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An Examination of the current knowledge of Contaminants in Mangroves: Hawaii and Globally

by

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In pursuit of

Master's Degree in Environmental Science and Management

Environmental Science and Management Department

Portland State University

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Abstract

The geographic range of mangrove forests is shifting quickly as they expand poleward in response to climate change while simultaneously being removed from their native extent to clear space for anthropogenic land-uses. Mangrove forests are also known to be sinks for anthropogenic contamination. Yet contamination research is under-researched in mangrove ecosystems, specifically the environmental fate, effect on biodiversity, and risk to human populations from contamination in the context of these changing conditions requires further research. The goal of this thesis is to address this data gap through analysis of contamination in the literature and through an investigational survey of mangrove ecosystems in Hawaii.

In chapter one, a review was completed for five classes of contamination in mangrove ecosystems, which was accomplished by summarizing other reviews and literature since those reviews. Four of these classes represent the most studied contaminants in mangrove literature, and include trace heavy metals, persistent organic pollutants, polycyclic aromatic hydrocarbons, and microplastics. The final category, pharmaceuticals and personal care products, is an emerging contaminant of concern that requires greater study in mangroves. This analysis identified several data-gaps that need to be addressed in the future. Pharmaceuticals and personal care products have received the least research despite biological activity at small concentrations. Research is concentrated in Asia and neglected in Africa and the Americas for all contaminant classes. Little discussion is given to whether the greater amount of research seen in Asia and Oceanic countries are due to the higher concentrations of mangroves in those regions or if there are other barriers preventing research. Some studies have noted that cost can be a prohibitive barrier to contaminant work, so efforts could be made to make contamination research more accessible. All contaminants were found to be widely present in mangrove ecosystems. Sources of contamination are correlated with proximity to waste water treatment plants, industry, and urbanized landscapes. Trace heavy metals and polycyclic aromatic hydrocarbons were found frequently at concentrations below the threshold to cause harm to plants, but may bioconcentrate in mangrove fauna. Persistent organic pollutants were found at levels that may cause harm to mangrove biota through long term exposure, and at some sites persistent organic pollutants were found at levels that might cause harm through short term exposure. Microplastics were found at variable levels, with some sites possibly being at concentrations that would cause harm to fauna, but more research is required in order to make that determination. In order to better assess the potential for environmental harm posed by contaminants, future research will need to consider multi-contaminant investigations. This is due to the potential for synergistic effects that different classes of contaminants can have when co-existing in the environment. Monitoring of contamination in mangroves should be increased, particularly given the ecologically or commercially important roles of mangroves, and in locations that have already been identified as hotspots for one or more of the contaminant classes. Finally, public outreach and involvement should be increased.

As mangroves are known to be an environmental sink for contamination where they are native, it is hypothesized that higher concentrations will be found in mangrove colonized coastal areas on Moloka'i, Hawaii where they are not native. In the second chapter this hypothesis was tested by comparing the microplastic and organic contaminant concentrations at coastal sites modified by non-native mangrove stands as well as unmodified coastline on Moloka'i, Hawaii in order to understand how mangrove invasion and land use interact to influence the distribution of contaminants along the coastline. Sediment, porewater, and mangrove plant tissues were investigated. MPs were found in sediment at an average abundance of 7.67 items/kg on the seaward side of mangrove transects, 10.11 items/kg on the landward side, and 8.49 items/kg along unmodified open coastlines. For porewater microplastics were found at an abundance of 10.89 items/L along the seaward side, 63.89 items/L along the landward side, and 9.84 items/L along the open coast transects. No relationship was found between mangrove presence and microplastic concentrations in porewater and sediment. However, a positive relationship was

observed between sediment microplastic and percent impervious surface. The most commonly found polymers via microscope Fourier transform infrared spectroscopy (u-FTIR) were polyethylene terephthalate (PET) (31%), polyamide (24%), and polypropylene (13%). Pesticide analyses was completed for sediment, porewater, and mangrove tissues (leaf, root, propagule). Six contaminants were found, the most common of which was the insecticide bifenthrin, which was found in 35 of the 37 sediment samples at an average concentration of 11.3 ng/g, 11 of the 11 root samples at an average concentration of 243.3 ng/g, and one of the five propagule samples at a concentration of 8.60 ng/g. There were two detections of Imidacloprid in porewater that had an average concentration of 37.1 ng/L. p,p'-DDE was detected in roots in two samples at an average concentration of 11.57 ng/g. The other three contaminants detected were p,p'-DDT, trifluralin, and permethrin and all were found at concentrations < 1 ng/g. The high concentrations of bifenthrin found in all of the roots when compared to the much lower concentrations detected in sediment suggests that mangrove roots are strongly accumulating some pesticides. Microplastics were low on Moloka'i when compared to global trends. This may be due to the low population on Moloka'i, or the isolated position of the Hawaiian Islands or of the sheltered location of Moloka'i in the center of the island chain. Future work should be done to determine if Microplastics are similarly low in more populated areas and how localized currents may control the distribution of contaminants along the Hawaiian Islands. The slight association of microplastics to the population center on Moloka'i and the lack of relationship between mangrove presence and plastics suggests that on Moloka'i urbanization is a more important factor for controlling the distribution of coastal microplastics than non-native mangroves. However, the high concentrations of bifenthrin bound to roots when compared to sediment concentrations from all sites suggests that mangrove presence may be playing a role in concentrating some types of pesticides such as pyrethroid insecticides. Further investigations should focus on determining the fate and cycling of root bound pesticides, which will allow for better management decisions around mangroves on Moloka'i.

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Chapter 1: Contamination in mangrove ecosystems: A synthesis of literature reviews across multiple contaminant categories

1.0 Introduction:

1.1 Background

Mangrove forests are coastal ecosystems found globally in tropical and subtropical latitudes and are known to be some of the most productive ecosystems on earth despite occupying only 0.5% of forest structure (Pérez et al. 2021; Hoque et al. 2015; Breithaupt et al., 2012). The benefits mangrove forests provide include ecologically important services such as: accreting sediment, providing shelter for fish and invertebrates, and affording roosting ground for migratory birds (Vo et al., 2012). Mangroves also provide crucial protection to the shoreline from extreme weather while slowing or preventing coastal erosion (MacFarlane et al., 2007; Swaidek, 1997). Despite the importance of mangrove ecosystems to the coastal environment, they face many issues. Currently, the single biggest reason for mangrove loss is direct deforestation to make room for urban development (Branoff, 2018; Giri et al., 2011). This deforestation is somewhat offset by global poleward expansion along the edges of mangrove range, induced by a warming climate (Saintilan et al., 2013). Additionally, there are direct replanting initiatives to restore mangrove extent but they are failing to reverse long term trends in habitat loss (Gatt et al., 2022). Currently, the total extent of mangrove forests is still decreasing at an overall rate of 1-2 percent per year (NASA, 2020). This trend of decreasing mangrove extent may continue at pace or worsen as coastal populations are expected to grow by 1.005 billion to 1.091 billion by 2050 (Merkens et al. 2016).

Mangrove forests compete with the human desire to live along the coast, and often coincide with population dense areas as ~ 1.2 billion people live within 100 km of the coast (John et al., 2022, Robin and Marcand, 2022; Small and Nicholls, 2003). As an increasing amount of mangrove forest comes under urban influence, it becomes increasingly important to fully understand how the urban environment may impact mangrove ecosystem functionality. While the most visible impact of urbanization is mangrove removal for land development and use in charcoal production, the urban landscape itself exerts numerous other pressures on urban mangrove forests (Branoff, 2018). One of the most profound ways the proximity to urban landscapes affects mangrove is the intensification of contaminants from sewage, runoff, and industrial effluent (Duke et al., 2007).

Pollution threatens mangrove biodiversity and ecosystem function, and contaminant loads in mangroves are exacerbated by their unique position at the interface of land and sea (Li et al., 2022; Freiss et al., 2019). This makes these ecosystems subject to inputs of contamination from both sides, such that mangrove ecosystems serve as physical and biogeochemical barriers to the movement of contaminants across the intertidal zone (Li et al., 2022). Mangrove areas adjacent to urban development are subject to wastewater discharge, industrial effluent discharge, stormwater runoff carrying contamination from urbanized areas, and more (Kulkarni et al, 2018). These contaminated outputs can be exacerbated by runoff from impervious surfaces that builds up directly behind mangrove forest in urban areas (Schleupner, 2008). On the seaward side, mangroves are subjected to impacts from activities such as fishing and shipping. The burdens mangrove forests face is borne out in the literature, with all kinds of common environmental contaminants detected in one or more ecosystem compartments.

