MICROPLASTICS AS CARRIERS OF PAHs RELEASED FROM OIL SPILLS: MEASURING INGESTION AND BIOACCUMULATION RATES IN COPEPODS

DEPARTMENT OF ENVIRONMENTAL STUDIES

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ABSTRACT

Microplastics (MP; plastics < 5mm) are a growing problem in the marine environment due to a large influx of plastics entering the marine environment through rivers, wastewater and litter from land. MP are known to adsorb nonpolar compounds in the water including polycyclic aromatic hydrocarbons (PAHs) released from oil spills. In this study, I quantified the ingestion rates of a marine copepod (*Calanus finmarchicus)* on clean polystyrene MP beads (12 um) and phenanthrene loaded MP beads (0.8 pg PAH pellet $^{-1}$). I measured the phenanthrene load in copepods ingesting phenanthrene sorbed MPs and copepods exposed to phenanthrene only to measure the role of MP as a vector for PAHs into marine food webs. Additionally, I examined the fecal pellets to check the presence of MPs and if phenanthrene was still adsorbed to them. The results showed that microplastics do not act as vectors as there was no significant difference between the body load of phenanthrene in copepods from ingestion of PAH sorbed MPs and the body load from passive uptake of phenanthrene in solution. Copepods ate significantly lower phenanthrene loaded MP (1.81×10^4 beads cop^{-1} day^{-1}) than microplastics only (2.67×10^4 beads cop^{-1} day^{-1}) suggesting that copepods can reject particles based on chemical content. Accumulation of phenanthrene within the copepods (GC-MS analysis) showed individual copepods can accumulate between 3.48 to 4.11 ng of PAH which potentially can biomagnify in the marine food web upon predation. Fecal pellet analysis showed that an average of 2013 MP beads are present with a mean phenanthrene load of 0.15 pg bead^{-1}. For the social science research, I conducted a literature review examining the current Indian plastic waste management strategies, legislations and its implementation. My results find that the Indian government over the years has progressively enacted and amended its policies to try to keep pace with the issue of plastic pollution. However, key challenges in the implementation such as poor enforcement, inadequate training for recycling lack of plastic alternatives and proper waste disposal infrastructure all contribute to the unsuccessful management of waste. Usage of plastic in road construction has shown to be a viable option in reducing plastic waste in India and also improving road conditions.

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CHAPTER 1: INTRODUCTION

Oil spills

On April 20, 2010, the release of uncontained hydrocarbons from the *Deepwater Horizon* oil rig caused lethal explosions and fire killed 11 crew members. It surpassed the magnitude of the Exxon Valdez oil spill in 1989 and is considered one of the largest oil spills (Ramseur, 2010). A federal estimate of 4.1 million barrels of oil was spilled in the Gulf of Mexico and 2.1 million gallons of dispersants were applied to the surface (Allan et al., 2012). This was not an isolated event - as oil spills have become increasingly more frequent in the last half century. Oil spills have become a category of man-made disasters that adversely affect the marine and human's ecological safety (Mei and Yin 2009). Spilled oil is difficult to recover and can stretch for thousands of nautical miles, polluting sea surfaces and beaches. For example, the *Amoco Cadiz* spill lost about a quarter of million tons of light Arabian and Iranian crude oil; *Ixtoc I* spill surpassed the *Amoco Cadiz* with an estimate of 1.4×10^6 tons (Teal and Howarth 1984); the *Solar I* oil spill had around 1.9×10^2 tons of oil in the initial spill affecting coral reefs and mangrove ecosystems and displacing around 25,000 people from the area (Mei and Yin 2009). It is essential to understand the sources and main causes of marine oil spills.

Figure 1.1. Aerial picture of the Deepwater Horizon Oil spill in the Gulf of Mexico (Viglione, 2020. Credit: Daniel Beltra)

Oil leaks from transport vessels, pipelines and drilling are identified as important sources of marine oil spills. Crude oil via marine transport has increased significantly throughout the years with the development of the global economy. The shipping industry has faced various problems such as aging ships, bad technology, collisions and marine disasters which have increased the frequency of oil spills. In normal shipping of oil, the intentional release of waste oil and oil mixture into the sea might make the maximum oil emitted into the sea by ships (Si 2002). A key cause of oil spills is the disregard towards marine ecological damage by oil industries in order to gain economic benefits (Mei and Yin, 2009). Oil owners carry out drilling operations and expand oil exploitation without paying attention to the ecological consequences faced by the marine ecosystem. Aging pipelines, refineries and oil wells and a lack of advanced transportation systems by many ports adds to the growing causes of increased oil pollution in marine habitats. Marine ecological safety has not been recognized as the biggest concern to national security due to oil being highly regarded by the world as a valuable energy resource. Thus, there may always be a sacrifice of ecological health to gain economic security and development.

Coastal communities face extensive socio-economic, environmental and psychological impacts due to oil spills, including effects on livelihoods and public health (Sumaila et al., 2012). Oil spills can lead to a quality decline in aquatic products which changes the demand-supply relationship in fishery industries and markets. Oil spills occurring in the spawning season or areas can reduce the reproduction rate of important fish (Mei and Yin 2009). After the *Exxon Valdez* oil spill, fisheries for salmon, herring, crab and shrimp were closed with some commercial fisheries closing through 1990. Herring and salmon species in the area never recovered ecologically or economically (Sumaila et al., 2012). Shallow water regions such as coastal swamps, mangroves and wetlands are regions that house abundant marine life and are very sensitive to oil spills. The 1986 *Galeta* oil spill near the Panama Canal saw dead mangroves along 27km of the coastline (Jackson et al.,1989) and the deterioration of surviving mangroves up to 6 years after the spill (Burns et al., 1993).

Crude oil is a complex mixture of both hydrocarbons, such as alkanes, cycloalkanes and aromatic hydrocarbons, and non-hydrocarbon compounds. Polycyclic aromatic hydrocarbons (PAHs) are considered to be the most toxic components of crude oil (Almeda et al 2013). PAHs are made up of two or more condensed aromatic rings and are characterized as generally environmental harmful pollutants. From the *Deepwater Horizon* incident, the oil released around 2.1 x 10^{10} g of PAHs. Elevated levels of PAHs were reported in Gulf Shores, Alabama in 2011, a year after the spill (Allan et al., 2012). Several researchers found that the PAHs had toxic effects on wildlife, such as immunotoxicity, embryonic abnormalities, and cardiotoxicity (Honda and Suzuki 2020). A study on marine medaka reported that a high dose of a PAH benzo[a]pyrene (BaP) adversely affected the expression of developmentally related genes (D. Sun et al., 2020). In the liver of Chinese rare minnows, data indicated that BaP may induce cell death (Zha et al 2017).

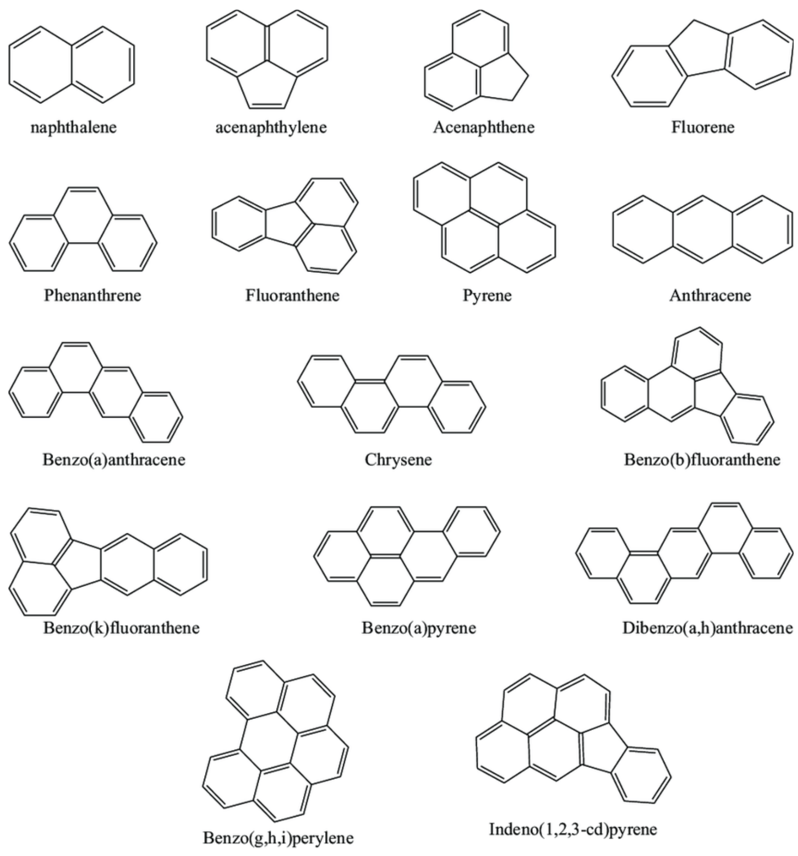


Figure 1.2. Structures of PAHs in crude oil (Hussain et al, 2018)

PAHs can enter the marine food web through ingestion of crude oil by planktonic species such as copepods. Copepods are small (0.1 - 10 mm) aquatic crustaceans. Copepods are one of the most abundant metazoan animals in the marine environment and drive the transfer of high energy lipids and fatty acids to higher trophic animals like commercially important fish species, birds and marine mammals throughout the year (Dahl et al., 2003). Copepods play an integral role in the marine biological pump (cycling of organic matter) by feeding in surface waters and producing sinking fecal pellets which are important sources of nutrients for deep water ecosystems (Turner 2015). Studies to date showing elevated copepod sensitivities to oil have ranged from simple *in vitro* LC50-type tests of straight crude and fuel oils, to more elaborate micro/mesocosm (e.g., Carman et al. 1995) and manipulative field studies (e.g., Fleeger and Chandler 1983). Other studies have reported that planktonic copepods tolerate low crude oil concentrations of <1ppm for days following an oil spill and can ingest these oil droplets (Spooner and Corkett 1979; Hebert and Poulet 1980; Almeda et al., 2014a) but can suffer sublethal effects such as reduced egg production and decreased fecal pellets production rates (Almeda et al., 2014). The negative impact ingestion of crude oil has on the copepod's reproductive success has immediate consequences on nauplii recruitment. Copepod nauplii (larva) are the main food of many fish larvae and their abundance determines the recruitment of commercially important fish species (Castonguay et al. 2008).

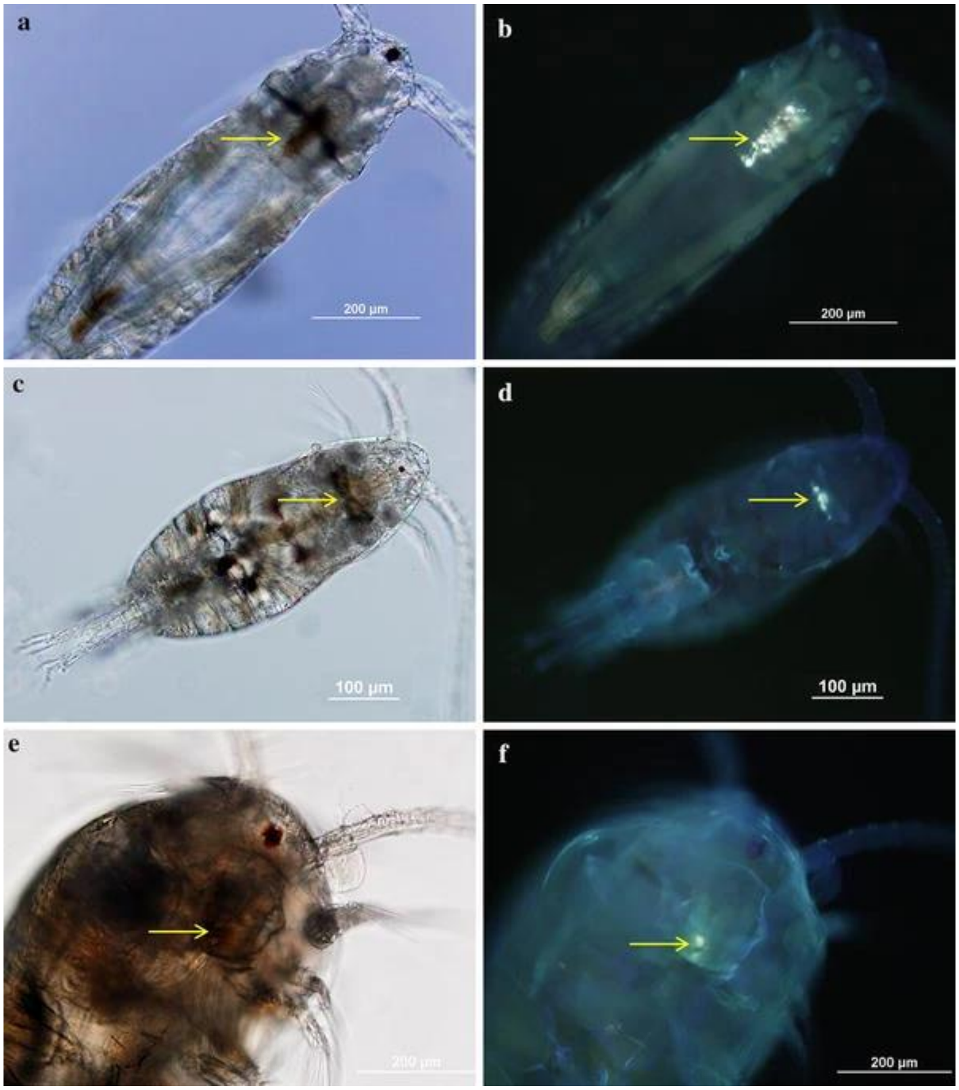


Figure 1.3. Microscopic images of copepods showing presence of crude oil droplets in digestive tracts (arrow indicates position). **a, b** *Acartia tonsa*, **c, d** *Parvocalanus crassirostris*, **e, f** *Temora turbinata* (Almeda et al 2014)

Little information on toxicities of PAHs to copepods is available in literature. Ott et al (1978) studied the toxicity of naphthalene to the calanoid copepod *Eurytemora affinis* and found significant effects on the life duration of the animal after chronic exposure. Narcotic effects were observed in copepod *Oithona davisae* at lower concentrations of PAHs (Barata et al 2005). *C. finmarchicus* in particular showed decrease in fecal pellet production and egg production with exposure to pyrene (PAH) (Hjorth and Nielsen 2011). Studies observed that highly soluble PAHs were excreted more rapidly whereas less soluble PAHs remain longer in the copepod bodies (Harris et al., 1977; Gyllenburg 1981; Cailleaud et al., 2009; Mitra et al., 2012). Less soluble PAHs are considered more harmful components of crude oil and can have highly mutagenic and carcinogenic effects to marine organisms that consume copepods in large amounts. (De Flora et al., 1991).

