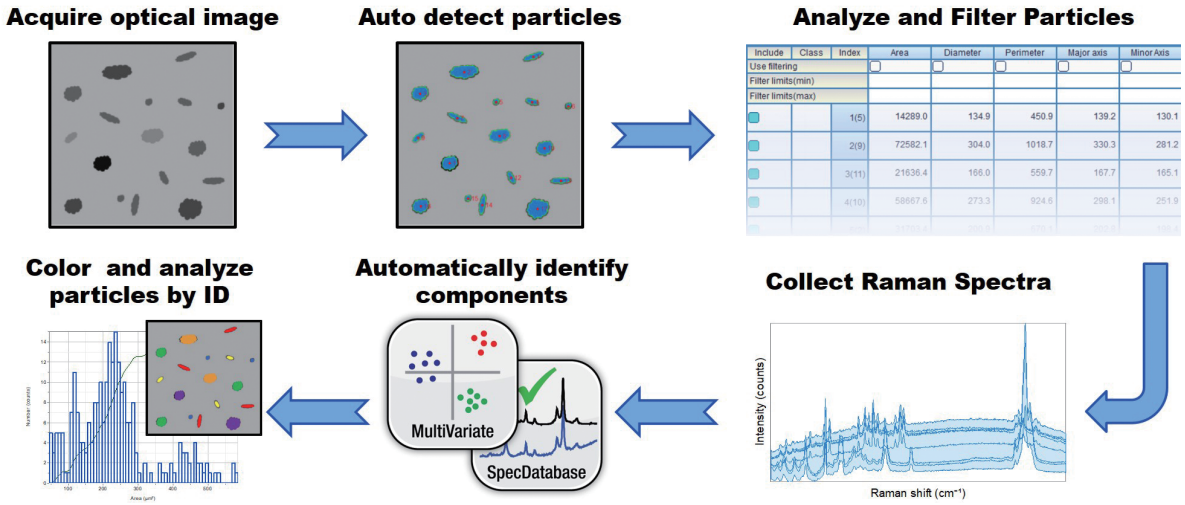

Figure 3 Example workflow for automation of microplastic measurements using Raman spectroscopy and HORIBA's ParticleFinder software module.

databases specific to microplastics can improve the quality of spectral matching and produce results that are more relevant to environmental samples, as microplastics encompass a diverse suite of polymers, additives, and dyes/pigments[8]. Existing libraries contain mostly pure polymers, which can only provide limited information. The development of SLoPP and SLoPP-E (Spectral Library of Plastic Particles, Environment) libraries by the Rochman Lab enable better spectral matching and provide much more information on collected samples, as the libraries include reference spectra from particles sourced from everyday products and from the environment.[35] Making these databases freely accessible to the microplastics community helps to ease the cost burden of commercial spectral databases, which frequently come with high annual subscription costs.

Toxicology

While it is important to understand the presence and nature of microplastics in our environment, it is also critically important to understand the impact of microplastics on our ecosystems and potentially on human health as well. There are a number of mechanisms through which microplastics may be harmful including physical and chemical pathways, as described above. Because microplastics can both sorb contaminants[36] and leach harmful additives,[37] bioaccumulation of these toxins may occur in marine organisms.[38] In addition, biomagnification, where toxins consumed by smaller organisms are concentrated in predators that consume them, can also occur, effecting the health of ecosystems across the entire food web. Examples of chemical contaminants in microplastics (sorbed contaminants, chemical ingredients, and chemical byproducts) are shown in Figure 4.[39]

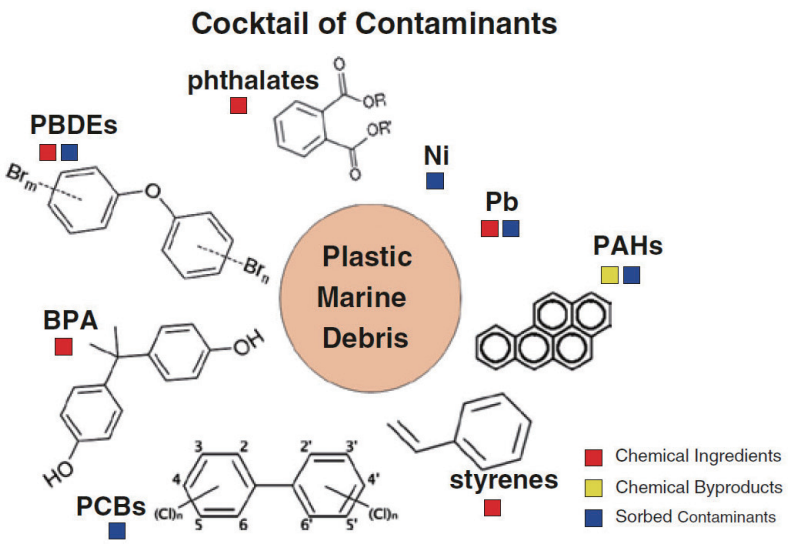

Figure 4 Cocktail of contaminants associated with marine plastic debris. Contaminants associated with marine debris include chemical ingredients (red squares), byproducts of manufacturing (yellow squares) and those that accumulate from surrounding ocean water in the marine environment (blue squares). Reprinted with permission from Marine Anthropogenic Litter (doi: 10.1007/978-3-319-16510-3).