Once contaminants enter the mangrove ecosystem, they may be retained due to the unique physical, chemical, and ecological characteristics of mangrove forest (Duke 2016). Mangroves produce extensive belowground root systems that enrich sediment with organic matter (OM) (Duke 2016). The

complex chemistry of mangrove sediment, including texture, pH, Eh/redox, tidal flux, salinity, anoxia, microbial communities, and root exudates regulate the movement of contaminants through mangrove ecosystems (Kulkarni et al., 2018; Hemkemeyer et al., 2015). For example, trace metals bind to suspended particulate matter that can be deposited on bottom sediment and buried (Caccia et al., 2003). Changes in pH and sulfide content can then remobilize trace metals into the ecosystem (Lacerda et al., 1993). Persistent organic pollutants (POPs) can potentially adsorb to organic matter or become degraded through microbial communities (Girones et al., 2021). The aboveground root structures slow the flow of water causing the sedimentation of suspended particles, though this varies across tree species and their associated above ground root structures (Deng et al., 2020; Krauss et al., 2014; Kathiresan, 2003). Ultimately, the tendency for mangrove areas to entrap contaminants, the frequency with which contaminants released from urban areas pass through these ecosystems, and the contaminants carried in by tidal action, all make mangrove sinks for pollution globally. As mangrove sediment is the primary compartment in which many contaminants settle, anthropogenic activities such as mangroves removal for urban development, aquaculture, or restoration through the removal of non-native mangroves may risk releasing buried contaminants back into the environment (Soper et al., 2019; Machado et al., 2002).

1.2 Contaminant Classes

This literature-review aims to explore trends in and make recommendations from current ecotoxicological literature reviews of mangrove ecosystems across major contamination categories. Five major categories of contaminants are represented in the literature: persistent organic pollutants (POP), microplastics (MP), polycyclic aromatic hydrocarbons (PAH), trace heavy metals (TM), and personal care products (PPCP), the latter an emerging contaminant of concern that does not yet have a robust body of research. The contaminants from each category that have been prioritized in the past are below (Table 1-1).

Table 1-1. Historically prioritized contaminants in mangrove research by category excluding the emerging contaminants of concern, PPCPs.

TM	POP	MP	PAH
Al	aldrin	polyethylene	Napthalene
Fe	chlordane,	polypropylene	Acenaphthylene
Ag	DDT	polyethylene terephthalate	Acenaphthene
Cd	dieldrin	polystyrene	Fluorene
Cr	endrin	polyvinyl chloride	Phenanthrene
Cu	heptachlor		Anthracene
Mn	hexachlorobenzen		Fluoranthene
Ni	mirex		Pyrene
Pb	polychlorinated		Benzo[a]anthracene
Zn	biphenyls		Chrysene
	polychlorinated dibenzo-p-dioxins		Benzo[b]fluoranthene
	polychlorinated dibenzofurans		Benzo[k]fluoranthene
	toxaphen		Benzo[a]pyrene
			Dibenzo[a,h]anthracene
			Benzo[g,h,i]perylene
			ndeno[1,2,3-c,d]pyrene

1.2.1 Trace heavy metals:

TMs can originate from mineralization and natural geochemical cycles. However, the high levels associated with contamination that can stress ecosystems are typically the result of anthropogenic influence (Kulkarni et al., 2018). TMs originate from a variety of anthropogenic sources including traffic, fertilizer, industry, and sewage (Wu et al., 2017; Lewis et al., 2011). They also enter mangrove environments from oceanic sources such as oil spills, shipping and transportation, and mining (Idaszkin et al., 2017; Ruiz-Fernandez et al., 2019). The sediment characteristics of mangrove ecosystems influence contaminant retention, with TMs being found at higher concentrations at mangrove sites that were fine-grained and rich in organic matter (Veerasingam et al., 2012). This means that mangrove ecosystems are typically a sink for these contaminants (Kulkarni et al., 2018). TM pollution is toxic to living organisms and may threaten mangrove biodiversity, stress ecologically sensitive species, and reduce species fitness (Antonovics et al., 1971; Lewis et al., 2011). TMs also have a high potential to bio-magnify, threatening fish and sea birds. This biomagnification also threatens human populations relying on protein from contaminated mangroves. Consuming food containing TMs can cause damage to human nervous systems and kidneys and have carcinogenic effects (WHO, 1996).

1.2.2 Persistent Organic Pollutants (POPs)

The designation “persistent organic pollutant” refers to families of chemicals that together comprise thousands of individual chemical species with long half-lives in soil, sediment, air, and water (Jones et al., 1999). Some of the most well-known POPs are organochlorinated pesticides (OCPs) such as DDT, many of which are banned or restricted in use but are still being detected in the environment decades after their use ceased (Ivorra et al., 2021). POPs encompass a diverse set of origins, chemical compositions, and environmental impacts. They can originate from agriculture (e.g., DDT, endosulfan, chlorpyrifos), halogenated flame retardants used in electronics and plastic production (e.g., PBDE and congeners), consumer products (e.g., PFAS and congeners), and other sources (Girones, et al. 2012). As many POPs are hydrophobic, they can adhere to mangrove sediment where they may be retained for a long period of time (Bayen et al., 2019). These compounds have characteristics such as lipophilicity that facilitate bioaccumulation up the food chain, putting at risk humans that rely on mangrove associated-species for sustenance (Beyen et al., 2005; Smith & Gangolli, 2002). 12 individual species of POPs were originally denoted as priority contaminants by the U.S. EPA (Table 1), and others are beginning to receive attention (Lohmann et al., 2007).

1.2.3 Microplastics (MPs)

Microplastics are plastic particles $\leq 5\text{mm}$ in length and result primarily from breakdown and weathering of larger plastic pollution, sewage effluent, and from the unintentional release of plastic pellets (Enfrin et al., 2020; Xu et al., 2020). They are found in several shapes, with fibers, fragments, spheres, foams, and films being the most common (Lazano et al., 2021). Microplastics are also chemically diverse, composed of different polymers depending on the formulation of the parent material (Table 1-1). The small size and light weight of these particles allow them to be easily transported along marine and tidal currents, through urban runoff, and via airborne deposition (Akdogan & Guven, 2019). They also vary in density, with more dense plastics settling to the sediment and lighter particles floating in the water column (Alomar et al., 2016). Further, more than 390 tons of plastic is produced yearly, and annual production is expected to increase (Hachem, 2023). 10 rivers are known to be responsible for about 90% of oceanic plastic, with the Yangtze River contributing the most (~1.5 million mT) (John et al., 2021). Due to these factors, microplastics are expected to continuously accumulate in all environments generally and in mangrove forests specifically (Maghsodian et al., 2022). Other sources of marine microplastics include fishing, marine and coastal tourism, urban runoff and wastewater discharge, and marine industries such as oil extraction (Thompson et al., 2009). Microplastics produce a variety of negative consequences to marine species that encounter them. Microplastics are easily confused for food, and fill space in stomachs and digestive systems (Bergmann et al., 2015). Additionally, the high surface area and non-

polar surfaces of microplastics allow them to adsorb persistent organic pollutants and trace metals, potentially providing a new vector for these contaminants to move through the environment (Koelmans et al., 2016). This may mean that humans who subsist on protein sourced from mangrove fisheries are at increased risk of exposure from co-occurring contaminants. The net impact of microplastics on species that consume them include lower reproductive and growth rates, weight loss, and diminished fitness (Lusher et al., 2013).