Marine plastic pollution

Ocean plastic contamination has become a global issue due to the increased reliance of plastic in our everyday lives. Scientists estimate about 8 million metric tons of plastic has entered the marine environment in 2010 (NOAA). There are many pathways of plastic entering the ocean. Land sources include almost 80% of marine plastics (Andrady, 2011). As 50% of the population lives within 50 miles of the coast, terrestrial plastic waste reaches the coastal region through many waterways (Moore 2008). Marine plastic causes complex problems, none of which is well understood: 1) Foul beaches worldwide, devaluing the experience of beachgoers which adversely impacts the tourism industry. 2) Entanglement of marine organisms which harms their physical health. 3) Ingestion of plastic fails to provide nutrition and can affect their feeding capacity. 4) Plastic items can act as sources and sinks to organic contaminants. 5) Degradation of plastic converts the plastic polymers into more fragmented units called microplastics and nanoplastics (Napper and Thompson 2020, Moore 2008).

Figure 1.4. Plastic debris floating on the surface of the ocean (Forbes 2019)

The marine environment has been showing increasing levels of microplastic concentrations. The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) defines microplastics as ‘plastic particles <5 mm in diameter, which include particles in the nano-size range (1 nm)’ (GESAMP, 2015; GESAMP, 2016). It is estimated that around 4.8 to 12.7 million tons of plastic enter the marine environment annually (Jambeck et al., 2015). Microbeads used in cosmetics and microfibers from clothes can enter the marine environment through waste effluents from sewage treatment plants (Thompson 2015; Napper and Thompson 2016). An important source of microplastics is the spillage of resin pellets that are the precursors to finished plastic products during transportation (Thompson 2015). Melting sea ice releases high levels of ice bound microplastics back into the environment (Obbard et al., 2014; Peeken et al., 2018). A larger abundance of microplastics has been detected near coastal areas and shelf-sea regions increasing the chances of copepods encountering microplastics in these regions. It is difficult to quantify microplastics in the marine environment as sampling methods depend on visual selection of items which introduces error including observer bias, misidentification or under-detection of particles that are too small. Another obstacle faced by researchers is that there is no widespread and comparable data that estimates the standing stock of plastic debris floating at the sea surface or sitting on the coastlines or seafloor (Law 2017).

Due to their size, microplastics are mistaken as natural prey by planktonic species and can be transferred up the marine food web. Microplastics obstruct feeding appendages and reduce food intake in zooplankton species (Cole et al., 2013). Copepod *Calanus helgolandicus* have been shown to select smaller-sized algal prey species when exposed to both microplastics and algae, indicating an alteration in feeding behavior. Microplastic exposed copepods had significantly smaller eggs and reduced hatching success than the copepods in the control group (Cole et al., 2015). Studies have shown ingestion of smaller microplastics (15um) by copepods *Calanus finmarchicus* more than larger microplastics (30 um) indicating smaller microplastics are more bioavailable (Vroom et al., 2017). Lower density polymers such as polyethylene and polystyrene are found to be at sea surface and are readily bioavailable to planktonic species (Wright et al., 2013). Lee et al (2013) found increased mortality rate in copepods chronically exposed to microplastics (average of 14 days).

Figure 1.5. Copepod *Centropages typicus* containing 7.3 μm polystyrene (PS) beads shown by fluorescence (Cole et al 2013)

Microplastics can adsorb organic compounds including PAHs which is when the PAH molecules stick to the surface of the plastic. Ingestion of organic contaminants that are adsorbed to microplastics may increase their bioavailability to zooplankton, leading to higher levels of organic pollutants within the animals (Almeda et al., 2013b). To evaluate its impact on the marine environment, there is a need to understand the predominant polymer type in the ocean and properties of microplastics that contaminants can sorb onto. Long term field experiments have researched the sorption of PAHs on commonly mass-produced plastics such as high-density polyethylene (HDPE), low- density polyethylene (LDPE) and polypropylene (PP) and found that they consistently sorbed greater concentrations. The results of this experiment have severe implications on the marine environment as HDPE, LDPE and PP account for 62% of all plastics globally and can consistently sorb greater concentrations of persistent organic contaminants which poses a health risk to the marine animals that ingest these plastic debris (Rochman et al., 2013). Contaminants such as polychlorinated biphenyls (PCBs) have been detected in plastic resin pellets collected from the coasts of Japan. A field adsorption experiment found a significant increase in PCBs concentrations in virgin polypropene virgin pellets indicating that the source of contaminants is seawater and adsorption to pellets acts as a mechanism of enrichment (Mato et al., 2001).

Purpose and Goals

The main purpose of this study is to track the fate of PAH sorbed microplastics in the marine environment. I investigated whether copepods *Calanus finmarchicus* ingest PAH sorbed microplastics and accumulate PAH or the microplastic and PAH get packaged into fecal pellets. This may have two implications: 1) Accumulation of PAHs in the gut may potentially transfer up the marine food web through ingestion of copepods by apex consumers or 2) Fecal pellets containing the PAH sorbed microplastic can sink down and be bioavailable to organisms residing in deeper waters (Fig 1.6). It is important to explore this question as (a), Microplastics and contaminants can induce a combined toxic effect in marine organisms and adversely impact their health. For example, the combined effect of cadmium and microplastics produced oxidative stress in juvenile discus fish (*Symphysodon aequifasciatus*), despite cadmium and microplastics alone showed no effects in survival and growth (Wen et al., 2018). (b) Microplastics could also increase the bioavailability of various contaminants. A study found that the medaka (*Oryzias latipes*) bioaccumulates PAHs and PCBs and exhibit hepatic stress and endocrine disruption responses after exposure to polyethylene pellets loaded with those pollutants in the environment (Rochman et al., 2013, 2014). (c) Copepods play a key role in the marine food web, serving as a link between primary producers and secondary consumers such as bigger fish, seabirds, seals and whales. The trophic transfer of MPs and contaminants could increase toxicity in the tissues of apex consumers and possibly affect humans as well. Studies have separately investigated the transfer of MPs and PAHs to animals such as jellyfish and ctenophores when fed to copepods (Almeda et al., 2013a; Costa et al 2020). (d) Copepod fecal pellets may also act as a vector to the vertical transfer of MPs and contaminants to deeper depths. Almeda et al 2014 showed that 90-100% of the copepod fecal pellets collected in their study contained large amounts of crude oil droplets.

The first objective for completing the natural science research included measuring ingestion rates of copepods exposed to clean microplastics and PAH sorbed MPs. Next, the PAH load in copepods in the ingestion experiment was compared to the body load of copepods exposed to PAHs only to identify if MPs increased the bioavailability in the animals. Finally, fecal pellets were collected to check the presence of PAHs sorbed MPs.

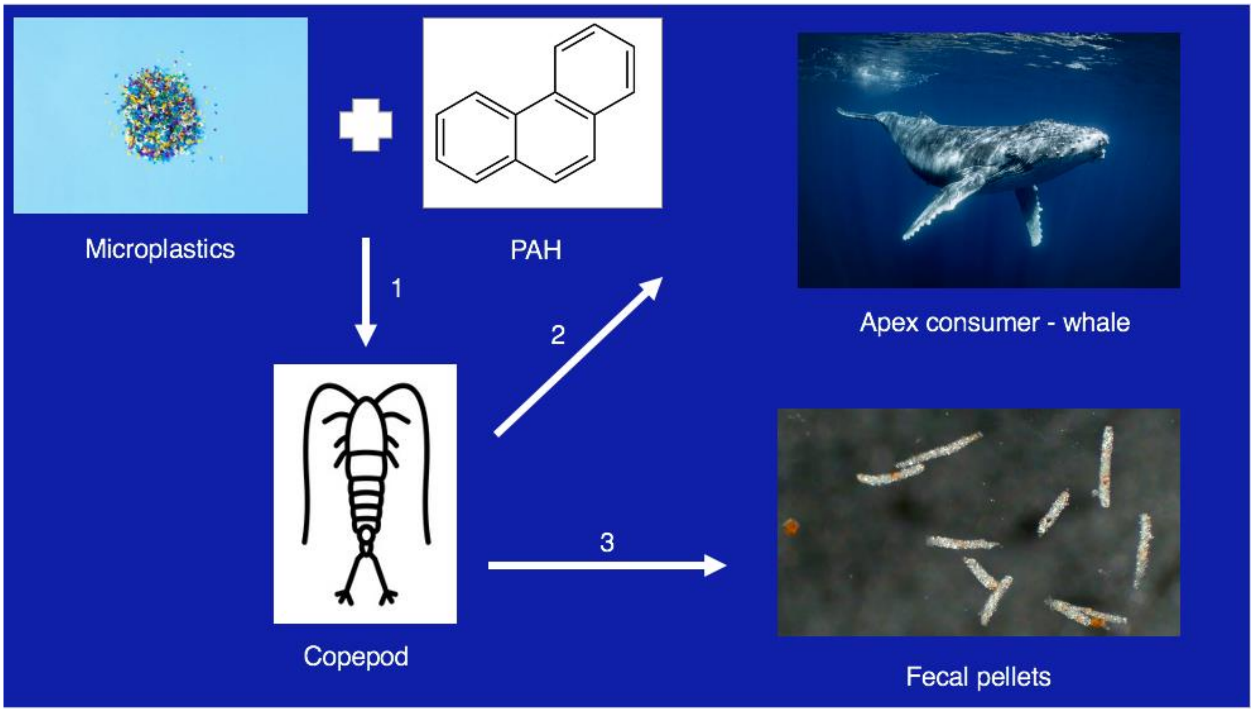


Figure 1.6. Pictorial diagram depicting the fate of PAH sorbed MPs once consumed by copepod: 1) transfer up the food web through predation of copepods, 2) packaged into fecal pellets and vertically exported to deeper waters

Indian Plastic Waste Management Legislations

Developing countries such as India contribute the most to marine plastic pollution. Currently, India is considered the 12th largest source of marine litter and is predicted to become the 5th largest by 2025. Ganga, an important river that provides water for 400 million people in India, has been documented as one of the top-five rivers dumping plastics into oceans. The country, as per 2017-2018 estimates, consumes 16.5 million tons of plastic annually (Financial Express 2020). A Central Pollution Control board report in 2019 puts the total annual waste generated at 3.3 million metric tons per year. Even though India boasts of a 60% recycling rate of plastics, there is a significant portion of plastic waste being dumped on the land and into the waterways. The seas near Mumbai, Kerala, and the Andaman and Nicobar Islands are amongst the worst polluted in the world (Bhattacharya et al 2018). Microplastic concentrations have been increasing in the coastal regions and rivers of India. A total of 3000 microplastic pellets were found and collected from six beaches along the Goa coast in 2015 (Veerasingam et al 2016). Average MP abundance of 72.03±19.16 microplastic particles/100g sediments were found in six beaches of the Puducherry coast in India (Dowarah and Devipriya 2019). Researchers have also reported that MPs found in India adsorb organic contaminants and concentrate them several orders of magnitude higher than the levels found in their surrounding environment (Veerasingam et al 2020). Plastic resin pellets in the beaches of Mumbai, Chennai and Sunderbans were assessed for the level of adsorbed organic pollutants (PCBs and DDTs). The PCB concentrations in the pellets from Chennai were higher than those in other tropical Asian countries (Ogata et al 2009). Similarly, MP pellets from Mumbai beach adsorbed various cyclodiene compounds in addition to PCB and DDT (Bentotage et al 2015).

India has taken clear measures to regulate plastic waste pollution and have formulated multiple legislations over the years. The Plastic Waste Management (PWM) rules in 2011 were issued by the Ministry of Environment and Forests and Climate Change (MoEF-CC) assigned the responsibility of plastic waste management to urban local bodies in India and set up a state-level monitoring committee (Bhattacharya et al 2018). The rules set a standard thickness for plastic carry bags and mandated retailers charge a fee to every bag made available. This rule was amended to the PWM rules of 2016 that stressed the phasing of multi-layered plastic packaging and implementation of extended producer responsibility (EPR) to producers, brand owners and importers in India. The rules were again revised to be known as the PWM rules 2018 which omitted the fee retailers had to pay if they made plastic bags available to customers. The latest 2021 PWM rules aim to phase out single use plastic products such as plates, cutlery, plastic decorations and more by 2022.

However, there are significant challenges to the implementation of these rules. The responsibility to enforce these legislations lies with feebly empowered agencies like the Central Pollution Control board and State Pollution Control boards. Monitoring the PWM rules are often the last thing on their checklist given the extensive tasks that the offices need to accomplish (The Wire 2021) A recent study across 8 state pollution control boards found staff shortage, high workloads, low motivation and accountability among the staff (NAAQS report 2020). There is no supportive infrastructure and incentive given by the government to produce alternatives to fill in the gap of plastic. The plastic industry is given a short time frame to phase out plastic products and manufacture plastic bags of the correct thickness which may result in the downsizing of manufacturing units and risk of unemployment. There is a lack of education of waste management practices including segregation, collection, and recycling which results in dumping of plastic waste in the environment (Times of India 2021). The objective of the social science research is to analyze the PWM rules in India and discuss the challenges in implementation through a literature review.

Preview

The second chapter covers the natural science section of this study focusing on if microplastics act as vectors to PAHs via ingestion by copepods. It begins with a brief introduction, methods used to carry out the research, results from data collection, stat analysis, ending with why the results are important and the implications they may have.

The third chapter is a literature review organized in a similar fashion like the second chapter but analyzes the plastic policies in India. It starts with an introduction, followed by methods explaining how the literature search was carried out, and lastly the results and discussion.

The final chapter is a synthesis of both questions that were explored in this study and how the two ideas connect with one another.

CHAPTER 2: NATURAL SCIENCE

Introduction

The rising demand for petroleum products has led to an increase in offshore oil drilling as well as in shipping using ocean waterways. Over the past 50 years, oil spills have become a major environmental concern. In a typical year, around 1.3 million gallons of oil are spilled from shipping vessels in U.S. coastal waters according to the U.S Department of Energy (Thompson 2010). Large oil spills can release an extraordinary quantity of oil and disrupt the local (100 sq. miles) or regional (1000 sq. miles) marine fauna for years after the original spill. When crude oil enters the water column, it partitions into hydrophobic (repellent to water) and hydrophilic (water-soluble) components. Although oil is often a complex suite of hydrocarbon compounds, the primary constituents of oil are polycyclic aromatic hydrocarbons (PAHs). The solubility of these compounds is generally very low and tends to decrease as the number of rings in the molecule increases (Borneff & Kunte 1983). Their chemical stability, low water solubility, and high sorption capacity contribute greatly to their persistence in the environment. PAHs are found to be acutely toxic and carcinogenic to marine organisms and humans. From the Deepwater Horizon oil spill in the Gulf of Mexico, the PAH concentrations in the water column increased up to 40 times above normal levels between May and June 2010 (Anderson 2017). Planktonic organisms can accumulate PAHs by two distinct routes: 1) The tissue of the organism is bathed in the crude oil (hydrophilic) and the PAHs are directly incorporated in nonpolar tissues or 2) indirectly through the ingestion of PAHs adsorb to particulate matter.