In a laboratory-based study by Rochman *et al*, the effects of bioaccumulation were tested using Japanese medaka. In this study, three groups of fish were studied; a control group, a group fed virgin LDPE, and a group fed LDPE that had been deployed in an urban bay. After one and two months of exposure, the amount of polycyclic aromatic hydrocabons (PAH), polychlorinated biphenyls (PCB), and polybrominated diphenyls (PBDE) were tracked in the tissue of the fish. The results showed that not only does bioaccumulation of chemical pollutants occur, but signs of liver toxicity and pathology arise in the groups fed both virgin and deployed LDPE, namely glycogen depletion, fatty vacuolation, and single cell necrosis (in deployed LDPE only). This study demonstrated that (1) bioaccumulation of chemical pollutants through exposure to microplastics occurs in aquatic organisms and (2) exposure to microplastics and associated chemicals may induce hepatic stress.[40]

While evidence for harm from microplastics in marine life has been well documented,[12,39-44] less well understood is the effect that microplastics may have on humans. It is clear that microplastics are found in food consumed, including a variety of species of marine organisms,[45] salt,[46] and canned fish.[47] An example of a potential route of exposure through seafood is shown in Figure 5.[14] What happens once microplastics are consumed is not entirely clear. It is likely that many microplastic fragments are passed as waste, but this does not negate the possibility of physical and chemical toxicity in humans. A well-known example of the effect of chemical toxicity from plastic packaging is bisphenol-A (BPA), a constituent monomer of polycarbonate. It was demonstrated that higher levels of BPA present in urine samples were associated with reported heart disease in American adults.[48] Further research is needed to understand the amount of microplastics consumed, associated chemical exposure, and what health effects arise from such exposure.[14]

Mitigation

To address the global problem of microplastics, governments around the world have begun to enact policies to deal with the staggering problem of plastic pollution. This includes the ban of microbeads from personal care products, the tracking of municipal or commercial waste, and commitments to reduce marine debris.[14] From an industrial perspective, companies have started to implement sustainability practices including manufacturing products from plastics collected from beaches[49], moving towards biodegradable or compostable materials,[50] and transitioning to durable, multi-use packaging.[51] Beach clean-up programs organized by non-governmental organizations (NGO) serve two purposes; to raise awareness of the problem of plastic marine debris and to help remove larger plastics that have the potential to become smaller microplastics.[52] For consumers, there are also strategies that can be used daily to reduce microplastics. While reducing plastic use in general is a good first step (especially single-use plastic items), other sources of microplastics can be addressed in different ways. For example, microfibers from textiles are likely a large contributor to micro-

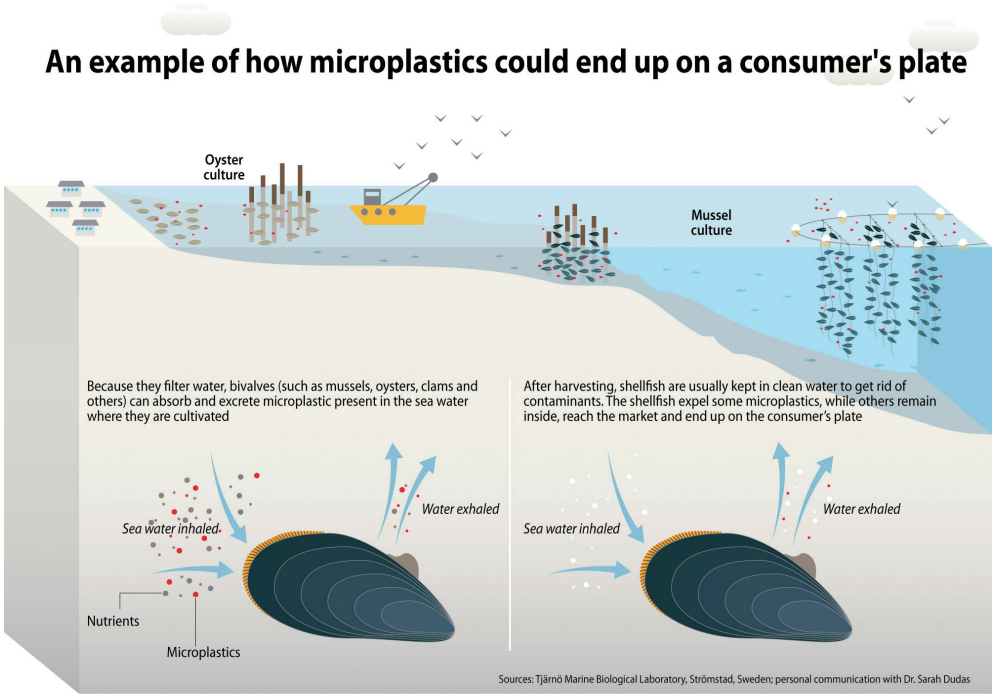

Figure 5 An example of how microplastics could end up on a consumer's plate. Reprinted with permission from Current Environmental Health Reports (doi: 10.1007/s40572-018-0206-z).

plastics emissions, where hundreds to thousands of microplastics are generated through the washing of a single garment. [53] Products like the Lint LUV-R filter and CORA ball can help to trap microplastic fibers before they reach wastewater treatment plants, and ultimately are discharged into bodies of water.[9]

Conclusion

Microplastic pollution is a global issue and one of the first steps in addressing the problem is to understand the nature of microplastics. This includes elucidating the major sources and sinks of microplastics in the environment, types of plastics and additives, and the particle morphologies and sizes. Optimization and standardization of laboratory methods for microplastic analysis is critical for reproducibility amongst labs including sample collection, extraction, detection, and identification methods. From this information, researchers can understand more about the sources of microplastics and how best to mitigate the threat to the environment and potentially to human health as well. The work of research laboratories like that of the Rochman group are critical to help drive standardized methodologies and a true understanding of the impact of microplastics and nanoplastics on our environment and health. Only through the development of harmonized reproducible methodologies will government agencies be able to provide the necessary recommendations to state and federal legislative bodies to put in place mandated monitoring and control programs.

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