1.2.4 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons are chemicals that occur naturally in coal, crude oil, and gasoline (CDC, Polycyclic Aromatic Hydrocarbons). There are 16 priority PAHs (Table 1-1) described by the US EPA due to their carcinogenic and mutagenic properties (ATSDR, 2005). Richter-Brockmann and Achten (2018) describe additional emerging PAHs of concern (anthanthrene, 7H-benzo[c]fluorene, and dibenzo[a,l] pyrene) that have higher carcinogenicity than the 16 priority PAHs. As PAHs are mostly produced through human activity, particularly combustion, they are ubiquitous in the marine environment (Tam et al., 2001). While they are persistent organic compounds, they are typically researched and reviewed as a separate category from other POPs. They can be produced through the combustion of oil, gas, wood, garbage, and domestic heaters. PAHs can be divided into high molecular weight (HMW) and low molecular weight (LMW) depending on the number of aromatic rings present in the compound, and LMW and HMW PAHs move through and react in the environment differently. For example, LMW PAHs are slightly more water soluble than HMW PAHs (Billah et al., 2022). HMW PAHs are less easily degraded and have a greater tendency to adsorb onto the surfaces of particles (Yu et al., 2005) The potential for bioaccumulation of PAHs is somewhat unknown, but other studies have demonstrated that other hydrophobic/lipophilic chemicals like PAHs have high bioaccumulation rates (Qiu et al., 2018). The hydrophobicity and low water solubility of PAHs also contribute to their retention in mangrove sediment (Ke et al., 2009). PAHs are highly persistent in mangrove ecosystems, with some studies estimating the half-life in sediment to be between three and ten years (Burns et al., 1993).

1.2.5 Pharmaceuticals and Personal Care Products (PPCPs)

Pharmaceuticals and personal care products are a category of contaminants distinct from the others, with high potential for environmental impact but an overall lack of research in mangrove forests (Bayen et al., 2016). Currently, there is no dedicated review exploring global distribution or environmental impact of PPCPs in mangroves. The U.S. EPA defines pharmaceuticals and personal care products as substances that contain hormones, products used to enhance personal health or the health of livestock, or products used for cosmetic reasons (U.S. EPA, 2013). This makes PCPs a diverse category containing many compounds, including chemicals not typically seen as potential environmental contaminants such as fragrances and caffeine. Many PPCPs enter the environment through sewage or from anthropogenic activities such as agriculture (Yang and Metcalfe, 2006). A primary challenge of PPCPs is that wastewater treatment plants may be ineffective at completely removing these chemicals from effluent (Radjenovic, 2009). The capability of wastewater treatment plants to remove these compounds depends on their specific characteristics, the methodology employed for wastewater treatment such as retention in polishing (*define or elaborate*) wetlands, and temperature. In the environment, PCPs tend to be hydrophilic, biologically active. And persistent. This is, in part, by design due to the need for these chemicals to last while performing their job (Radjenovic, 2009). PPCPs can manifest a variety of toxic and damaging effects on biota. These toxic impacts vary by substance but include endocrine disruption, reduced reproduction rates, feminization of male fish, and toxic effects on human populations (Froehner et al., 2011). PPCPs, particularly endocrine disruptors, may induce significant ecotoxicological harm at low environmental levels (Nawaz & Sengupta, 2019). Antibiotics entering the environment also influence bacterial communities in various ecosystem compartments, and antibiotic resistant bacteria have been observed to be increasing in industrialized areas (Montesdeoca-Esponda et al., 2021). Further,

PPCPs are widely distributed in the marine environment and infiltrate multiple ecosystem compartments with mangrove sediments in particular being viewed as both sinks and sources of contaminants (Bayen, 2012).

2. Goals and Methods

2.1 Goals

This literature review seeks to answer:

1. What common lessons from the research into different environmental contaminants can be applied broadly to improve ecotoxicological understanding in mangrove ecosystems?
2. Where are the priority data gaps in contamination research in mangroves?
3. How can managers combine knowledge from differing contaminant categories to more holistically manage mangrove ecosystems?

2.2 Methods:

In order to answer the above objectives this review synthesizes the knowledge from the most recent literature reviews to determine: where has contamination research been focused geographically, what contaminants have received the most research, what is known about the ability of mangrove ecosystems to be damaged by or remediate contaminants, where contamination research could be improved, and what data gaps exist that need to be addressed. Geographic distribution of research is current as of June 2023. Google scholar and web of science were used to search for relevant literature, checking the first 25 pages of results using keywords “review, “mangrove”, “contaminant”, combined with each of the five categories of contaminants: “polycyclic aromatic hydrocarbons”, “personal care products”, “trace metals”, “persistent organic pollutants”, and “microplastics”. This study includes recent literature reviews, published during or before 2023, excluding reviews published before 2013. Topical reviews discussing the role of mangroves in remediating contaminants are included. Using the criteria described above, eight literature reviews, including six global reviews with two on microplastic contamination, one on persistent organic pollutant contamination, one on trace metal pollution, and two on polycyclic aromatic hydrocarbons – are discussed. Three topical reviews exploring bioremediation of PAHs, MPs and OCPs were included. No reviews were found summarizing what is known about global distribution of PPCPs in mangrove ecosystems or the role of mangrove ecosystems in remediating PPCPs. However, with limited articles published on PPCPs in mangroves, all articles were included.

3. Results and Discussion:

3.1 Sediment

3.1.1 Extent of study by location and hotspots

The reviews indicate that contamination research within mangrove forests is relatively spread out geographically, but favors Asia overall, particularly for MPs (Figure 1-1). Of the four contaminant categories with reviews available, trace metals have received the most research in mangrove sediment and surface water with 181 papers either discussed in the review or published since the review was made available; POPs have 55 such papers, MPs 72 papers, and PAHs have been covered in 55 papers. Microplastic research is relatively new, the most recent review of MPs in the mangrove environment was published in 2021; and ~34 of the 43 papers have been published since 2017 (Deng et al., 2021). PPCP research investigating mangroves is under-represented in the literature, with only 8 papers published, the oldest in 2011. Most PPCP research has taken place in China and India (Figure 1-1).

There are several locations which can be viewed as hot spots for multiple contaminants such as China (MPs, POPs, OCPs, PAHs), India (PAHs, TMs, POPs), Malaysia (MPs, TMs, PAHs), Mexico (OCPs), and Brazil (TMs, POPs, PAHs). However, sites identified as having higher contamination also correlate to areas that received more investigations (Figure 1-1), highlighting the need for research into under-reported areas to better assess where contamination is distributed in mangroves globally. Individual sites can also be viewed as hot spots for specific contaminants, such as Guayaquil, Ecuador, which was contaminated by Pb, Sn, Cd, Ag, Mo, Zn and Ni and was called out as being a location with the highest TM concentrations (Kulkarni et al., 2018). However, the fragmented nature of contamination research means that there have not been any investigations into the concentrations of other contaminants at the Guayaquil site. As there has been no global review of PPCPs, and only limited investigation generally, this review will provide an initial reporting of what is known about PPCP contamination in mangroves. Of the seven studies targeting PPCPs in mangrove abiotic compartments, one was in China, three were in Brazil, two were in Indonesia, and one was in India (Table 1-2).

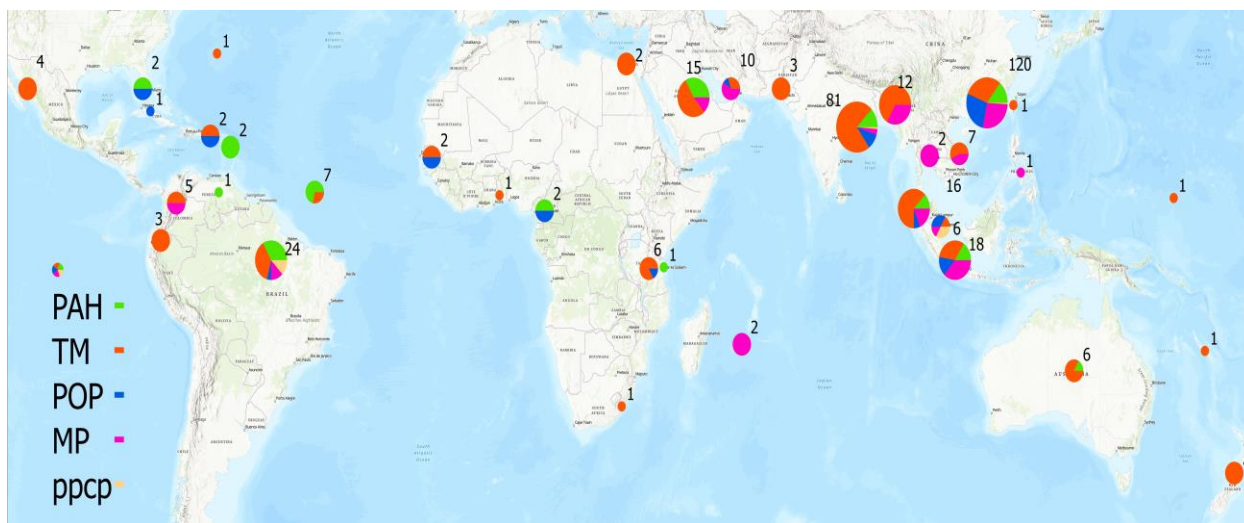


Figure 1-1 Locations (total publications across classes by country) of research in mangrove abiotic compartments for TMs, POPs, PAHs, PPCPs, and MPs

3.1.2 Overall concentrations in sediment

Contaminants from every category are represented in mangroves sediments. However, the extent and ecological threat varies by contaminant class. For example, Kulkarni et al., 2018 found that the majority of global mangrove extent is contaminated by some level of metal pollution. However, many sites with metal pollution tend to be below levels of environmental concern, though some hotspots exist (Kulkarni et al., 2018). Which metals were contaminating mangroves was variable, and was typically determined by source of pollution (Kulkarni et al., 2018). Cu and Pb were the metals found most frequently and are of highest concern, followed by Ni, Cr, and Cd (Kulkarni et al., 2018).