Particulate matter includes biological particles such as phytoplankton and anthropogenic particles such as microplastics. Microplastics (MP) are a growing ecological concern. The world's oceans are littered with large plastic debris. The discovery of staggering amounts of plastics collecting in the gyres of the North Pacific and Atlantic Oceans as well as along our coasts focused growing attention on determining the fate of these plastics as they break into smaller pieces. Microplastics (smaller than 0.5 mm) are likely the most numerically abundant plastic debris in the ocean today (GESAMP, 2016). Microplastics are known to accumulate in the coastal pelagic zones of the Atlantic Ocean. Indeed, seawater from the Gulf of Maine, among other regions, contained microplastics in 61% of the samples (Barrows et al., 2018). MPs are an excellent adsorbent of hydrophobic organic contaminants. Due to their non-polar surface and high adsorption coefficients, polyethylene and polystyrene microplastics are recognized to adsorb high amounts of HOCs present in the marine environment (Hüffer and Hofmann 2016). Concentrations of PAH ranging from 282.4 to 427.3 ng g^{-1} were found adsorbed onto PE and PS microplastics on the surface water of the Feilaixia Reservoir (Guangdong Province, China) (Tan et al., 2019).

In addition, microplastics are within the food size spectra of many planktonic species including copepods. These zooplankton play an integral role in the marine food web as they provide a link between primary producers and higher trophic level animals. Studies have shown copepods face adverse effects in regard to their fitness after ingestion of microplastics such as altered feeding capacity, reduced spawning, smaller eggs produced and increased mortality (Cole et al., 2015; Rodríguez-Torres et al., 2020). It is unclear whether ingestion of PAH sorbed microplastics by copepods occurs as well. The potential implications of copepod consuming PAHs sorbed to plastics is that they can become a sink for PAHs along with other harmful chemical contaminants present in the marine environment. Trophic transfer of contaminants and microplastics in the marine food web can lead to their increased concentrations found in tissues of fish and whales which might pose a health risk to humans who depend on a seafood diet. Alternatively, they may transfer PAH sorbed microplastics to the deep ocean via fecal pellets.

In this study, I investigated whether the copepod *Calanus finmarchicus* accumulates PAHs through ingestion of PAH sorbed microplastics. I addressed three objectives, to assess the: 1) ingestion rate of microplastics and PAH sorbed microplastics by copepods; 2) amount of PAH present in the copepods body and 3) presence of microplastics and/or PAH inside fecal pellets. I predicted that copepods would ingest microplastics and PAH contaminated microplastics. PAH would desorb from the MP and get absorbed into the gut of the copepod and the MP would be packaged into the fecal pellet.

Methods

Study system

I conducted my research at Bigelow Laboratory for Ocean Sciences from late August to November 2020 and late May to July 2021. The laboratory is situated in East Boothbay, Maine and oversees the Damariscotta estuary which opens up into the Gulf of Maine, one of the most biologically productive marine ecosystems. The Gulf of Maine is one of the world's most dynamic environments and includes deep basins and shallow banks. This semi-enclosed sea is characterized by powerful tides (3m+) which mix the influx of North Atlantic waters with fresh water which drain a large watershed spanning the states of Maine, New Hampshire and Massachusetts. The high nutrient input and large-scale mixing fuels one of the world's most diverse and productive ecosystems. Over 3,000 marine species and birds call the Gulf of Maine home. Coastal marshes and estuaries serve as nurseries and feeding grounds for fish, crabs, and shellfish, and abundant plankton provide the base of a rich food web all the way up to large fish, seals and whales. The Gulf of Maine provides a sanctuary for more than 30 species at risk, including the roseate tern and the critically endangered North Atlantic right whale.

In this study I chose the copepod *C. finmarchicus* as the model organism to investigate the fate of organic contaminants in the open ocean. In addition, phenanthrene was chosen as a model PAH and polystyrene (PS) microbeads (d = 12 um) as the microplastic (Fig 2.1).

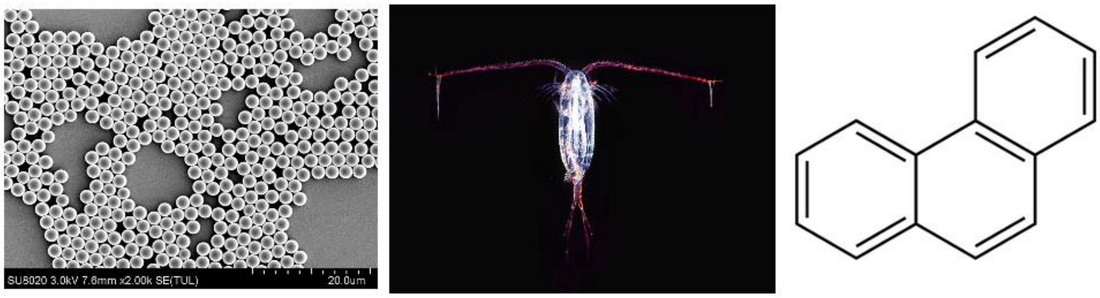
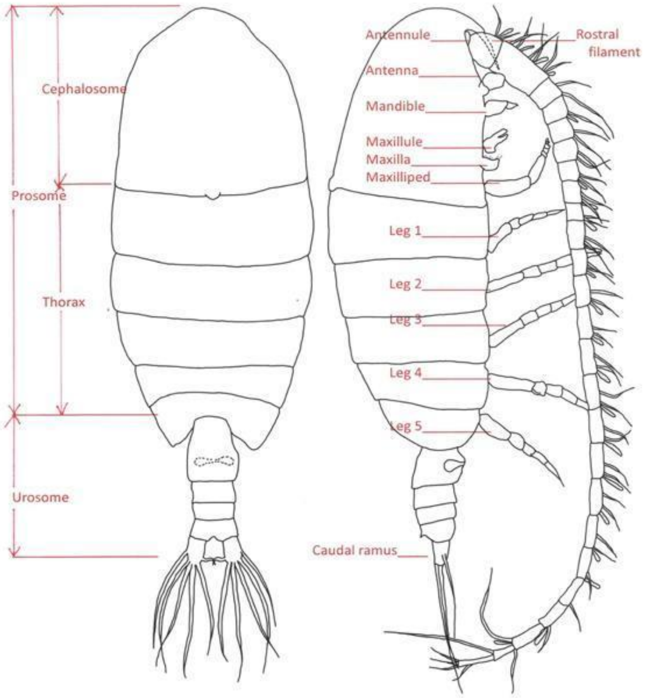


Figure 2.1. Research subjects - Polystyrene microbeads, *Calanus finmarchicus* and phenanthrene

Copepods are the most abundant mesozooplankton taxa in the marine environment. Copepods form a subclass belonging to class Hexanauplia in the subphylum Crustacea and are divided into 10 orders. The abundant, free living orders are Calanoida, Cyclopoida, Poecilostomatoida and Harpacticoida. The copepod body has two principal sections (Fig 2.2): the prosome (cephalic segments plus six thoracic segments) and the urosome (four to six tubular segments). A single median compound eye which is bright red in color can be found in the center of the copepod's head. Copepods in the order of Calanoid have a rounded head that is fused with the first few thoracic segments in the prosome. The thoracic appendage is modified to form maxillipeds which assist in feeding. These copepods have a rudimentary heart but no blood vessels and no gills; they absorb oxygen directly into their bodies (Miller 2004). Most Calanoida are particle feeders ingesting particles up to 100 um in diameter. Their cephalic appendages produce a water current to entrain and capture food. Water with the algal prey is drawn into the chamber between the maxillipeds and the water squeezed through the setae leaving behind the algae. This chamber can retain particles as small as 5 um diameter (Dürbaum and Künnemann 1997).

Calanus finmarchicus is a copepod species belonging to the order of Calanoida. *Calanus* is a large copepod, typically 2 - 3 millimeters long, and is the biomass dominant mesozooplankton in the Gulf of Maine. *Calanus* lives anywhere from the ocean surface down to about 4,000 meters deep and in a wide temperature range from −2 °C to 22 °C. The copepod primarily feeds on different forms of phytoplankton including diatoms, dinoflagellates and ciliates. It is predominantly found in the North Atlantic Ocean. In the Gulf of Maine *Calanus finmarchicus* is a major food source for the endangered right whale, lobster larvae, herring, cod and mackerel populations.



Text-fig. 1. Dorsal, lateral and ventral views of *Calanus sinicus*

Figure 2.2. Body structure of Calanoida copepods (Chang-Tai et al 2020)

Polystyrene (PS) is a transparent aromatic polymer formed as a result of polymerization of styrene monomers (Kik et al 2020) and has a specific gravity of 1.05 g cm^{-3} (Andrady 2011). The global production of PS was 15.5 million metric tons in 2018 (Agboola and Benson 2021). PS is used in the production of CDs, toys, utensils etc. It is an abundant plastic polymer found in the marine environment and can exist as large pieces or smaller fragments such as microplastics and nanoplastics (Erni-Cassola 2019). PS microplastic is a good sorbent of hydrophobic organic contaminants due to its polymer properties. It has a nonpolar surface and is hydrophobic which increases the sorption of organic contaminants to the microplastic (Agboola and Benson 2021). PS has an amorphous structure which means the carbon chains in the polymer are more disordered. This promotes more movement of organic molecules around the structure of the polymer, hence influencing greater sorption (Hartmann et al 2017). Polystyrene can act as a source and sink for PAHs in the marine environment (Rochman et al 2013).

Phenanthrene is a PAH with formula $C_{14}H_{10}$ and consists of three fused benzene rings. It is a colorless crystal like solid. Phenanthrene is used to make dyes, pesticides and explosives. Research has shown that exposure to phenanthrene has been related to behavioral effects in marine organisms, such as slow respiration in amphipods (Gauthier et al 2016), reduced reproduction and even mortality in copepods (Lotufo et al 1997, Louati et al 2014). Due to its low molecular weight and high hydrophobicity, phenanthrene can be adsorbed by microplastics (Chen et al 2018). A study calculated the partition coefficient of phenanthrene between seawater and polystyrene which was 5.39. This value means that phenanthrene is more likely to adsorb to PS due to hydrophobic interactions than remain in seawater (Lee et al 2014).

Material preparation

I collected copepods 5 km offshore (at station 4; Fig 2.3) of the Damariscotta Estuary from the research vessel IRA-C from May to July 2021. I collected the zooplankton using a 0.75 m ring net (300 um mesh) using oblique tows from 100 m depth to the surface at a speed of 2 knots. In the laboratory, I sorted and transferred *C. finmarchicus* specimens to a 20 L bucket containing 0.2 um filtered seawater. Copepods were maintained at 12°C on an algal diet of *Rhodomonas salina* (CCMP768) and *Thalassiosira weissflogii* (CCMP1336).

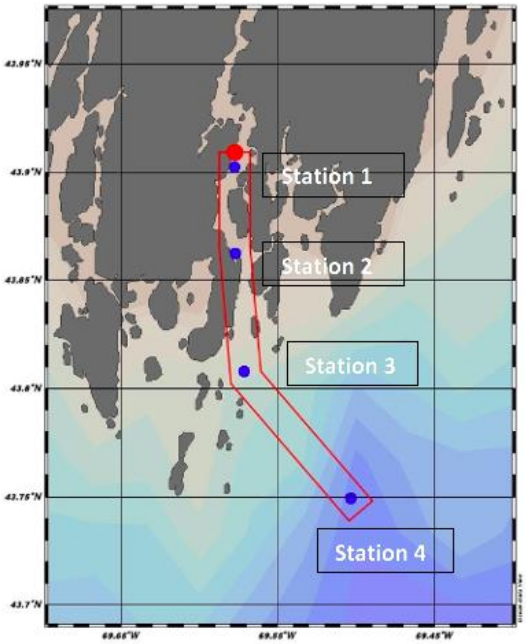


Fig 2.3. Map view of stations in the Damariscotta Estuary

Corpuscular (Corpuscular Inc, NY USA) supplied PS microbeads (C-PS-12.0 - 12 um). To prepare a bead stock solution of 10^6 beads mL^{-1} concentration, I transferred autoclaved ultra-filtered seawater (0.2 um filter) to a 40 mL bottle. I then pipetted 10 mL of the polystyrene microbead solution into the bottle and measured the bead concentration using a miniaturized coulter counter called ORFLO- Moxi (ORFLO Technologies, ID USA). I pipetted 5 mL of the stock solution into a 500 mL jar to prepare a concentration of 10,000 beads mL^{-1} to feed the copepods.

I prepared a 10 g mL^{-1} phenanthrene stock solution in dimethyl sulfoxide (DMSO) and then made a 1:100 dilution. I assembled serial dilutions of 5, 10, 50 and 100 ug L^{-1} of phenanthrene with filtered seawater. I analyzed the standard concentrations in the fluorometer. The fluorometer plot showed three distinct peaks with a large discernable peak at 345 nm. Using emission values at 345 nm, I plotted a calibration curve against the sample phenanthrene concentrations (Figures in Appendix A).

Experimental design

Phenanthrene load on beads:

I used 2 independent methods to measure the amount of phenanthrene on the PS bead. The first method measured the disappearance of phenanthrene from the background solution. This had the inherent flaw due to losses of the phenanthrene to sorption on the glassware. The second method measured the load on the bead using a GC-MS. Using this method, I was concerned that we could not fully extract the phenanthrene from the PS bead.

Method 1: I conducted a time series experiment to find the amount of phenanthrene that adsorbs to microplastics. I pipetted a concentration of 10^4 PS beads mL^{-1} in a 25 mL jar consisting of 50 ug L^{-1} phenanthrene solution. I filtered the beads out at six timepoints – 30 mins, 1 hour, 4 hours, 16 hours and 23 hours and analyzed the background phenanthrene concentration in the fluorometer. I calculated the concentration of phenanthrene at each timepoint using the equation of line from the phenanthrene calibration curve. The control for the experiment consisted of 50 ug L^{-1} phenanthrene solution in a 25 mL jar.

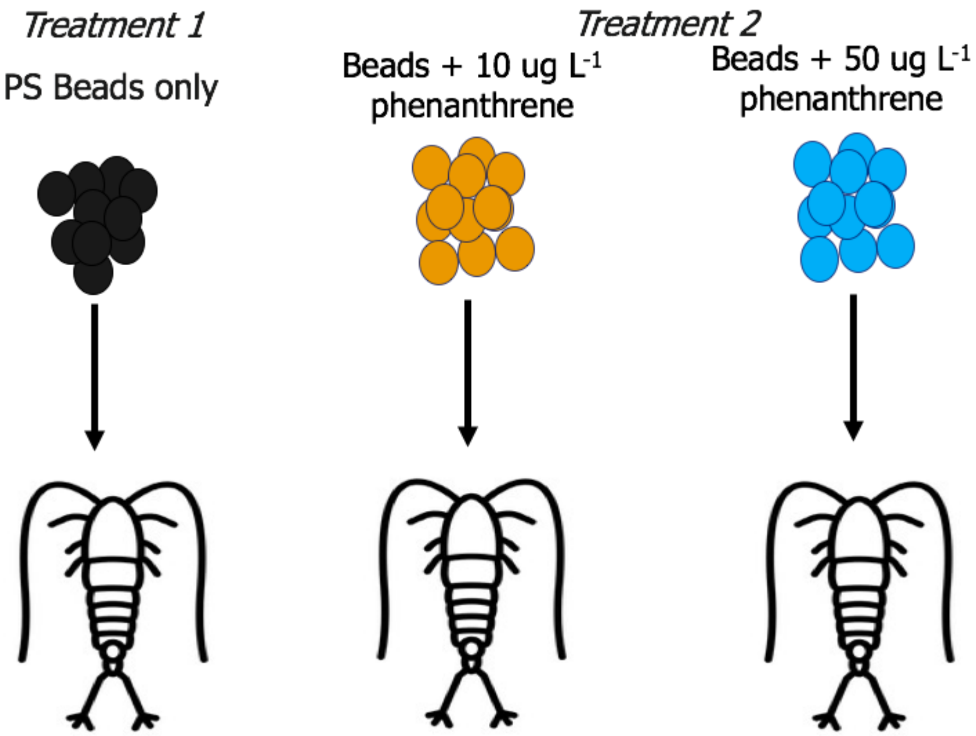
Method 2: I also tested the phenanthrene load on the beads using a GC-MS. I prepared a 100 mL bottle consisting of 20,000 beads mL^{-1} and 0.2 um FSW and added 50 ug L^{-1} of phenanthrene. I filtered 10 mL (x3) of the solution using 0.5 um Whatman glass fiber filters after 24 and 48 hours. I placed the filters in 10 mL of hexane for 24 hours for the phenanthrene to partition from the beads to the hexane. I again filtered the hexane to remove the beads using a 20 mL syringe with a luer lock attached to it. Additionally, I collected the aqueous solution (without beads), added 4.7 mg L^{-1} o-terphenyl standard (oTP) and conducted liquid-liquid extractions with 5 mL of dichloromethane. With nitrogen gas, I blew the aqueous and hexane sample to 1 mL. I measured the concentration of phenanthrene in the aqueous solution and the filtered hexane on the Agilent 7890B gas chromatography mass selective detector (GC-MS).

Ingestion experiment:

I performed ingestion experiments with two treatments - 1) PS microbeads only and 2) phenanthrene adsorbed to PS microbeads. Experimental design for each ingestion treatment consisted of five 25 mL jars each containing five adult copepods. The animals were allowed to feed and excrete for 23 hours. I stored the 25 mL jars in two 200 mL vials and tightly sealed them with a cap and mounted them on an algal wheel in the incubator to keep the beads in suspension (12°C). For the second treatment, 50 ug L^{-1} of phenanthrene was added to 10,000 beads mL^{-1} of PS microbeads in a 500 mL bottle and was kept for 23 hours to allow for adsorption onto the beads.

a) Pilot experiment:

I conducted a pilot ingestion experiment in 2020. I fed the copepods an average polystyrene bead concentration of 5.8×10^3 beads mL^{-1} for both treatments. The beads sorbed phenanthrene from two concentrations: 10 ug L^{-1} and 50 ug L^{-1} for the second treatment. The control treatment consisted of PS beads and phenanthrene sorbed beads with no animals. I used a coulter counter called ORFLO-Moxi to calculate bead concentration before and after the ingestion experiment.

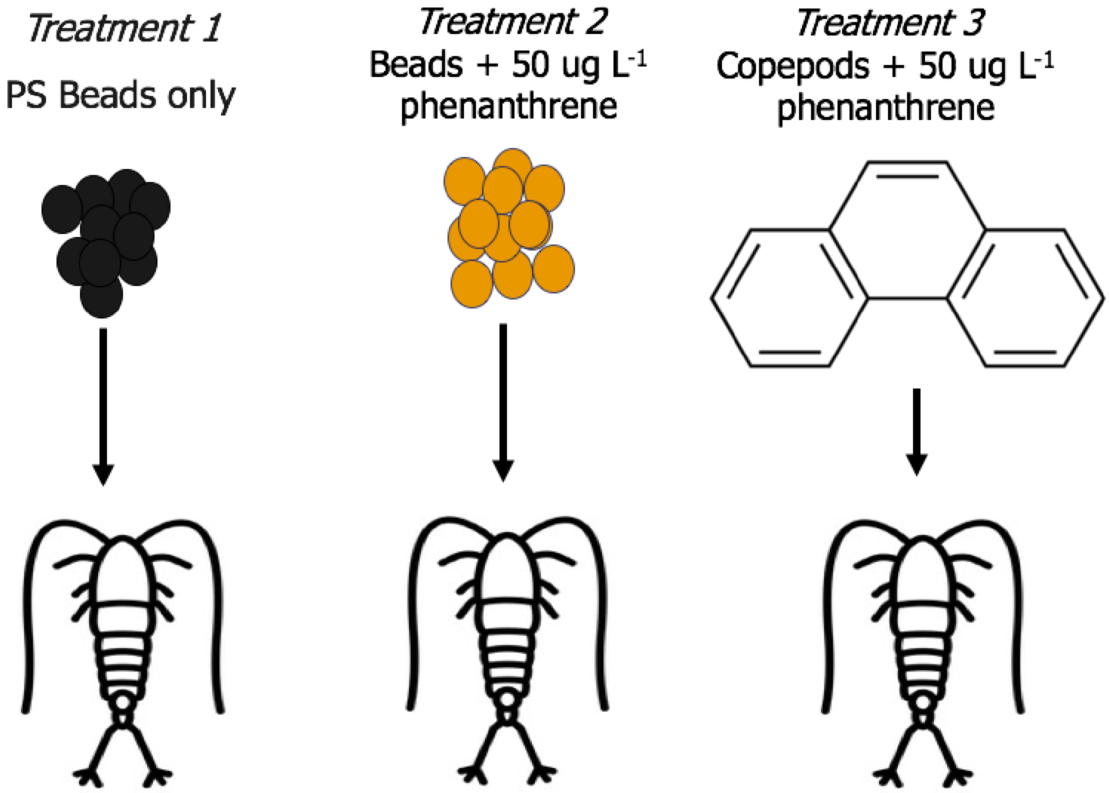


b) Secondary experiment:

I replicated the ingestion experiment in 2021 with a few differences. I fed the copepods a higher average concentration of PS beads at 9.0 x 10^3 beads mL^{-1}. PS beads sorbed phenanthrene from a concentration of 50 ug L^{-1} for the second treatment. ORFLO-Moxi was used to calculate bead concentration before and after the ingestion experiment.

I conducted another experiment of just copepods swimming in filtered seawater and phenanthrene. From the 500 mL jar consisting of 10^4 beads mL^{-1} and 50 ug L^{-1} phenanthrene, I filtered out 25 mL (x5) using a 0.5 um glass fiber filter to remove the beads.

The aqueous solution that only contained phenanthrene was transferred to five 25 mL jars. I placed 5 copepods in each jar and let them swim around for 23 hours.



Phenanthrene load in copepods:

I selected live copepods that were fed phenanthrene sorbed PS beads carefully from each experimental jar and placed them into 1 mL tubes and stored them in the freezer at -80^{o}C. I added five frozen copepods from each replicate jar to 2 mL vials consisting of Si/Zr beads, 750 uL of hexane and Na/Ethanol solution and 5 uL of o-terphenyl. The animals were decimated by bead-beading and sonication, each process taking about 5 minutes. I pipetted the top hexane layer containing the phenanthrene into a clean 2 mL vial and desiccated using anhydrous NaOH. I repeated the hexane extraction twice. Using nitrogen gas, I concentrated the hexane to 1 mL, then pipetted 20 uL using a glass syringe to a GC vial and added 5 uL of internal standard (1:250 PAH) for analysis using the GC-MS.

I conducted a sensitivity test first to quantify the minimum number of copepods needed for the GC-MS to detect phenanthrene. I selected copepods that ingested phenanthrene loaded beads in 2021 for the test. I was able to pick 1 and 2 copepods from one jar each and 3,4 and 5 copepods from three jars each. I used the procedure written above to measure the phenanthrene in the copepods.

Fecal pellet collection and phenanthrene load:

Using a microscope, I identified an average of 10 fecal pellets from four jars of treatment 2 which were copepods ingesting phenanthrene adsorbed PS beads. I calculated the length and width of each pellet using ImageJ. I used two methods to determine the number of PS beads inside each pellet separately from the extraction process. I first used a sonication machine to break seven fecal pellets open, releasing the beads trapped inside. I then used the Moxi to measure the bead concentration. Additionally, I broke 13 pellets using a tweezer and manually counted the number of beads under a microscope. I calculated the volume of each pellet and plotted a curve against the number of beads. Using the equation of the curve I calculated the number of beads in each fecal pellet collected from the jars.

To determine the phenanthrene load on each pellet I conducted fecal pellet analysis similar to the copepod extraction. Fecal pellets from 4 replicate jars were collected and combined to ensure that the concentration reached levels that were detectable by the GC-MS. I sonicated the pellets to release the beads and transferred the entire contents to a 2 mL vial containing 900 uL hexane and no oTP. Using nitrogen gas, I reduced the hexane volume to 20 ul pipetted into a GC vial for GC-MS analysis.

Statistical analysis

I used a one-way ANOVA to measure statistical differences in average ingestion rates of copepods fed – microplastics only, 10 ug L^{-1} phenanthrene sorbed MPs and 50 ug L^{-1} phenanthrene sorbed microplastics (pilot experiment 2020). I used student T-test analysis (two-sample assuming equal variances) in Excel to measure difference in ingestion rates between copepods fed microplastics only and 50 ug L^{-1} phenanthrene sorbed microplastics (secondary experiment 2021).

I used the Frost equation (1972) to calculate average ingestion rates of copepods. First, I calculated the constant (k) for prey concentration, in this case PS beads, which was done by the control treatment. The control had only PS beads and phenanthrene adsorbed PS beads with no animals and was measured using the ORFLO-Moxi before (T1) and after 23 hours (T2). The formula: $C_2 = C_1 * e^{k\,(T2 - T1)}$ was used where: C_1 is initial PS bead concentration at T_1 and C_2 is final bead concentration at T_2.

For the pilot and secondary experiments, bead concentration ingested by the copepods were calculated by the formula: $C_2 = C_1 * e^{(k - g)(T2-T1)}$ where: C_1 is initial PS bead concentration at T_1 before the copepods were added and C_2 is final bead concentration after the copepods ate for 23 hours at T_2. Thus, the average concentration of PS beads is calculated by:

$$\boxed{[C] = \frac{C_1 * [e^{(k - g)(T2 - T1)} -1]}{(T_2 - T_1)(k - g)}}$$

Calanus obtains its food by means of filtering maxillae but the true filtering rate (volume of water passing through the maxillary filter per unit time) cannot be directly measured. Therefore, we measure the volume swept clear which is defined as the volume of ambient medium from which cells are completely removed by copepods to achieve the measured ingestion rate. It is calculated by - $F = V/N$; V is volume of beaker and N is number of copepods (Harvey 1937). The average ingestion rate was then calculated by = $[C] * F$

Results

Experimental design

Phenanthrene load on beads*:*

The objective of the time series experiment was to calculate the amount of phenanthrene that adsorbs to polystyrene beads by analyzing background phenanthrene concentration at 6 checkpoints. In a span of 4 hours, phenanthrene dropped to around 0.22 ug L^{-1}, a 92% decrease from the control phenanthrene concentration of 28.9 ug L^{-1} resulting in a loss of 0.065 ug. This suggests that most of the phenanthrene gets adsorbed to the PS beads (Fig 2.6). Adsorbed phenanthrene was calculated by dividing 0.065 ug with the 10^4 number of beads present in the 25 mL jar which was 2.6 pg $bead^{-1}$.

The GC-MS data showed that beads need 48 hours or more for the phenanthrene to adsorb to the beads. 24 hours – beads had 0.6 pg of phenanthrene while 48 hours beads had 0.8 pg of phenanthrene on them. Future work should allow a longer period of time for the PS beads to equilibrate.

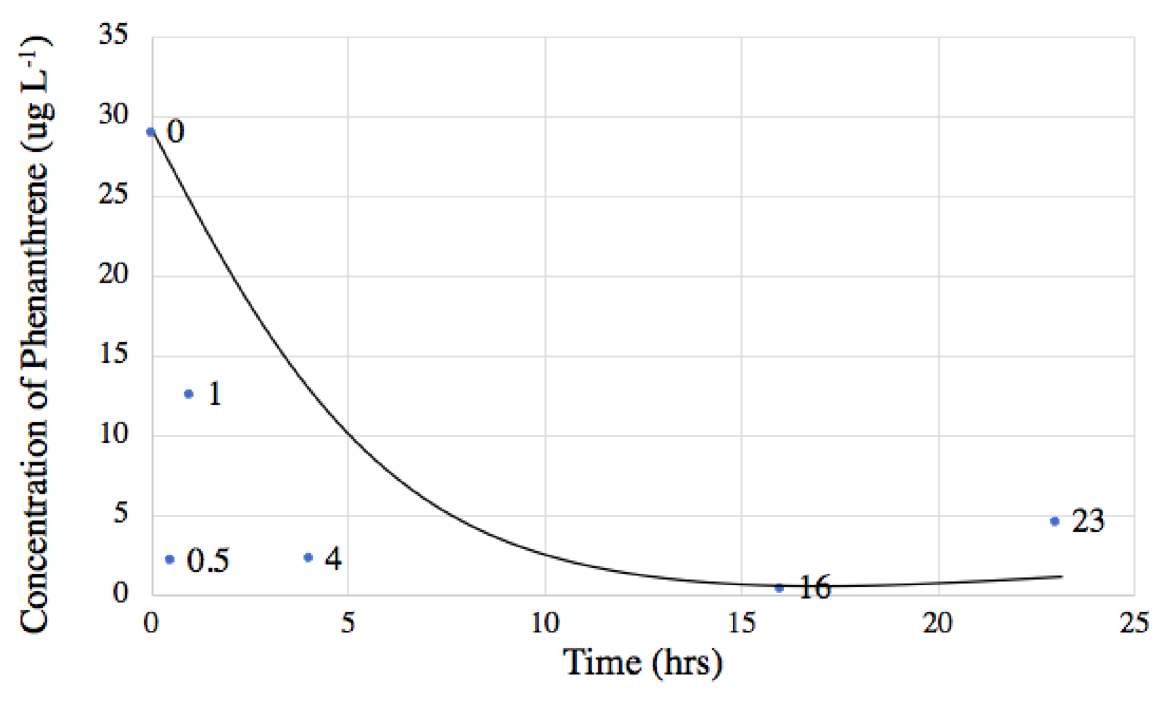


Fig. 2.6. Concentration of background phenanthrene solution (ug L^{-1}) decreasing with time. The control (0 hours) had an initial concentration of 28.9 ug L^{-1} which dropped to 2.2 ug L^{-1} in 4 hours and 0.4 ug L^{-1} in 16 hours.