The reviews on POPs by Girones et al. (2021) and OCPs by Ivorra et al. (2021) came to some common conclusions while differing in certain areas. Girones et al. (2021) found POPs (γ -HCH, DDTs, Endosulfan, HCB, PCBs, PBDEs) globally, and frequently in concentrations that may pose a threat to mangrove biota by the standard of the sediment quality guideline Norwegian Environmental Quality Classification System (NEQCs). By this standard, the concentrations at many sites were above the level at which chronic exposure would cause harm to resident biota. At some sites γ -HCH, endosulfan, and PCPs were found at concentrations high enough to cause damage to biota following short-term exposure (Chen et al., 2020; Bhupander and Debapriya 2012; Alegria et al., 2016; Oliveira et al., 2016). The only pollutant that was not found at environmentally toxic levels were HCBs. In contrast to other research

Ivorra et al. (2021) found that on average OCP concentrations (methoxychlor, HCB, heptachlor, HCH, endosulfan, DDT, chlordane, aldrin) were 2.2 times lower in mangrove areas than non-mangrove areas in regards to sediment. This review used a different sediment quality guideline, effect range low (ERL) and effect range median (ERM) to contextualize the contamination levels in mangroves. Below the ERL, biological toxicity effects are rare. Above the ERM it can be assumed that toxicity effects are common. While both reviews agreed that many sites have concentrations at rates that may damage biota, Ivorra et al., (2021) found no concentrations above the ERM standard while Girones et al., (2021) did find sites that were acutely contaminated when comparing the concentrations reported in the reviewed studies to the Norwegian Environmental Quality Classification System (NEQCs) sediment quality standard.

PAHs are broadly present in mangroves in low to moderate levels, with some hotspots (Robin & Marchand et al., 2022; Billah et al., 2022). Robin & Marchand (2022) also used the standard ERL and ERM. The highest concentrations of PAHs were found in Hong Kong, Brazil, and the Sundarbans, India (Billah et al., 2022), though for these areas and elsewhere, contaminated mangrove sites were below the ERL (4022 ng/g) or between the ERL and ERM (44,792 ng g⁻¹) for PAHs. Billah found that PAHs ranged from low (0–100 ng/g dry weight) to moderate (100–1000 ng/g dry weight) levels in mangroves globally.

Mangroves are postulated to be sinks for MP contamination, and reported concentrations reflect the potential for mangroves to sequester MP contamination (Deng et al., 2021). Concentration of MPs in mangrove sediment is typically reported as items/kg. MP concentrations are fairly variable from study to study, with some finding as few as 12 items/kg in Singapore to as many as ~7900 items/kg in the Pearl River Estuary, China (Deng et al., 2021). The locations of greatest MP pollution in mangroves includes Iran (3252 items/kg), several sites across multiple studies in China (5783.3-7900 items/kg), and Malaysia (963 items/kg) (Maghsodian et al., 2022). Notably, there is no sediment quality standard for MPs in marine and freshwater sediment to contextualize these MP ranges (Redondo-Hasselerharm et al., 2023). Redondo-Hasselerharm et al., (2023) discuss a possible standard for freshwater sediment, using food dilution and translocation through tissues as biological risk factors and MP volume and surface area as the metrics for establishing threat levels. According to their analysis, MP concentrations threaten five percent of freshwater benthic organisms at 1698.5 items/kg. This suggests that MPs are a danger to organisms, at least in the most contaminated mangrove forests.

The research into PPCPs in mangrove forests is limited, though concentrations from published studies are presented below (Table 1-2). PPCPs such as antibiotics, fragrances, estrogens, and caffeine were investigated in China, Singapore, Brazil, and India (Table 1-2). The most investigated compounds were triclosan, an antifungal/antibiotic present in consumer products, the estrogenic hormone estrone, and caffeine, with three investigations each (Table 1-2). The highest detection was triclocarban (0-1318 ng/g) in Brazil, other detections ranged from not detected to 137 ng/g for triclosan in Brazil. However, the lack of research into mangroves makes it difficult to understand if these values represent environmentally harmful concentrations.

Table 1-2. Countries of study and concentration ranges for PPCPs in mangrove sediment (ng/g).

PPCP Chemical	Singapore Bayen (2016)	Singapore Bayen (2019)	Brazil Froehner (2011)	Brazil Chaves (2020)	Brzsil Beretta (2014)	India Ramasway (2011)	Macao Moreira (2021)	China Liu (2020)
17 α -estradiol							0 - 1.01	
17 β -estradiol							0 - 1.49	
Atenolol							0 - 9.84	
Bisphenol A	<0.4 - 81						1.38 - 19	
caffeine	<0.1 - 1.12			0 - 20	0 - 23			
Carbamazepine	<.001 - 1.3				0 - 0.62			
Chloramphenicol	N.D.							0 - 26
Chlorotetracycline								0 - 57.2
Ciprofloxacin								0 - 94.2
Diazepam					0 - 0.64			
Diclofenac	N.D.				0 - 1.06			
Enrofloxacin								0.6 - 19
Erythromycin					0 - 2.29			0 - 8.3
Estriol							0 - 0.17	
estrone	N.D.		0 - 49.27				0 - 9.05	
Florfenicol								0.5 - 85.5
Galaxolide		1.02 - 59			0 - 52.5			
Musk moskene		<0.23 - 3						
Ibuprofen					0 - 18			
Nifedipine				0 - 75				
Nimesulide				0 - <3				
Norfloxacina								3.6 - 80.3
Ofloxacin								0 - 36.6
Oxytetracycline								2.6 - 30.2
Propranolol				0 - 2				
Roxithromycin								0 - 11.4
Sulfadiazine	N.D.							15.6 - 115.4
Sulfadimidine								0.2 - 9.5
Sulfamethoxazole	<0.01 - 0.22							0 - 36.9
Tetracycline								2 - 22
Tonalide		<0.6 - 45			0 - 27.9			
Triclocarban				0 - 1318				
Triclosan	<0.4 - 15			0 - 137		0 - 85.3		

3.1.3 Physical characteristics control contamination in the environment

The reviews identify physical features and processes of mangrove ecosystems that affect contaminant concentrations, with some common themes. Mangrove forests and the anoxic sediment that they form have unique characteristics that cause it to be a sink for contaminants (Billah et al., 2022; Maiti and Chowdhury, 2013). For example, mangrove sediment can be a sink for environmental trace metal pollution and PAHs due to adsorption and desorption, chemical and biological factors, and precipitation and diffusion, as well organic matter content (Chatterjee et al., 2009; Girones et al., 2021). Certain factors also are found to increase the resident times of contaminants. For example, sediment can become a

reservoir for PAHs for years or decades when bound to clay fractions or high OM content material (Ukalska Jaruga and Smreczak 2020). OM content also has a relationship with MP distribution, though the exact nature of this relationship appeared to be conflicting. Some studies found more MPs where sediment had higher OM and less sand and other studies found no relationship between these factors (Maes et al., 2017; Vianello et al., 2013; Li et al., 2019). Macro-organic matter such as dead and decaying roots may also have a relationship to contaminants, for example dead roots were found to correlate to higher levels of the POP toxaphene due to the development of many cracks and fissures that offer additional sites for adherence (Girones et al., 2021; Gallagher et al. 1979).