Ingestion experiment:

a) Pilot experiment:

In the pilot ingestion experiment, we found no significant difference between ingestion rates of *C. finmarchicus* in the experimental treatments (Fig 2.8; one-way ANOVA $df_{total} = 17$, $f_{stat} = 2.48$, $p = 0.12$). Copepods in the PS beads only treatment (n=8) consumed on an average of $3.63 \times 10^4 \pm 1.42 \times 10^3$ beads copepod^{-1} day^{-1}. In the second treatment, copepods consumed around 9.28 ± 2.76 x 10^3 beads copepod^{-1} day^{-1} (n=5; 10 ug L^{-1} phenanthrene) and 4.50 ± 3.83 x 10^3 beads copepod^{-1} day^{-1} (n=5; 50 ug L^{-1} phenanthrene).

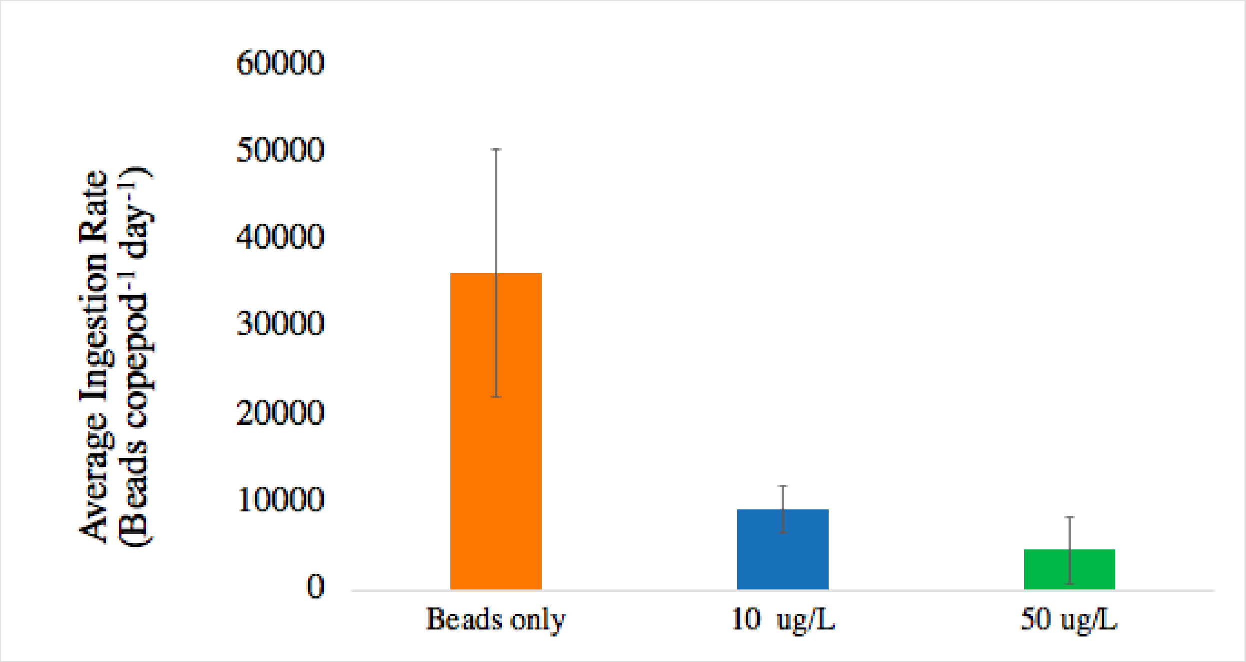


Fig. 2.7. Ingestion rates of copepods (beads copepod^{-1} day^{-1}) fed on – 1) PS beads only, 2) PS beads in 10 ug L^{-1} phenanthrene solution and 3) PS beads in 50 ug L^{-1} phenanthrene solution. Values are averages (± SE) of 5 replicate experimental jars.

b) Secondary experiment:

In the second trial of the ingestion experiment, copepods ate significantly lower phenanthrene adsorbed PS beads (50 ug L^{-1} phenanthrene) than the PS beads (Fig 2.8; t test df =10, t =2.42, p =0.017). Copepods ate an average of 2.67 x 10^4 ± 3.67 x 10^3 PS beads copepod^{-1} day^{-1} (n =5) and 1.81 x 10^4 ± 1.88 x 10^3 phenanthrene adsorbed PS beads copepod^{-1} day^{-1} (n =8).

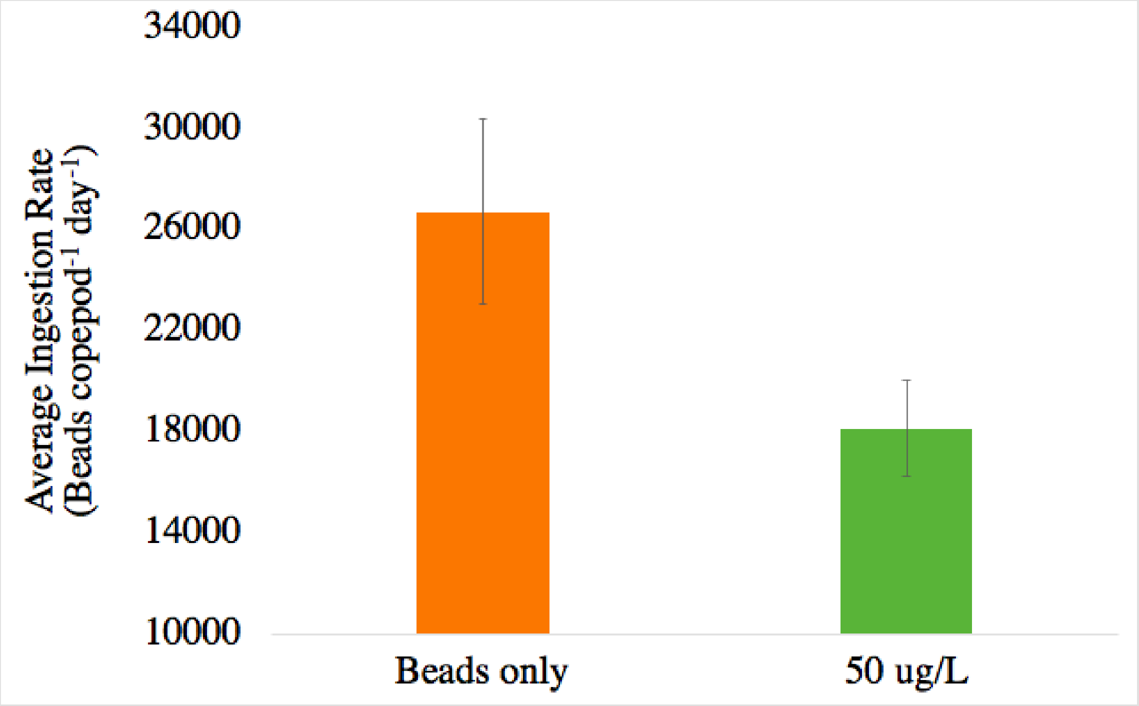


Fig. 2.8. Ingestion rates of copepods (beads copepod^{-1} day^{-1}) fed on – 1) PS beads only and 2) PS beads in 50 ug L^{-1} phenanthrene solution. Values are averages (± SE) of 5 replicate experimental jars.

Phenanthrene load in copepods:

The sensitivity test concluded that a minimum of two copepods are needed for the GC-MS to detect phenanthrene levels in the copepods (Fig 2.9). Two copepods can accumulate an average of 7.9×10^3 pg phenanthrene, three copepods 1.1×10^4 pg, four copepods 8.9×10^3 pg and five copepods 3.2×10^4 pg.

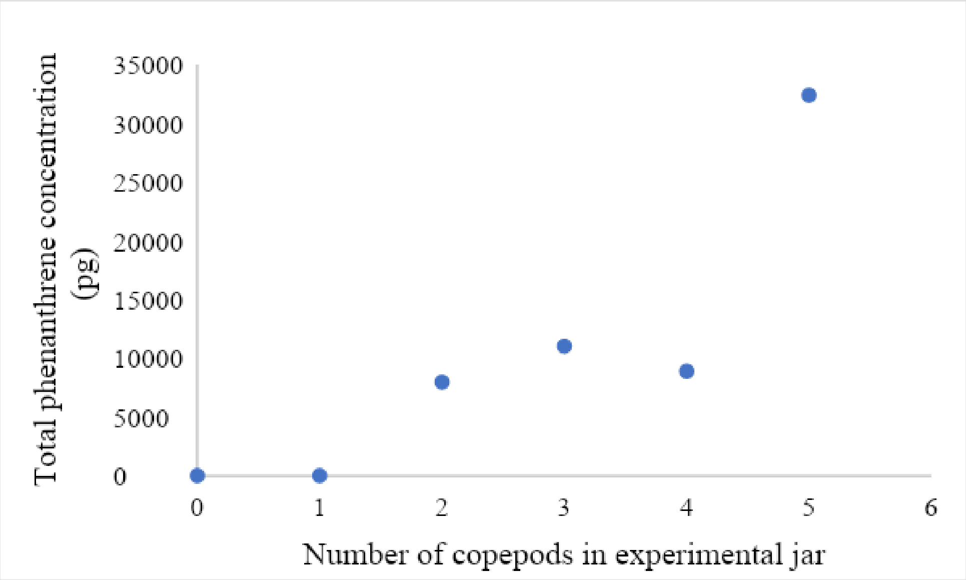


Fig. 2.9. Sensitivity test showing that a minimum of two copepods are needed for the GC-MS to quantify phenanthrene load in the bodies. Values are averages for phenanthrene concentration in 3,4 and 5 copepods (n=9) in experimental jar.

Copepods that consumed beads only had a phenanthrene concentration of 0 pg copepod^{-1} while copepods that ingested phenanthrene adsorbed to the beads had a mean body load of 4110 ± 1545 pg copepod^{-1}. Copepods that were only exposed to phenanthrene had an average concentration of 3485 ± 230 pg copepod^{-1}. There was no significant difference in the body load between copepods that ingested phenanthrene adsorbed beads and copepods exposed to phenanthrene only (t= 0.56, df= 10, p= 0.29). However, a one-way ANOVA showed that there was a significant difference between the 3 treatments (df_{total} = 18; f = 3.56; p = 0.05) (Fig 2.10).

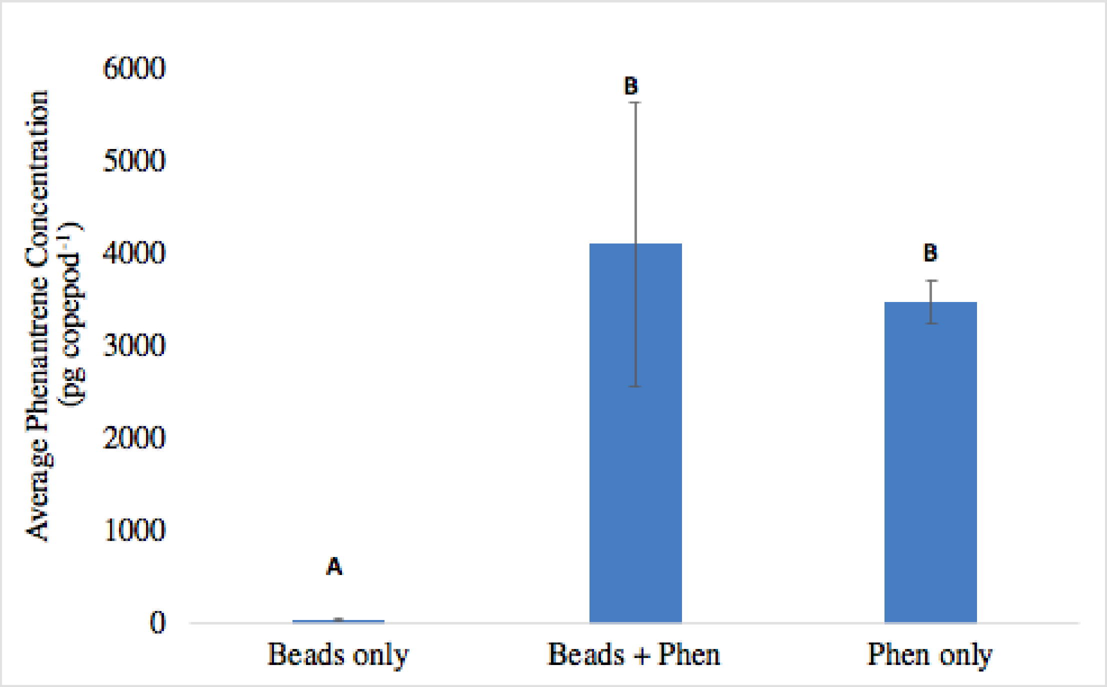


Figure 2.10. Average phenanthrene concentration (pg copepod^{-1}) of the three treatments: copepods ingesting PS beads only, PS beads with phenanthrene and copepods only exposed to phenanthrene. Error bars are SE

Phenanthrene load in fecal pellets:

The average length and width of fecal pellets from the four jars was 0.32 ± 0.018 mm and 0.058 ± 0.003 mm respectively. Average number of PS beads calculated from the first method using the Moxi was 300 beads pellets^{-1}. In the second method, beads were calculated using the equation of the curve. The average number of beads in the four jars was 2013 ± 92 beads. The phenanthrene concentration sorbed onto the beads present in the pellets was 0.15 ± 0.046 pg bead^{-1}.

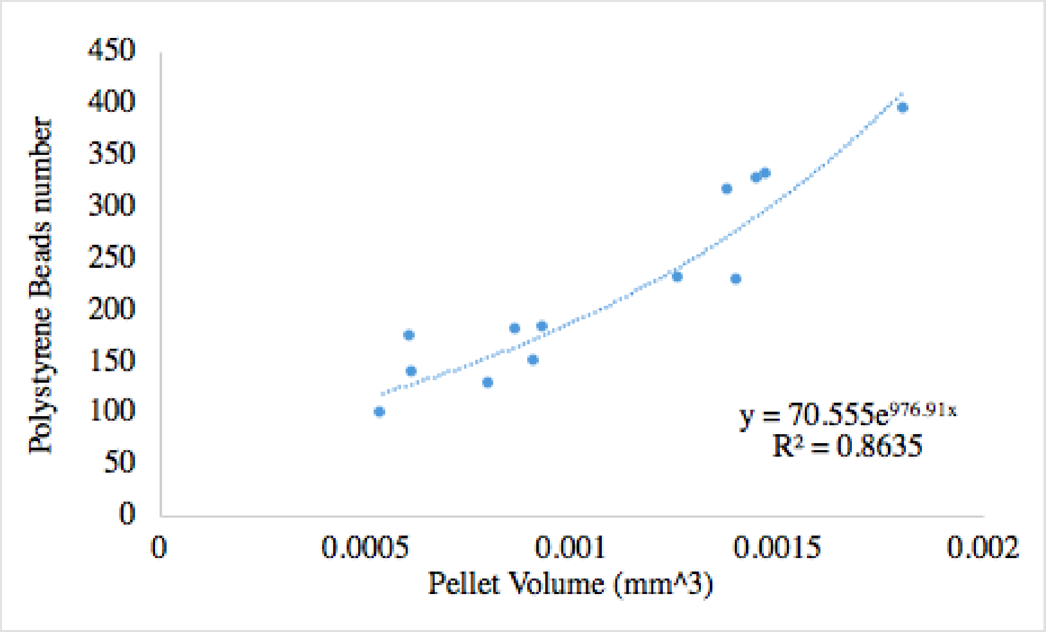


Figure 2.11. Number of beads in the 13 fecal pellets plotted against the pellet volume (mm^3)

Discussion

In the present study, I found that microplastics (MPs) did not act as vectors to PAHs upon ingestion by copepods. I found that the phenanthrene load in copepods ingesting phenanthrene adsorbed MPs was not significantly different to the load in animals only exposed to the 50 ug L^{-1} phenanthrene. These results are in line with previous studies that have researched the potential of microplastics acting as vectors to contaminants. A study investigated the combining effect of polystyrene microplastics and dibutyl phthalate (DBP) in the marine copepod *T. japonicus* demonstrated that sorption to the MP reduces the bioavailability of DBP and results in decreased toxicity of the chemical to the copepod (Li et al 2020). The presence of PE microplastics did not increase the bioaccumulation of 4-n-

Nonylphenol and 4-Methylbenzylidene-camphor and their toxicity in copepod *A.clausi* (Beiras et al 2019).

Other studies also suggest that the uptake of PAHs through dietary means (e.x - adsorbed to particulate matter) by zooplankton is not a significant route of exposure compared to the passive uptake of PAHs from surrounding waters. In the Berrojalbiz et al (2009) study, no significant differences were observed in bioaccumulation values between food (contaminated phytoplankton) uptake and water uptake in copepods exposed to various PAHs of the same concentrations. Similarly, Arias et al (2016) found no significant difference between contaminated algae and passive uptake of three PAHs - phenanthrene, anthracene and pyrene in copepod *Pseudodiaptomus marinus.* A different study reported that freely dissolved PAHs were more significantly bioavailable to copepods *C. finmarchicus* and *A. tonsa* under co-exposure with MP (polyethylene and polystyrene)-sorbed PAHs (phenanthrene and fluoranthene) (Sorenson et al 2020)

My findings indicate that copepods can transfer phenanthrene concentrations between 3.48 to 4.11 ng and approximately 600 polystyrene microbeads (present in the gut at any given time) up the marine food web. Microplastics and PAHs can implicate the health of marine animals such as anchovies, cod and right whales that ingest large amounts of copepods and other zooplankton species. Evidence of trophic transfer of microplastics is highlighted in the Cedervall et al (2012), where polystyrene nanoparticles were fed through a food chain from algae, to zooplankton to goldfish with noticeable effects on the feeding behavior of the goldfish when exposed to microplastics. Only two studies have performed experiments that track the fate of contaminated microplastics in the marine food web in order to understand the potential for bioaccumulation and biomagnification. Batel et al (2016) demonstrated the trophic transfer of benzo[a]pyrene (BaP) contaminated microplastics in an aquatic food chain consisting of shrimp nauplii and zebrafish. The study indicated that the BaP may actually desorb in the intestine of the zebrafish and transfer to the liver, which can cause potential health hazards. However, Tosetto et al (2017) explored the effects of PAH contaminated polyethylene microplastics using amphipods and frill gobies and found no significant behavioral change in the gobies in response to PAHs.

Studies that have investigated accumulation of PAHs in copepods and zooplankton have reported similar values to my study. Phenanthrene concentration of 10 ug g lipid^{-1} was found in Calanus copepods during the exposure time of 192 h (Jensen et al 2012). *C. hyperboreus* females were incubated in phenanthrene (111, 50 and 10 nM), pyrene (57, 28 and 6 nM) and benzo(a) pyrene (10, 5 and 1 nM) for three days. The highest concentrations in the copepods were 129, 30 and 6 nmol PAH g female^{-1} (Nørregaard et al 2015). Copepods exposed to 500 ng L^{-1} PAHs had concentration of 613 ± 40 ng g^{-1} dry weight (Cailleaud et al 2009). Zooplankton were found to have total PAH concentrations between 5 to 5440 ng g^{-1} dry weight in Gaoping coastal waters, Taiwan (Hsieh et al 2019) and 2 to 3500 ng m^{-3} concentration in East China Sea (Hung et al 2014).

Ingestion rates of copepods in the MPs only and phenanthrene sorbed MPs treatments were between the order of magnitude of 10^3 to 10^4 beads copepod^{-1} day^{-1}. Microplastics can reduce feeding capacity of copepods and stunt their growth and reproduction which consequently affect their population size. Adult female *C. helgolandicus* can readily ingest three different types/shapes of microplastics - fluorescent PE microspheres, nylon fibers/fragments, and polyester fibers. Exposure to the fibers had more of an impact on copepod's feeding and fitness (Coppock et al 2019). 3-4-day exposure to nylon fibers decreases algal ingestion rates of *Scripsiella trochoidea* and *Thalassiosira rotula* by *C. finmarchicus* (Cole et al 2019). Egg production in calanoid copepod *P. crassirostris* reduced after 5-day exposure to PET microplastics and also resulted in depletion of population size of 75% in contrast to their control experiment at higher concentrations of MPs (Heindler et al 2017). Lee et al (2013) exposed copepod *T. japonicus* to high concentrations of 0.5- and 6-µm PS microbeads and observed significantly reduced fecundity over two generations. This was attributed to depleted nutrition and inhibited fertilization caused by ingestion of microbeads.

Figure 2.12. Copepod Calanus finmarchicus (has a broken antennae) with 12 um polystyrene microbeads in the gut. (Credits - David Fields)

An interesting result I observed in the ingestion experiment was that there was a 32% decrease in ingestion of phenanthrene adsorbed beads compared to the PS beads only. This can imply that copepods can selectively feed and reject particles based on their chemical content. One study reported that three copepod species discriminated between toxic and non-toxic *Alexandrium spp* by chemosensory means, which suggests that selective behavior governs grazing response of copepods exposed to toxic prey (Teegarden 1999). A study reported that *C. finmarchicus* in presence of toxic dinoflagellate – *Alexandrium spp* and non-toxic closely related species *A. tamarense* only ingested the non-toxic dinoflagellate (Turriff et al 1995). The toxicities of sediment-associated phenanthrene, fluoranthene and diesel fuel to the estuarine copepod *Schizopera knabeni* were investigated. Copepods actively detected and avoided PAH amended sediment (Lotufo 1997).

Fecal pellet analysis showed that the pellets contained an average of 2013 ± 92 PS beads with a mean phenanthrene load of 0.15 ± 0.046 pg bead^{-1}. Fecal pellets could potentially facilitate the movement of PAHs and MPs to deeper waters. This is similar to the findings of a study that showed pellets collected in sediment traps in Dabob Bay, Washington quantitatively account for 100% of the PAH fluxes, implying that they control the removal of PAHs to the sediments (Prahl and Carpenter 1979). Fecal pellets are an important nutrient source for deep water ecosystems. Small pellets are likely to be important for recycling of organics in the water column, whereas large pellets that sink faster are more efficient for the transport of organic material to depth (Wassmann, 1998). Fecal pellet production rates of actively feeding copepods may exceed 10 pellets per hour. Such rates, combined with the abundance of copepods in some ecosystems, can mean that a significant component of contaminated microplastics captured is transformed into much larger packages represented by the fecal pellets. Many of these rapidly sinking pellets may exit the surface layers of the ocean, and either reach the sea floor of the continental shelves or enter the bathypelagic zone.

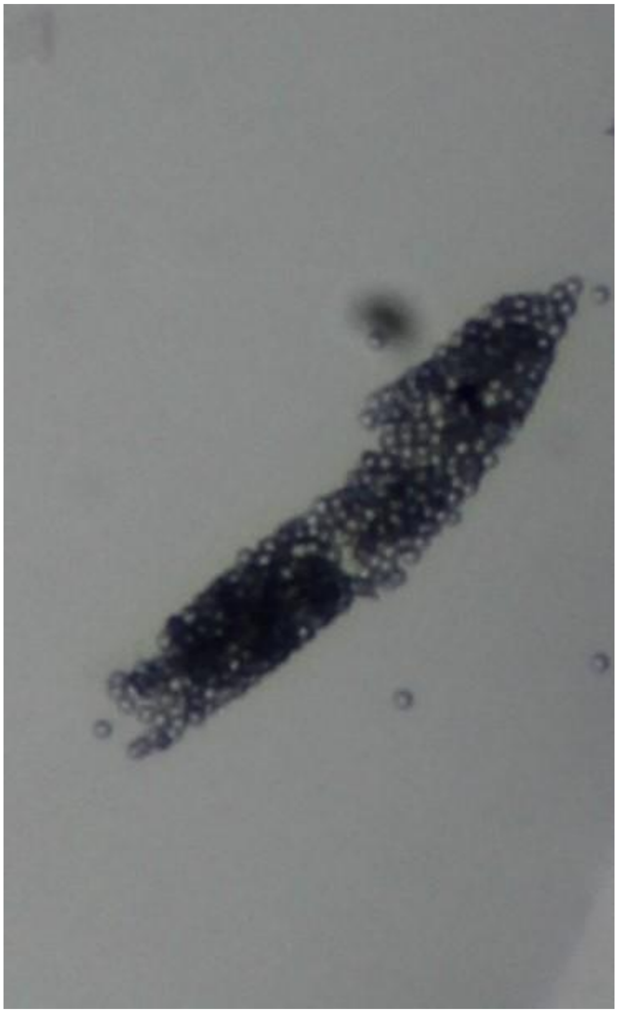


Figure 2.13. Picture of fecal pellet of copepod containing PS microbeads (Credits - David Fields)

My research subjects – *Calanus finmarchicus*, polystyrene microbeads and phenanthrene were models to track the fate of PAHs in the marine ecosystem. Future work in this field could include using different microplastics, copepods and containment which can lead to different results. For example, a study did find polyethylene microbeads having a vector effect on triclosan on the marine copepod *Acartia tonsa*. Similarly, Bellas and Gil (2020) have also demonstrated that PE microplastics can boost the toxicity of chlorpyrifos (CPF), an organophosphorus insecticide, in the copepod *A. tonsa*. Using environmentally relevant concentrations of microplastics and PAHs to determine the real level of risk in another future approach as my study used high concentrations of MPs and phenanthrene. In the natural environment, copepods exposed to MPs sorbed pollutants may have already accumulated the same pollutants in their tissues. Using pre-contaminated animals may influence bioavailability of MP- sorbed pollutants. A trophic transfer study could be conducted to compare PAH concentrations in predators ingesting copepods that contained PAH sorbed MPs and copepods only exposed to PAH solution.

To mitigate marine plastic pollution, stringent legislations on plastic waste management need to be enforced globally. The current governance for reducing plastic pollution has been ineffective. International efforts like the United Nations Law of the Sea convention and the Honolulu strategy, 2011 have only imposed general obligations for reducing marine plastic pollution, leaving it entirely upon the countries to adopt their own laws and legislations that address this issue. However, the globalization of the plastic industry since the 1950s has made it harder for countries to prevent oceanic plastic pollution. Asia is by far the single biggest source of macroplastic pollution (Dauvergne 2018). Studies have reported India, a developing Asian country, as a source of about half of marine plastic waste and the biggest source of primary microplastic pollution due to washing of synthetic clothing (Boucher and Friot 2017; Dauvergne 2018). Plastic consumption in India for major plastics was at a total of 16 million metric tons in 2019 (Statista 2021). Consequently, the Central government reported that the amount of plastic waste generated was more than 3,400,000 tons in the same year (NDTV 2021). The government has formulated multiple legislations over the years to regulate the manufacture, consumption and disposal of plastics in India, but its efficacy remains unanswered. In the next chapter, I will be discussing the plastic legislations in India, and the accomplishments, shortcomings and challenges in the implementation. I will also be discussing the plastic waste management strategies undertaken by the government.

CHAPTER 3: SOCIAL SCIENCE

Introduction

India, an emerging Asian economy, is an important producer and consumer of plastics (Dhanshyam and Srivastava 2021). India generates approximately 9.46 million tons of plastic waste annually. This figure is based on the Central Pollution Control Board (CPCB) projection that an estimated of 25,940 tons of plastic waste is generated per day (Business Standard, 2021). Currently, India's per capita consumption of plastic is quite low at 11 kg compared to countries like the US and China. But the projected high growth rates of GDP and continuing rapid urbanization suggest that India's trajectory of plastic consumption and plastic waste is likely to increase. The plastic processing industry is estimated to grow from 13.4 million tons (MT) in 2015 to 22 MT a year by 2020, and nearly half of this is single-use plastic, according to a Federation of Indian Chambers of Commerce and Industry (FICCI) study (Fig 3.1; The Economic Times, 2019).