Sediment texture and grain size can influence contaminant distribution. MP films and fragments accumulate in muddy sediments and foam shaped MPs accumulate more in sandy sediments (Zhou et al., 2020). For trace metals, fine grained sediments had greater concentrations at some sites (Soto-Jiménez & Páez-Osuna, 2001; Kulkarni et al., 2018). Likewise, Fe and Mn can form complexes with metals to form either oxyhydroxides or mix with organic carbon to create sulfides. This complexing can reduce the bioavailability and toxicity of metals (Kulkarni et al., 2018). One study concluded that sediment texture may be an important factor for controlling the deposition, fixation, and degradation of PPCPs (Beretta et al., 2014). For example, fine grained sediments had higher concentrations of fragrances when compared to coarse grained sediments (Beretta et al., 2014).

External processes can also control the distribution of contaminants. For example, factors that influence MP distribution include tidal flooding during full and new moons, and current velocity during flood and ebb tides (Lima et al., 2016; Zhang et al., 2020). Hydrodynamics, and coastal morphology can impact the deposition of MPs, with MP build up being favored where tidal activity is weaker and where there is a higher, flatter coastline (Ivar do Sul et al. 2014). Ivorra et al., (2021) found a connection between tidal flushing and lower OCPs in mangrove areas when compared to non-mangrove areas. It was speculated that this is due to upwelling forces from tides that suspend sediment particles with adhered OCPs. Some studies demonstrate that metal pollution can vary seasonally, with Zn and Hg being detected in higher levels before the monsoon season but Cu and Cd being higher after the monsoon phase (Clark et al. 1997, Sankar et al., 2010). Other extreme weather events may affect the distribution and species of heavy metals, with one study detecting them at higher levels after a tsunami (Seralathan et al., 2006; Babu et al., 2007).

3.1.4 Sources of contamination to the environment

While distribution of contaminants in mangroves is controlled by complex physical and biological factors, high concentrations are correlated with anthropogenic and industrial activity for all categories. For example, Guayaquil, Ecuador and other locations that were highly polluted with TMs are in close proximity to heavy industrial action (Kulkarni et al., 2018). For PAHs and POPs there was a correlation with industrial effluent such as electroplating plants, and electronic waste recycling (Robin & Marchand, 2022; Fusi et al., 2016; Nozar et al., 2013). Non-industrial effluent, such as urban sewage and WWTP effluent can be a source of MPs and is a primary source of PPCPs into the environment (Deng et al., 2021; Bayen et al., 2019; Chaves et al., 2020).

While industry and WWTPs are some of the main sources for contamination in mangrove ecosystems, there are other activities that can produce contamination. Agriculture is a source of many POPs. PAHs can originate from combustion of biomass, wood, coal, and rice husk, as well as from black carbon, vehicular emissions, and contaminated runoff from urban areas (Billah et al., 2022). PAHs can also originate from oceanic sources such as from shipping and oil spills, from oceanic oil production and transport (Billah et al., 2022). Oceanic MPs may originate from tourism, shipping, active fishing gear, “ghost” or abandoned fishing gear, and adjacent aquaculture (Clark et al., 2016; Frère et al., 2017; Li et al., 2018b).

3.1.5 Data Gaps

Common recommendations for sediment research include the need to understand the biogeochemical cycling of contaminants in mangroves, particularly when it pertains to tidal flushing and how contaminants move through the sediment-water-plant interface. In their review, Ivorra et al. (2021) credited tidal flushing as a possible reason for lower contaminants in mangrove ecosystems than non-mangrove coastal ecosystems. Robin & Marchand (2022) recommended the use of hydrological modeling to track the flux of PAHs to adjacent environments and highlight the need to better understand how organic matter influences the sequestration and sorption of contaminants, including how MPs sorb other contaminants (Table 1-3). As sediment can be released from mangroves during storm events, understanding the linkages between mangroves and adjacent marine ecosystems, which may be sensitive to contaminants, is an important area of future research. Several of the reviews highlighted the need to direct attention towards emerging contaminants including perfluorooctanoic acid and related compounds (for POPs), and 7H-benzo[c]fluorene and dibenzo[a,l]pyrene (for PAHs) (Girones et al., 2021, Billah et al., 2022).

A significant hindrance to MP research in mangrove ecosystems is the lack of an established sediment quality guideline or standard through which to indicate when MP concentrations are at environmentally harmful levels. Redondo-Hasselerharm et al. (2023) provides one such standard for freshwater sediments, while also drawing attention to how the lack of standardization in collection, MP count, and MP identification methodologies for sediment MPs hinders the development of scientifically sound standards.

Reviews have suggested that global contamination in mangrove environments needs to be better understood and documented (Prasannakumari Meera et al., 2021; Girones et al., 2021). However, the asynchronous nature of contamination research has made a coherent picture of global hotspots for pollution difficult. Future research should focus on targeting the locations of greatest contamination for each category and surveying them for a more complete suite of contaminants. In this way, the most at-risk mangroves can be efficiently identified.

3.2 Flora and Fauna

3.2.1 Extent of study by location and hotspots

The particular characteristics of mangrove ecosystems facilitate a unique mixture of flora and fauna, both resident and transient (Yeo et al., 2021). Fish from adjacent ecosystems such as coral reefs come to mangroves to use the sheltering roots provided by mangroves as nursery habitat, burrowing crabs and filter feeds such as oysters colonize the mud and root structure of mangroves, and both migratory and non-migratory birds utilize the ecosystem (Buelow & Sheaves, 2015; Nagelkerken et al., 2008;). Human beings also extract protein from mangroves, making them a part of the mangrove food web. Because of this, it is important to understand how contamination might be cycling through the flora and fauna of mangrove ecosystems. There have been fewer studies examining contaminant concentrations and toxic effects in mangrove flora and fauna when compared to the number of studies on sediment contamination (Figure 1-2). Most research in this environmental compartment has occurred on TMs (N=87), followed by MPs (N=31), PAHs (N=23), and PPCPs (N=2). The total number of studies for POPs in plants and animals was not reported. Geographical research still favors Asia over other regions (Figure 1-2). For PAHs, hotspots were found for fauna in Guanabara Bay, Brazil (N=62,398 ng/g), and Gangasagar, India (N=5919.23 ng/g). The remaining values reported for PAHs in fauna were ~1300 ng/g or below. The highest values for MPs in biota were found in Indonesia in fish, the maximum for which was 511.33 items/individual in the digestive tract and 372.63 items/individual in the respiratory organs. The next highest value was 9.0-59 MPs/individuals in the Beibu Gulf, China. Currently no data available for MPs

in mangrove plants (Deng et al., 2021). There is not enough research on PPCPs in mangrove biota to identify hotspots.

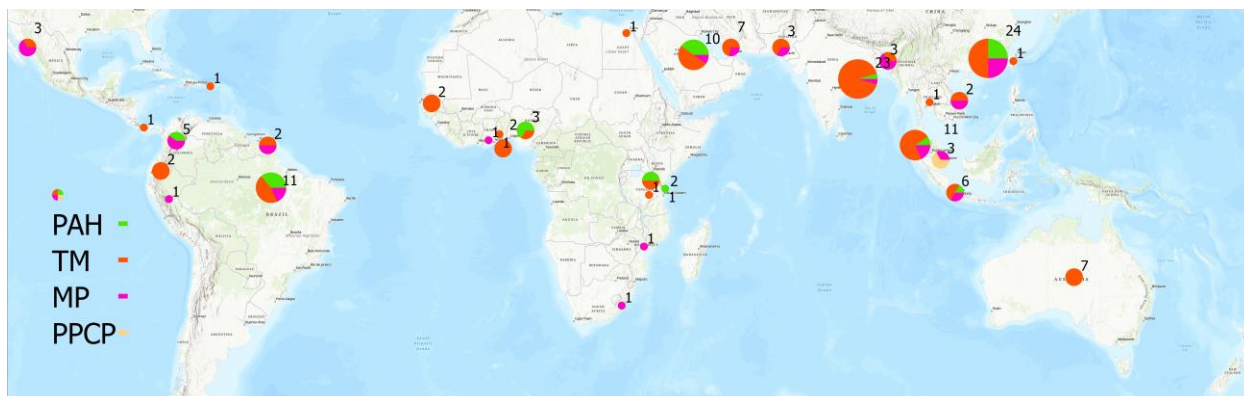


Figure 1-2. Locations (total publications across classes each country) of research in mangrove biotic compartments for TMs, POPs, PAHs, PPCPs, and MPs

3.2.2 Flora

Plants are known to accumulate contaminants, and past studies have demonstrated the toxic effects of POPs and PAHs on plant physiology (Girones et al., 2021; Billah et al., 2022). For example, plants can be adversely affected by POPs through damage to cell ultrastructure and impairing the biosynthesis of important molecules such as proteins (Zhang et al., 2017). PAHs can damage plants via inhibiting growth and damaging the cell membrane (Naidoo & Naidoo, 2016; Zaalishvili et al., 2002). There are also potential synergistic effects between contaminants, such as PAHs potentially inhibiting production of metallothioneins, proteins important to the detoxification of metals (Gauthier et al., 2014). Despite the potential harm that contaminants can cause, reviews demonstrate that natural processes of mangrove sediment can mitigate toxicity. For example, radial oxygen loss from mangrove roots triggers the precipitation of an Fe plaque through oxidation that can immobilize some POPs such as PBDEs (Pi et al., 2017). Mesocosm studies have indicated that up to 25% of PBDEs were immobilized by these plaques (Pi et al., 2017). Similar mesocosm studies found that the Fe plaque immobilized up to 20% of PAHs and that less than 1% of total PAHs were detected in plant tissues (Pi et al., 2017). Plants also employ antioxidant enzymes that can alleviate the oxidative stress that POPs can cause (Liu et al., 2017; Wang et al., 2014).