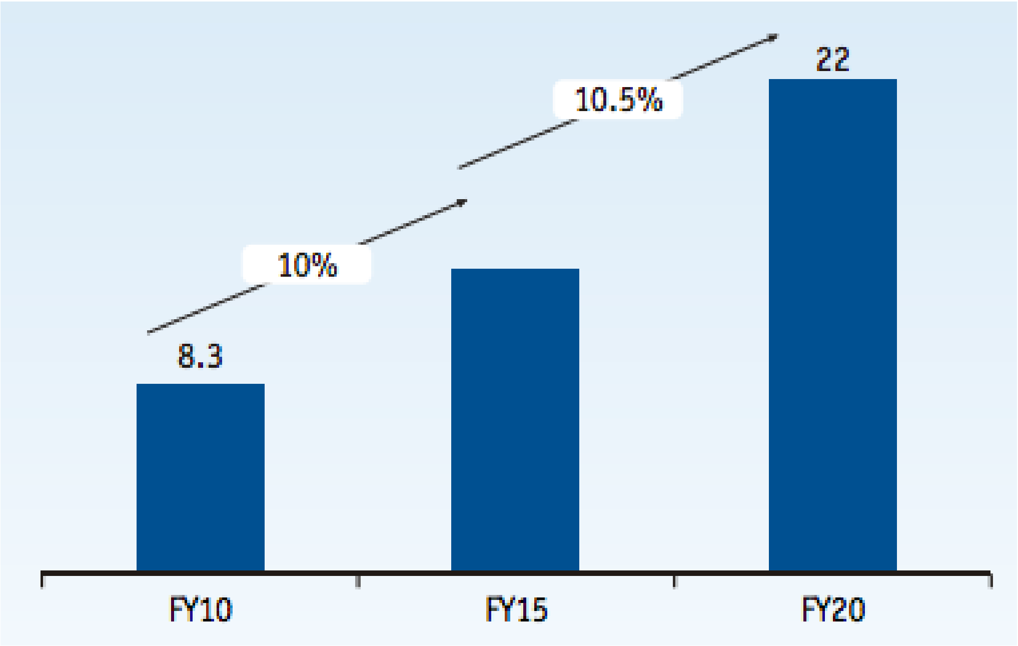


Figure 3.1. Growth of plastic processing industry from 8.3 MT in 2010 to 22 MT in 2020 (FICCI study 2019)

There are significant challenges to plastic waste management in India as there is no source segregation, little recovery and a significant proportion of waste gets dumped on land and in waterways (DownToEarth, 2019). The plastic waste processing capacity of India is a meager 15 percent of the waste generated (The Economic Times, 2019). Being a land-scarce country with high population density, the capacities of dump yards and landfill sites are inadequate (DownToEarth, 2019). A 2020 study by The Center for Science and Environment, a Delhi-based think tank, found 3,159 garbage mountains containing 800 million tons of plastic rubbish across India (BBC 2021). Nearby communities are at risk of inhaling gases such as nitrogen dioxide (NO_2) and sulfur dioxide (SO_2), which makes them vulnerable to chronic obstructive pulmonary disease and allergic rhinitis (Singh et al 2021).

In 2017, a 70-acre landfill in Ghazipur, Delhi collapsed leaving two people dead and 5 injured. This landfill impacts lives of more than 300,000 people living within 10 km area; polluting local air, groundwater and additionally poses a serious threat to Yamuna river which is just 7 km away (Fig 3.2; Swachh India 2017). Plastic in landfills degrades into smaller pieces and can leach into the soil and water table. Additives and other organic chemicals adsorbed to plastics can contaminate groundwater and cause major health hazards.

Figure 3.2. Ghazipur landfill picture (above) and map view (below) in Delhi, India (Hindustan Times 2019; Ramanathan and Mina 2019)

The water bodies in India are another major source of plastic waste. India is ranked 12th in the world in plastic marine debris generation, contributing about 0.09 - 0.24 million metric tons of plastic waste which is mainly transported by rivers, streams and wastewater (Jambeck et al 2015). The presence of microplastics has been detected in the coastal regions, rivers and lakes in India. A study that examined microplastic particles at 25 beaches along the southeast coast of India showed high abundance of polyethylene and polypropylene MPs in the beaches. The gut content analysis of important fish species revealed MP ingestion in 10.1% of commercially important fish (Karthik et al 2018). The Netravati River which empties in the Arabian Sea had a microplastic abundance of 288 pieces m-3 (Fig 3.3). Microplastics were mostly transparent and white colored due to the decay of plastic bags (Amrutha and Warrier 2020). The Ganga river system is considered the second largest plastic pollution contributing catchment in the world; around 0.12 million tons of plastic is discharged per year. A study reported that 100-400 MPs per kg of the river sediments were present in samples collected across 7 locations in lower and estuarine reaches of Ganga, majority of which were polyethylene terephthalate and polyethylene polymers (Sarkar et al 2019). Surface waters of Ganga contained 0.026-0.051 microplastic particles per liter, 90% of which are plastic fibers. Combined with flow from Brahmaputra and Meghna tributaries, Ganga releases 1-3 billion microplastic pieces into the Bay of Bengal daily (Fig 3.4; Napper et al 2021).

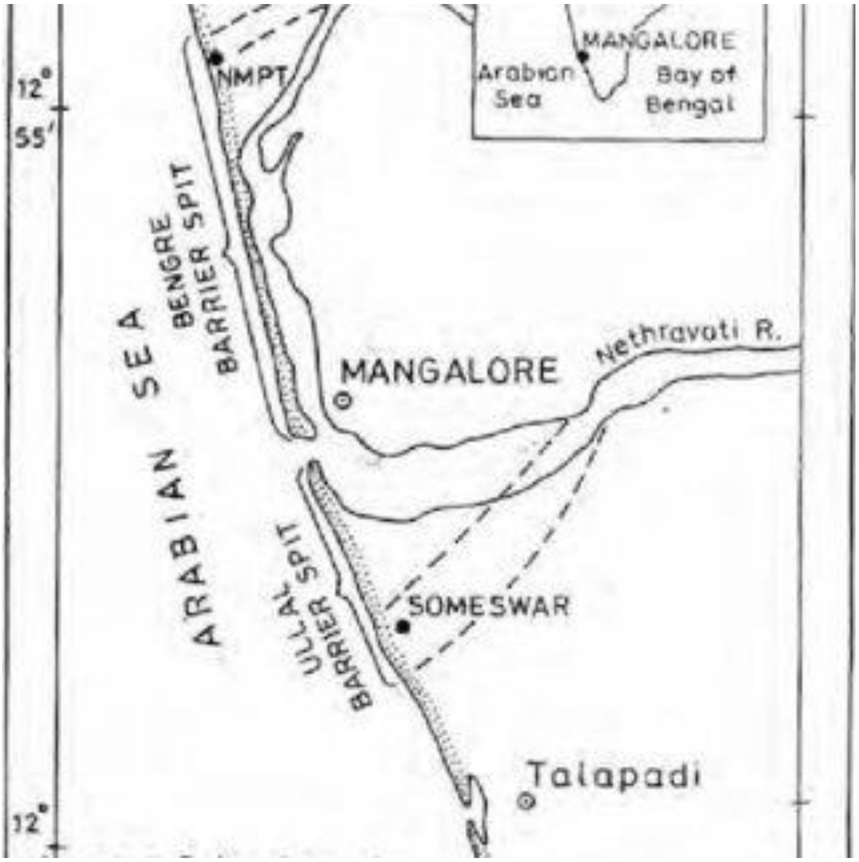


Figure 3.3. Map location of Netravati River, India flowing into the Arabian Sea (Balakrishna and Manjunatha 1999)

Figure 3.4. Map location of Ganga river flowing into the Bay of Bengal (Financial Times 2015)

India has been very active in formulating regulations for environmental protection since the 1960s. As India adopted liberalization and globalization as a policy in 1991 (The Hindu, 2016) there has been a significant growth in Indian GDP as compared to pre- 1991. This created a big shift in Indian demographics where a significant number of people moved to urban areas and created a market for plastic products (Talyan et al 2008). Since 1990, the demand for plastic in India multiplied over 20 times and was over 18,000 kilotons per annum in 2018 (Statista 2022).

Recognizing the need to regulate the manufacturing, consumption and disposal of plastic, the government has come up with multiple legislations over the years. One such example is the restrictions on single use plastics (SUP) implemented in 1999, where the sale of thin polythene bags was prohibited. The first government legislation on plastic was the Recycled Plastics Manufacture and Usage Rules in 1999 that aimed to regulate the food item packaging in recycled plastics and manage littering. The rule was amended in 2003 to prohibit the manufacture, storage, shipping or selling of recycled or virgin plastic bags less than 20 microns thick (ICPE, India 2020). The Plastics Rule in 2009 mandated that packets and containers should be manufactured with virgin plastic without the use of colorants (Kataki et al 2022). The Plastic Waste Management (PWM) 2011 rule mandated that state pollution control boards regulate plastic production and disposal in their respective states. This was revised in 2016 to introduce the concept of extended producer responsibility which was a progressive step in the right direction. The amended PWM rules which will come into effect in July 2022 will have two major shifts: 1) a gradual but firm phase-out of single use plastics and 2) central law applicable all over India. The latter point is important to note as the central government, until now, had encouraged states to enact laws to phase out SUPs (Times of India 2021). The enactment and implementation of these state laws by respective state governments has been slow and therefore the Center decided to step in (Times of India 2021).

These legislations were put in place by the government to narrow the gap between plastic production and waste management in India, yet this doesn't seem to be the case as presented by the empirical evidence above. This problem will become more acute in the coming years. The plastic industry in India is considered to be one of the largest in the world, valued at $26 billion and had an estimated volume of 16 million metric tons per annum in 2019 (Statista 2021). However, in the same year, more than 3,400,000 tons of plastic waste was generated. Plastic waste in India has more than doubled in the last 5 years with an average annual increase of 21.8% (NDTV 2021). Waste management strategies such as recycling and plastic road construction as specified in the PWM rules have been implemented to some extent but still face challenges. The purpose of this study is to critically analyze the state of plastic waste management policies in India, with the goal of identifying successes, failures of regulatory approaches and delineating future recommendations. In this chapter, I discuss the PWM rules in depth, their potential in reducing plastic pollution and the challenges in effective implementation of these rules. I also discuss the strategies used in India for plastic waste management including recycling and use in road construction.

Methods

A systematic literature review of peer-reviewed and grey literature was conducted to assess the current plastic waste management legislations and implementation in India. Several databases such as Google scholar, Google search, Web of Science and Science Direct were used for this review. Research articles (published between 2010 to 2022) were identified by searching the following keywords – ‘India’ ‘Plastic waste management rules’, ‘plastic problem’, ‘microplastics’, ‘ban on plastic’, ‘EPR’, ‘plastic industry’, ‘waste management’, ‘recycling’ and ‘plastic roads’. Data from newspapers such as the Times of India, Hindustan Times, Down To Earth, Business Standard and Economic Times were included.

I have attempted to summarize the following information: 1) National plastic legislation in India, 2) Challenges to the implementation of rules, 3) Plastic waste management strategies including recycling and road construction.

Results and Discussion

My research in the Indian legal framework regarding plastic manufacturing and its disposal shows that the government over the years has progressively enacted and amended its policies to try to keep pace with the issue of plastic pollution. We see that there is scope of improvement for the PWM rules to be inclusive of concerns of all stakeholders while balancing its aspiration of becoming a pollution free and developed country.

Legislations

The Plastic Waste Management (PWM) came into force in 2011 and was issued by the Ministry of Environment and Forests (MoEF), Government of India (GoI). Respective State Pollution Control Boards (SPCBs) were authorized to enforce provisions related to manufacturing, recycling and disposal of plastic. Urban local bodies (ULBs) were tasked with enforcement of provisions related to the use, collection, segregation, transportation, and disposal of plastic waste. Plastic bags needed to be labeled in order to indicate their thickness, registration number of the manufacturer and the material of the bag (recycled plastic or compostable). A minimum thickness of 40 microns was set for plastic bags, and a mandate for retailers to charge a fee for each bag available. Some of the salient features of the rule were the ban of plastic packaging for pan masala and tobacco and the explicit recognition of the role of waste pickers (PWMH 2011).

The 2016 Plastic Waste Management was much more comprehensive. The minimum thickness of plastic bags was increased from 40 to 50 micron which would increase manufacturing costs to deter retailers from supplying the bags for free. The rules specified that non compostable plastics needed to be greater than 50 um thick and the manufacture of non-recyclable plastics had to be phased out within two years. Retailers were liable to be fined for selling customers with non-standard plastic bags around forty-eight thousand rupees. The rules introduced the term Extended Producers Responsibility (EPR) which was a progressive step for the government. The concept for EPR is that producers are responsible for the reduction of the environmental impact of their products. Producers, importers and brand owners in India had to establish a system for collection of plastic waste generated due to their products. They also had to design and implement waste collection either individually or in collaboration with ULBs. Producers also needed to share their EPR plans and file informational reports on their plastic waste collection efforts with state pollution control boards (Bhattacharya et al 2018; PWM 2016).

The 2016 rules were revised to be the Plastic Waste Management (Amendment) Rules 2018. The forty-eight thousand rupees fine for retailers in the 2016 rules were omitted. The term ‘non-recyclable multilayered plastic’ was substituted by ‘multilayered plastic which is non-recyclable or non-energy recoverable or with no alternate use’. There was an attempt to establish a centralized registration system by mandating brand owners and producers operating in two or more states to register with the Central Pollution Control Board (CPCB) instead of their respective SPCBs (Bhattacharya et al 2018; PWM 2018)

In 2018, the Prime Minister of India announced that single use plastic products would be phased out. In 2019, the government released a draft notification to impose a nationwide ban by July 1st, 2022 (Times of India 2021). This will be done in three stages. Thickness of plastic carry bags will increase from 50 to 75 microns to 120 microns with effect from 31st December 2022. Manufacture of the products such as earbuds with plastic sticks, plastic sticks for balloons, plastic flags, candy sticks, ice-cream sticks and thermocol decoration would be prohibited by January 1st, 2022. Finally, plastic plates, cups, glasses, banners and cutlery will be banned from July 1st, 2022. Waste management infrastructure will be carried out by the Swachh Bharat Mission. States have been requested to create a Special Task Force for elimination of SUPs. A national taskforce has also been constituted for the same.

Challenges

Although the PWM rules were in the right direction in managing plastic production and waste in India, the implementation of these rules has been ineffective (Bhattacharya et al 2018). The latest PWM rule, 2022 raised a lot of questions on whether the ban against SUPs has been well thought out. As mentioned in the introduction, the ban is the first federal law tackling SUP pollution. For a long time, the task of identifying and regulating the usage of single use plastics was delegated to the state governments and thus, no effective broad-based, cohesive strategy was evolved at the federal level for the states to follow. Additionally, there was and is no concerted effort at the government level to identify and build an ecosystem for SUP alternatives. There is still neither a supportive infrastructure for production of SUP alternatives nor any tax-based incentivization for their production. Appropriate marketing effort is also lacking (Hindustan Times 2019). Without taking efforts to complete the required groundwork, bans have been summarily imposed. The authorities have not provided enough time and support, financial & otherwise, to plastic alternatives, to allow the industry to gear up and thrive against the competition from plastic (Times of India 2021). At the same time, since the production of SUP is concentrated in micro enterprises, there is a political cost associated with closure of such units which the governments have balked at. When the announcement of the ban was first made in 2019, there was no clear guideline of which products come under the definition of single-use plastic. This led to a 4.5 lakh job loss in the plastic sector (Business Today, 2019). SUP, in Indian context, is largely used by economically lowest strata – be it the vegetable vendor on the street, small grocers catering to the needs of daily wage earners etc. All these vendors who cater to the below poverty line (BPL) segment and just above the BPL segment cannot afford to lose the customer by insisting costly SUP alternates nor these customers have the ability to pay for such costly alternates. Many times, the cost of packaging (using SUP alternates) is equal or almost equal to the value of goods being transacted (Bhattacharya et al 2018).

EPR is an important concept that could help deal with India’s plastic waste, especially when it comes to multilayered plastic (MLP). According to the CPCB, a MLP refers to any material used for packaging that has at least one layer of plastic as its main ingredient in combination with one or more layers of paper and aluminum foil. MLPs cannot be recycled and therefore don’t fetch waste pickers in India much money. EPR comes into play here because companies can incentivize these workers to recover 100% of the waste, including MLPs by provision of infrastructure and technology (Pulla 2018). Unfortunately, the PWM 2016 rules leave much room of ambiguity of the EPR guidelines. The rules ask producers, importers and brand owners of plastic packaging to establish their own system for waste management. There was a lack of clarity as to who among the organizations involved in the product value chains used the plastic packaging material and will take responsibility for the plastic waste generated. Furthermore, producers were required to share their EPR reports with the SPCBs, but it meant that companies having a multistate presence would have to communicate with multiple SPCBs (Pani and Pathak 2021). This was rectified in the 2018 rules, with the establishment of a centralized registration system with the CPCB. Two years after the 2016 rules, only 45 of the thousands of companies in India shared their EPR rules with the regulator (Pulla 2018). It was difficult for producers to meet the PWM obligations due to a couple of reasons: 1) lack of infrastructure for formal recycling, 2) very few waste-to-energy plants, 3) less than adequate uptake of plastic waste as raw material by various industries such as power plants, cement manufacturers (Pani and Pathak 2021).

Plastic waste management in India

Regarding strategies on plastic waste management in India, the PWM legislations have stated that: 1) Plastic waste which can be recycled shall be channelized to registered plastic waste recyclers and 2) Local bodies shall encourage use of plastic waste for road construction (plastic which cannot be recycled) (PWMH 2011, PWM 2016, PWM 2018)

Recycling:

India has the best recycling status, recycling 38% more plastic than global average (Gopinath et al. 2020). A 2013 estimate by CPCB declares that 60% of plastic waste is recycled with a majority taking place in the informal sector that consists of waste pickers (MoEFCC, 2018; MoHUA, Govt. of India 2019). 65% of plastic waste is recycled at authorized facilities, 15% by the unorganized sector and 10% at home (Chattopadhyay and Kumar, 2020). Only high-density polyethylene (HDPE) and polyvinyl chloride (PVC) plastics are recycled in India (NPC 2019). The country has around 3500 numbers of organized recycling units for plastics along with an additional 4000 unorganized recycling facilities (Satapathy 2017). 3.6 million tons per year of plastic recycling is carried out, currently providing employment to almost 1.6 million people (Satapathy 2017). Recycled plastics have great potential to address pre-existing plastic waste problems in India. Some key product sectors that contain recycled plastics include construction, furniture, clothes and so on. Fabric is being developed from the recycling of PET bottles by the National Chemical Laboratory in Pune. The fabric is being used for making T-shirts, scarves and denim. The jersey for the Indian Cricket Team is made up of recycled PET bottles (Bhattacharya et al 2018).

Even though the recycling rate in India is high compared to other countries, there have been setbacks that impede the success of production of recycled plastic products. One of the main causes for inefficient recycling is the lack of segregation at the source of generation (Kataki et al 2022). Plastic identification codes and container codes are not found on any packaging materials which leads to inability to identify the types of plastic present in the waste (Satapathy 2017). This is due to the recycling process mostly being carried out by the informal sector. There is no training available on separating plastic and no proper sorting facility that is operated by trained workers (Kataki et al 2022). As a result, the quality of the recycled plastic products is inferior compared to other plastic products in the market. Plastic recycling requires technology to separate and target plastic in a product for recycling. Unfortunately, India lacks developed and tested technologies or appropriate technical competency for plastic waste recycling (Satapathy 2017). Additives in the plastic waste also create problems as they are difficult to remove from the plastic polymer. This makes the plastic viscous and unable to be recycled (Bhattacharya et al 2018; Kataki et al 2022). Additionally, the Indian consumer may less likely purchase recycled plastic due to inconsistency with quality (Bhattacharya et al 2018). The lack of adequate government policies, regulations and funding, and tax incentives to support entrepreneurs in setting up recycling facilities are observed as barriers in the Indian context (Kataki et al 2022).

Road construction:

India has been leading the world in experimenting with plastic-tar roads since the early 2000s (BBC 2021). This method of plastic waste recycling for road construction was first introduced by R. Vasudevan, from Tamil Nadu, India (Kataki et al 2022). His earliest plastic roads in the premises of his college were appraised by the Central Pollution Control Board (CPCB) for having no potholes and slowed down deterioration (Vasudevan et al 2012). Incorporating plastic waste into roads instead of incinerating it saves three tons of carbon dioxide for every kilometer of road (Vasudevan et al 2012). The Government of India in 2015 made it mandatory to use plastic waste alongside bituminous mixture during the construction of roads near large cities of more than 500,000 people (Plastindia 2018; MoEFCC 2018). Jambulingam Street in Chennai was one of India’s first plastic roads built in 2002 (Bhattacharya et al. 2018). So far, 703 km of National Highways in India have been constructed with plastic waste (Times of India 2021). 15 states/Union Territories are using plastic waste for polymer bitumen road construction (CPCB annual report on PWM rules, 2019).

The process of making plastic roads involves shredding of plastic, heating the shredded plastic along with aggregate mix and bitumen in the mixing chamber at a temperature of 160oC (Biswas et al 2020). One of the advantages of plastic roads is that different types of plastic can be added to the construction including: carrier bags, disposable cups, hard-to-recycle multi-layer films and polyethylene and polypropylene foams (BBC 2021). Several laboratory and field studies have confirmed the superior performance of plastic-bitumen roads. Behl et al (2014) established that polyvinyl chloride, a hazardous polymer that is hard to recycle and releases dioxin gas when incinerated, can be safely used as a modifier along with bitumen. Plastic in asphalt mix is also beneficial in preventing excessive rainwater drainage (Bindu and Beena 2010).

Conclusion

The present study demonstrates the ineffectiveness of the PWM rules in narrowing the gap between plastic production and plastic waste management in India due to various challenges in their implementation. The absence of a strategic plan, larger corporation's non-compliance with EPR, inadequate training for recycling, poor enforcement of the legislation all contributes to the unsuccessful management of plastic waste. As noted above, plastic in road construction is a viable option in reducing waste in India and improving road conditions all across India. Proper education and training on recycling can improve the recycling rate, employ more wastepickers and reduce waste being dumped on landfills and in waterways. India can model their plastic legislations based on bans and policies in other countries. For example, Rwanda has been plastic bag free since 2008 (Behuria 2021). In 2002, Ireland introduced a 15 Euro cent tax on plastic shopping bags which previously was provided free of charge to customers. The number of bags was reduced by approximately 94%. Litter surveys also found that between January 2002 and April 2003 the number of ‘‘clear’’ areas in Ireland (i.e., areas in which there is no evidence of plastic bag litter) had increased by 21% (Convery 2007).

CHAPTER 4: SYNTHESIS

In the previous chapters, I have discussed essentially two separate parts to my study: first being on the fate of microplastics and PAHs in the marine ecosystem through ingestion by copepods and secondly on the plastic waste legislation and implementation in India. In this chapter, I will try to link both these seemingly different parts which will help us understand the criticality of the problem of plastic pollution in developing countries with a special emphasis on India.

The results of the natural science portion of my research concluded that microplastics do not act as vectors to organic contaminants such as PAHs upon ingestion by copepods. The conclusion we can draw is that the accumulation of PAHs through ingestion of contaminated microplastics does not significantly differ compared to passive uptake of PAHs from surrounding waters. However, my data shows that copepods can consume high amounts of microplastics and PAH sorbed microplastics and can act as vectors in the marine environment. On average, copepods have around 600 microplastic beads in their gut at any given time which can be passed to higher trophic levels. This means that the marine animals that rely on copepods as a part of their diet are also ingesting large amounts of microplastics through the copepods ingested. For example, right whales in the Gulf of Maine consume up to one ton of copepods per day. Assuming the average ingestion rate of copepods is 10,000 MPs copepod^{-1} day^{-1}, whales consume up to 10^{13} MPs day^{-1} which can negatively impact their health.

To study the effect of this problem, I have taken up situation in India for my study. This is because of 2 major reasons. By the sheer size and the population of India, it’s hard to overlook the effect of Indian situation on the entire world. Moreover, India being on the cusp of being a ‘developed country’ with aspirations of achieving 5 trillion GDP, the plastic consumption of its burgeoning middle class is going to rise substantially in the near future.

If you look at the situation in India, there is a large abundance of copepods in India, accounting for more than 70% of the mesozooplankton community in Indian seas and estuarine systems (Jagadeesan et al., 2013, Jagadeesan et al., 2017; Jyothibabu et al., 2018; Arunpandi et al., 2017, Arunpandi et al., 2021a). Studies have also shown that there is a significant consumption of microplastics by copepods in the west coast of India (Rashid et al 2021b, Rashid et al 2022). Microplastics in India have been shown to adsorb various organic contaminants such as PAHs, PCBs and DDTs from surrounding waters. A scenario where the contaminant sorbed microplastics potentially bioaccumulating in copepods and transferring up the food web may possibly be happening in water bodies in India, given the rise in plastic usage and resultant rise in microplastics. Commercially important fish in India that predate on copepods maybe at risk of accumulating these contaminants thereby affecting the fishing industry and posing a major health hazard to the health of people who consume seafood. In Goa, researchers have found high concentrations of microplastics in two popular seafood delicacies - shellfish and finfish (Saha et al 2021). Thus, the challenge of plastic pollution in the water bodies in India has to be addressed on priority. In this context, we need to take a critical look at the future of plastic consumption in India and its effective disposal. This ties in with the second part of my study.

The easiest way for plastic disposal is dumping in the vacant lands which leads to the plastic waste flowing into the streams and rivers and other such water bodies during the monsoon season. This has been happening for many years now. The same goes with the industrial waste and its disposal. The quickest, easiest and cheapest way is to dump it in the stream/river nearby. This is a lethal combination. Plastic waste management in an aspirational country like India can prove to be particularly challenging if not accompanied by a robust legal framework to regulate the production, the societal mindset and efficient structure for effective disposal.

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APPENDIX A

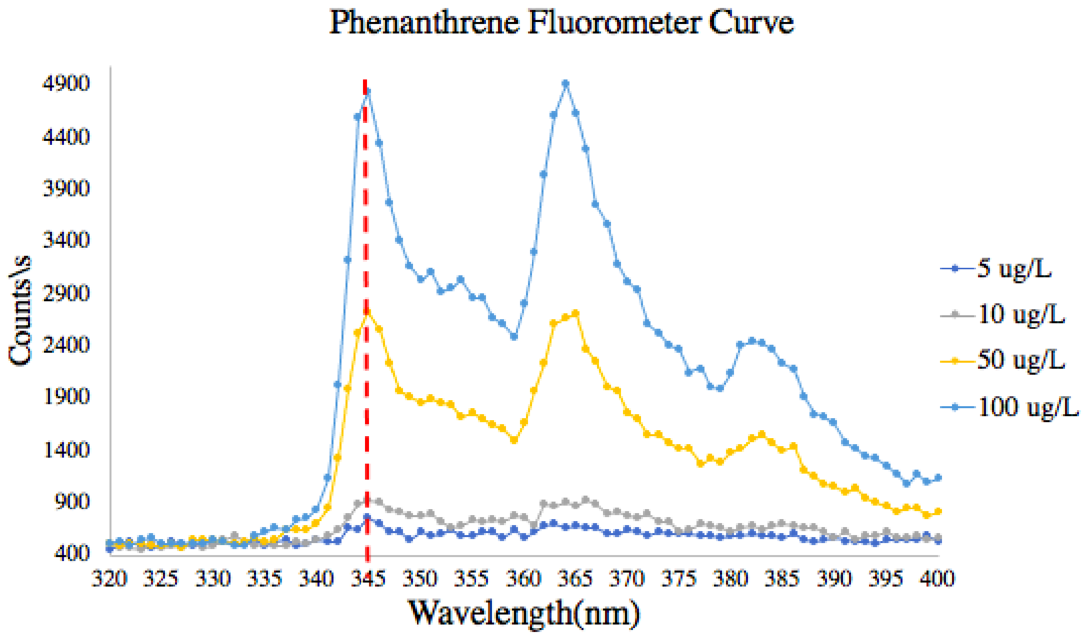

Fig. 1. Fluorometer curve of phenanthrene standards 5 ug L^{-1} to 100 ug L^{-1} with three discernable peaks at 345 nm, 365 nm and 385 nm.

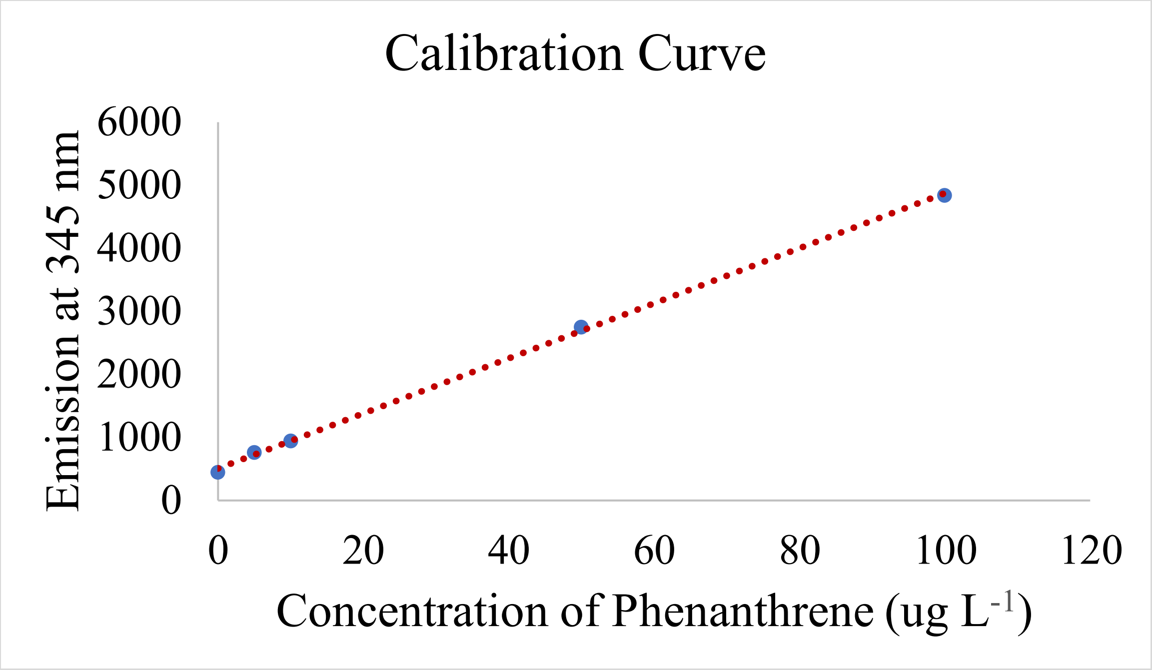

Fig. 2. Calibration curve of phenanthrene standards with emission values at 345 nm plotted against the concentration of phenanthrene (ug L^{-1})