Plants can become contaminated with PAHs, POPs, and TMs through absorption or translocation (Billah et al., 2022; Girones et al., 2021; Kulkarni et al., 2018). However, concentrations of contaminants and the routes through which they are accumulating within plants is still being investigated (Girones et al., 2021; Robin & Marchand, 2022). For example, PAH was found to accumulate in leaves through atmospheric deposition which represents an understudied pathway for organic contamination (Billah et al., 2022; Simonich & Hites 1995; Orif & El-Maradny, 2018). For TMs, a poor correlation between metals in leaf tissues and adjacent sediment indicates that plants may selectively uptake metals or that metals have low bioavailability (Kulkarni et al., 2018; MacFarlane et al., 2003). However, when a higher metal concentration is found in leaves, the samples tend to have been collected from areas of high overall concentrations of sediment metals (Kulkarni et al., 2018). Young leaves and fine nutritive roots show higher concentrations of metals than mature leaves and other plant parts, with roots demonstrating the highest concentrations overall (Kulkarni et al., 2018; Nath et al. 2014). Mangroves such as *A. germinans* demonstrated some ability to concentrate heavy metals into senescent leaves, indicating phytoextraction potential for heavy metals (Maldonado-Román et al. 2016). However, this may indicate that mangrove leaf litter can be an avenue for these metals to re-enter and be dispersed through the environment (Kulkarni et al., 2018). The greater accumulation of metals in root tissues than other plant parts suggests

that metals have low mobility once in plants, with Cu demonstrating the highest mobility (Kulkarni et al., 2018).

Girones et al. (2021) found that mangroves were capable of limited absorption of various types of POPs. For example, mesocosm studies found that mangroves usually only absorbed 0.5% to 1% of POPs into their tissues (Chen et al., 2015; Chen et al., 2017; Farzana et al., 2019; Li et al., 2020). Additionally, the efficiency with which mangrove plants uptake contaminants via their roots is highly variable by species, with conflicting reports on how factors such as the lipophilicity of the contaminants themselves factor into plant uptake (Girones et al., 2021; Qiu et al., 2019b; Li et al., 2019). When investigating POPs in plants, the translocation factor (TF) or, the concentration of a contaminant in aboveground tissues divided by the concentration of the contaminant in roots, differed significantly between experiments in the field versus laboratory experiments (Girones et al., (2021). Laboratory experiments found no significant correlation between TF and lipophilicity, whereas some field studies detected a correlation (Gaeckle 2016), possibly because plants are exposed to air and water in the environment in a way that is not replicable in laboratory conditions. These contradictions highlight the difficulty of getting accurate results from mesocosm experiments. Similarly, in laboratory settings and in situ experiments, transfer of PAHs from roots to shoots seemed limited (Jia et al., 2016; Lu et al., 2011; Orif & El-Maradny, 2018). No research papers have Investigated PPCPs in mangrove flora

3.2.3 Fauna

Contaminants from all categories were found in mangrove fauna (Kulkarni et al., 2018; Girones et al., 2021; Deng et al., 2021; Billah et al., 2022; Bayen et al., 2012). MP numbers in fish and invertebrate tissues are highly variable by individual and species, ranging from 0 to 511 items/individual (Deng et al., 2021; Huang et al., 2020; Not et al., 2020). 13 studies have reported on PAH concentrations in mangrove fauna (Billah et al., 2022) with concentrations ranging from .028 (ng/g) in fish sampled from Zanzibar to 62,398 (ng/g) in bivalves sampled from Guanabara Bay, Brazil (Billah et al., 2022; Nudi et al. 2007). Only two research articles discussed PPCPs in mangrove fauna, both of which were in Singapore: Bayen et al., (2016) found BPA in the bivalves *P. viridis* and *P. expansa* (25.9– 207.1 ng/g). Bayen et al., (2019) studying fragrances in mussels found methyl triclosan (0.4-114 ng/g), traseolide (<1.3-45.9 ng/g), galaxolide (7-727 ng/g) phantolide (<0.8-13.2 ng/g), celestolide (<1-81.0ng/g), tonalide (<11-638 ng/g).

Factors influencing contamination in mangrove fauna include external factors such as season and weather and physiological/morphological factors such as feeding behavior, body length and weight, and habitat. For example, fauna can be exposed to PAHs via direct exposure in sediments or water, or via ingestion of contaminated bivalves, litterfall, and other OM (Zuloaga et al., 2009). MP and TM contamination seemed to preferentially accumulate in gills and digestive tracts, while few studies found MPs in muscle and livers (Kulkarni et al., 2018; Baskaran and Prabhahar, 2013; Barboza et al., 2020). As discussed above, contamination can vary seasonally, and this is reflected in faunal contamination, where metal concentrations in fish during the monsoon season exceeded the safe permissible limits set by the FAO (Kulkarni et al., 2018; Baskaran and Prabhahar, 2013). While there are some areas where metals exceed food safety standards or may pose a danger to benthic organisms, Kulkarni et al. (2018) conclude that the overall concentrations of metals were below what would be considered a threat to mangrove ecosystems or associated human populations.

For factors influencing MP accumulation in mangrove fauna, the relationship between fish body length and weight and number of MPs is inconclusive with some studies suggesting a correlation and other studies finding none (Deng et al., 2021; Huang et al., 2020; Not et al., 2020). The physical density of MPs influenced accumulation in biota, with lighter plastics remaining suspended in the water column while heavier particles sank to the sediment where they became more available to filter feeders and burrowing

animals (Deng et al., 2021). Some studies found that carnivorous species had a higher number of plastics than omnivorous or herbivorous organisms, while other studies indicated that omnivorous species had higher MPs (Garcés-Ordóñez et al., 2020; Hastuti et al., 2019; Markic et al., 2018). Larger body mass and being at a higher trophic level also contributed to high OCPs in mangrove fauna, however there was an exception found for mudskippers, an herbivore (Ivorra et al., 2021), with dermal adsorption listed as a possible reason for their high OCP concentrations (Bayen et al., 2005).

3.2.4 Data Gaps

From the reviews gathered, the toxicity of the different contaminants on mangrove flora and fauna remains unclear. Most of the reviews demonstrate the potential for each contaminant class to have a toxic effect on mangrove biota while highlighting the need for further research to confirm the real-world consequences of pollution in mangrove ecosystems (Table 1-3). More work is needed to understand how plants concentrate, adsorb, or breakdown contaminants (Table 1-3) (Kulkarni et al., 2018; Girones et al., 2021; Deng et al., 2021). The cycling of contaminants through the environment and how they interact with each other is poorly understood. For example, MPs can adsorb other contaminants such as organic chemicals and represent a pathway for chemicals to be introduced into the mangrove biota (Deng et al., 2021). Further, POPs and PAHs have some ability to translocate to leaves, even if the contaminants are mostly immobilized at the roots (Girones et al., 2021; Robin & Marchand et al., 2022). This means as mangroves absorb or adsorb contaminants, they may turn into a source of contamination following their natural expiration, or the shedding of senescent leaves (Girones et al., 2021). Understanding how contaminants move through biotic and abiotic components is particularly important for MPs and POPs, which are reported more frequently at values that may be environmentally harmful in sediment (Giornes et al., 2021; Deng et al., 2021; Redondo-Hasselerharm et al., 2023).

The lack of information on how contaminants may affect mangrove flora and fauna individually and synergistically, is a significant data gap. Additionally, the weaknesses of mesocosm studies suggest the need for more real-world investigations into the toxic effect of contaminants on mangrove flora and fauna (Girones et al., 2018). Finally, studies across contaminant classes highlight the need to identify an effective bioindicator species, with multiple species including bivalves and mangrove trees proposed as candidate species (Table 1-3). The mangrove species *Avicennia marina* was indicated to good candidate species for the biomonitoring of TMs (Wilda et al., 2020) as *A. marina* has a good capacity to absorb the metals Hg, Cr, Cu, Cd, Pb, Mn, and Zn through their roots while minimizing toxicity (Wilda et al., 2020). *A. germinans* was also listed as a species appropriate for TM phytoremediation (Kulkarni et al., 2018).

3.3 Bioremediation in mangrove ecosystems

3.3.1: Extent of study by location

Mangrove ecosystems are known to remediate contaminants through microbial metabolism or phytoremediation (Giornes et al., 2021). Recent global reviews touch briefly on the role of mangrove microbiota and flora in the remediation of contamination and two recent topical reviews address this subject in more depth. In the review for POPs, 14 papers addressed microorganismal-mediated degradation of pentachlorophenol (PCP) and PBDE only (Girones et al. 202021), 10 studies discussing microbiota associated bioremediation of PAHs (Filgueiras de Almeida et al., 2021), and six studies about MPs addressed biodegradation by microbes native to mangroves (Prasannakumari Meera et al., 2022).

3.3.2 Results

Mangrove microbiota have the ability to break down organic chemicals by dehalogenation, a process by which the toxicity of organic pollutants can be reduced (Girones et al., 2021; Luo et al., 2015).

Some compounds such as BDE-209 can be transformed almost completely to a less toxic variant, and when sterile and non-sterile conditions were compared, remediation was higher in non-sterile conditions in all reported cases (Girones et al., 2021; Yang et al., 2016). However, microbial degradation of organic contaminants in mangroves is complex and controlled by a variety of physical and chemical factors such as tidal cycles and salinity (Filgueiras de Almeida et al., 2021; Hemkemeyer et al., 2015). For example, a higher PBDE reduction rate occurred in mangrove sediments exposed to daily tidal changes (Tam et al., 2016). This is borne out in the review of PAHs, which found that degradation in mangrove ecosystems was variable, with studies finding differing rates of breakdown (years to decades) and different percentages of total degradation (e.g., 42.5-74.5%) (Burns and Yelle-Simmons 1994; Govarthanan et al. 2020). One factor that influences microbial metabolism and degradation is the presence and ratios of electron acceptors such as oxygen (O_2), and sulfate (SO_4^{2-}) (Filgueiras de Almeida et al., 2021; Souza et al. 2018; Hemkemeyer et al., 2015; Pan et al., 2017). POPs may compete with other electron accepting compounds common in the mangrove environment such as Fe_3+ and SO_4^{2-} and to a lesser degree NO_3^- and CH_4 . One of the more important factors controlling dehalogenation is the quantity of electron donors and acceptors present in the sediment (Chen et al., 2015; Ding & He, 2012; Giornes et al., 2021; Cheng et al., 2019). Further, the rate of dehalogenation in naturally anaerobic wetland sediment can vary by contaminant species, sediment characteristics, and microorganism community (Zanaroli et al., 2015; Giornes et al., 2021). Some authors found that highly halogenated POPs such as PBDEs were not degraded at all following 30 days of aerobic incubation (Zhu et al., 2014; Arbeli, 2009). Contradicting this, other studies found that PCPs experienced dehalogenation under both aerobic and anaerobic conditions (D'Angelo & Reddy, 2000). Sediment characteristics such as high OM may interact with PAHs and POPs to cause them to become biologically unavailable, hampering microbial degradation (D'Angelo & Reddy, 2000; Zhu et al., 2014a). Though this relationship is complex, one study found that organic carbon content was positively correlated with PCP degradation under methanogenic conditions (D'Angelo & Reddy, 2000).

A. germinans was also listed as a species appropriate for the phytoremediation of TMs (Kulkarni et al., 2018). Wetland plants may improve the biodegradability of POPs by dehalogenation in aerobic conditions through radial oxygen loss from their roots (Colmer et al., 2003; Carvalho et al., 2011; Huesemann et al., 2009). However, the authors noted that most of the studies investigating remediation of POPs by mangroves and wetland plants were done via mesocosm studies, with field studies often less conclusive and/or contradictory (Giornes et al., 2021). Mangrove plants contribute to the remediation of POPs in the environment at the sediment-root interface (Girones et al., 2021). Plants can secrete exudates such as organic acids and inorganic minerals that increase microbial activity in ways that improve bioremediation of POPs such as PBDEs (Hu et al., 2019). Root exudates may also assist with biodegradation by acting as biosurfactants, which encourage the mobility, desorption, and bioavailability of POPs (Farzana et al., 2019; Huesemann et al., 2009). These exudates can also contribute to phytoremediation by causing the desorption of contaminants from the sediment, allowing for easier uptake by plant roots (Ivorra et al., 2021; Jia et al., 2016). Wetland plants may improve the biodegradation of POPs that can be de-halogenated in aerobic conditions through radial oxygen loss from their roots (Colmer et al., 2003; Carvalho et al., 2011; Huesemann et al., 2009).

3.3.3 Data Gaps

The role of mangrove flora and microbiota in remediating contaminants requires more investigation to understand how mangrove microbes metabolize contaminants, which species and genes are associated with that process (Table 1-3), how environmental conditions affect population dynamics (table 1-3) and which environmental factors affect microbe species distribution and metabolic activity (Girones et al., 2018; Prasannakumari Meera et al., 2022; Billah et al., 2022). As there are differing results between field and lab studies, longer term research could be done, supported by lab research, in order to better assess contamination in complex environmental conditions.

Table 1-3. Areas for future research as suggested by reviewed literature.

<i>Suggestion</i>	<i>TMs</i>	<i>POPs</i>	<i>MPs</i>	<i>PAHs</i>	<i>PPCPs</i>
Identify Bioindicator from mangrove flora or fauna	- novel bioindicators (Kulkarni et al.,)			- crabs, fish, mussels suggested (Robin and Marchand) crabs and barnacles suggested (Billah et al.,)	
How OM impacts contaminant sorption and bioavailability			- How sediment OM impacts contaminant sorption potential of MPs (Deng et al.,)	- OM component impact on PAH sequestration (Robin & Marchand) - bio-availability of PAH in sediment, compare intra- species bioaccumulation (Billah et al.,)	
accumulation, and toxic effect on flora, fauna, and biota	- Investigate predators and humans harvesting from mangrove (Kulkarni et al.,)	- toxic effects on microbiota, absorption, behavior, toxic effect on plants (Girones et al.,)	- flora (Deng et al.,) - fauna with different life cycles, mangrove transient species, flora (Maghsodian et al.,)	- export of PAHs from mangroves to adjacent ecosystem (Robin & Marchand) - Bioconcentration from air to flora, adopt fish embryo toxicity assessment, bioaccumulate up food chain (Billah et al.,)	- ecological risk (Bayen et al.,)
Remediation	- plants and microbiota (Kulkarni et al.,)	- improve phyto remediation technologies (Girones et al.,)	- investigate in-situ, mp-biofilm adhesions & how biofilms degrade MPs, biofilm traps (Prasannakumari Meera et al.,)	- metabolic routes, effect of physicochemical and nutritional patterns for microbiota (Filgueiras de Almeida et al.,)	- microbial degradation of estrogens (Froehner et al.,)
Genetics and metabolism behind microbial degradation		- which species degrade POPs and how (Girones et al.,)	- which species and genes, adaptive characteristics related to MP exposure, research into improving degradation through gene editing, use multi-omics (Prasannakumari Meera et al.,)		
Increase and standardize monitoring	- focus on macrobenthos, perform more modeling studies (Kulkarni et al.,)	- include emerging emphasis, near cities and garbage recycling (Girones et al.,)		- (Robin & Marchand) - establish sediment quality guidelines, emerging PAHs, dated cores/historical assessment (Billah et al.,)	- more PPCP monitoring of coastal ecosystems globally (Beretta et al.,) - emerging contaminants (Chaves et al.,)

<i>Human risk from mangrove contaminants</i>	- investigate toxic effect in predators + associated human pops (fisherman, etc.) raise public awareness to support sustainable resource actions and restoration (Kulkarni et al.,)			- legacy and emerging (Billah et al.,)	
<i>Multidisciplinary research and/or multi-contaminant study</i>			- MPs adsorption of other contaminants; how aging affects MP adoption (Deng et al.,) - standardize multidisciplinary research (Maghsodian et al.,)	- Other contaminants interaction with microbiome, PAH effect on mangrove metal tolerance (Robin & Marchand)	
<i>Reduce contaminants entering into mangrove</i>	- (Kulkarni et al.,)		-Maghsodian et al.,		
<i>Biogeochemical cycling</i>	- cycling of metals through water-sediment-plant interface (Kulkarni et al.,)	- wetland physical characteristics effects on POP cycling (Girones et al.,)		- Hydrological modeling of flux of PAHs to adjacent ecosystems (Robin & Marchand)	
<i>Other Suggestions</i>	- Support sust. resource management & increase aquaculture (Kulkarni et al.,)		- use of mangrove microbiota for bioplastic production (Prasannakumari Meera et al.,)	- how environmental conditions affect microbiota population dynamics (Filgueiras de Almeida et al.,)	

4.0 Recommendations for Future Research and Conclusions

4.1 Recommendations for future work

There are common themes and conclusions across the reviews that can be synthesized to guide future contamination research. For example, the need for standardization of monitoring and sediment quality guidelines was mentioned by multiple reviews, particularly for MPs (Deng et al., Billah et al., Redondo-Hasselerharm et al., 2023). This suggests an opportunity to explore the creation of standards that account for multiple contaminants. Further, priority mangroves should be subject to more extensive surveys, when possible; this will establish valuable baseline information. As many contaminants originate from point sources such as WWTP, these baselines will provide valuable information to measure future response from mangrove ecosystems following improved effluent treatment programs. Mangroves in particular are effective monitoring locations, because, as is established above, they serve as endpoints

sequestering most kinds of contaminants. As contamination research can be prohibitively expensive, contaminants from categories found infrequently could be dropped or surveyed at less frequent intervals.

The proximity of human populations to mangroves, and the reliance of many human communities on mangrove ecosystems for sustenance was discussed in multiple reviews (Table 1-3). Billah et al. (2022) talked about the human element in the context of emerging PAH contaminants that are more toxic than legacy PAH contaminants. These emerging contaminants are associated with urban activities such as industry which increases their likelihood of endangering humans fishing in urban adjacent mangroves. Kulkarni et al. (2018) recommended that TM bioconcentration be investigated in predators and human fisher-people.

As a more holistic approach to environmental contamination, humans could be considered an important ecosystem compartment as humans are vulnerable to the bioconcentration of contaminants from mangrove derived protein (Hossain et al., 2021). Further, the single-contaminant focus of ecotoxicological research undermines the ability to classify the most vulnerable human populations as multi-contaminant hotspots remain mostly unidentified. Kulkarni et al., (2018) brought up the importance of raising public awareness for contamination research and engagement, but the literature on its use and success is scarce both for ecology generally and ecotoxicology specifically (Field et al., 2007). Engagement around contamination issues can be difficult as it can lack visibility to the public, and when identified the prohibitive cost of analysis can leave few options for remediation (Arriaza et al., 2018).

The reviews identify the geographic inconsistency of available data with research concentrated around Asia and Oceania (Figure 1-1, 1-2). Why these geographic biases exist is barely addressed in the reviews, though one obstacle to more in-depth research in mangrove ecosystems maybe the prohibitive cost of analysis which can restrict research to well-funded government agencies while excluding NGOs and student research. For example, Amoah et al. (2006) noted that monitoring of pesticide residue was minimal due to expense for the responsible authorities. Abbas et al. (2004) discussed the prohibitive expense for some developing countries to monitor aflatoxin. And Frazzoli et al., (2020) observed that in sub-Saharan African, constraints on scientific funding limited toxicological studies. Whatever the reason, the lack of participation globally causes several challenges. It prevents chemicals from being studied and understood more holistically, particularly emerging contaminants of concern, making achieving a more thorough understanding of how mangroves may degrade and remediate contaminants difficult; and it prevents an understanding of the global distribution of mangroves contaminants. Understanding why these research barriers exist is important, and in the future, contamination research and mitigation would benefit from being made more accessible.

4.2 Conclusions

Mangrove flora have defenses that mitigate the harm of some contaminants such as POPs and TMs, can remediate or immobilize PAHs and some POPs, and TMs and PAHs were highlighted as low risk to mangrove plants at most sites due to low concentrations (Kulkarni et al., 2018; Girones et al., 2021; Billah et al., 2022; Robin & Marchand 2022). For mangrove fauna, POPs and MPs were more frequently found at environmentally harmful levels, and PPCPs lack sufficient investigation and reporting to determine whether existing levels cause damage to biota. Assessing the danger that environmental contamination poses to humans and mangrove ecosystems is undermined by the lack of research into synergistic effects between different types of contaminants. Thus, multi-disciplinary and multi-contaminant research should be considered to better understand how contaminants may be interacting. Increasing the efficiency and lowering the cost of contamination monitoring by, for example, selecting biomonitoring species that are adequate across multiple contamination categories may help to address the lack of contamination research in mangrove ecosystems.

The reviews called attention to the regional data gaps that exist for the distribution of contaminants in mangrove ecosystems, suggesting the need for expanded monitoring of environmental pollution. However, little discussion was given to the reasons why some areas such as China and Malaysia receive more research, and what barriers may exist that prevent the expansion of contaminant monitoring. Future research should address these gaps and provide methodologies for increasing access to contamination research. Finally, contamination research in mangrove ecosystems may benefit from considering the human association to mangrove forests. Community outreach, monitoring, and investigation into the consequences of contaminated mangrove biota on human health may draw attention to contamination research and increase funding and opportunities.

Chapter 2: Influence of Non-native *Rhizophora mangle* on Coastal Contamination on Moloka'i Hawaii

1.0 Introduction:

Mangrove ecosystems are unique brackish forests that provide numerous and valuable ecosystem services in tropical and subtropical latitudes and are responsible for a disproportionate amount of coastal biodiversity (Saenger et al 1983; Lefcheck et al., 2018). This is due in part to the complex and 3-dimensional root baffles formed by mangrove root systems, which provide shelter for numerous species of fish and invertebrates (Nagelkerken, 2009). These baffles also slow the flow of water, which reduces coastal erosion while contributing to the accretion of sediment and sheltering upland areas from storm surges and extreme weather events (Augustinus 1995; Badola & Hussain 2005; Gilbert & Janssen, 1998). Mangrove root systems enrich the belowground organic matter (OM) of coastal areas, at the same time, sequestering contaminants (Kristensen et al., 2010; Vane et al., 2009) and sheltering downstream ecosystems such as reefs and kelp forests from contaminated runoff (Barbier et al., 2011).

Despite the ecological value that mangroves provide, they are in decline globally due, primarily, to forest clearing to liberate space for urban development, aquaculture/ agriculture, or as a source of charcoal (Saenger et al., 1983; Fortes, 1988; Malik 2016). Further, mangroves are frequently proximate to urbanized areas, which applies other pressures on mangrove ecosystems such as the intensification of anthropogenic contamination (Defew et al., 2005; Girones et al., 2021; Kulkarni et al 2018). Mangroves possess physical characteristics that lead them to be an endpoint for many of these contaminants including the physically complex aboveground and extensive belowground root structure produced by mangroves that trap waste and adsorbs organic contaminants (Kristensen et al., 2010; Martin et al., 2020).

Unfortunately, ecotoxicological research in mangroves lags in many areas, two such under-researched categories are microplastics and organic contaminants (Deng et al., 2021; Bayen, 2012). Plastics are one of the pollutants frequently found in coastal environments generally, and mangrove ecosystems specifically (Luo et al., 2021). Plastic enters the coastal environment from harbor activities, urban runoff, sewage, and circulates through the environment via sea currents and tidal activity (Thushari & Senevirathna, 2020; Sbrana et al., 2022). Microplastics (MPs) are defined as any plastic equal to or less than 5 mm in length, with larger pieces being defined as macroplastics. When present in the environment plastics can be consumed by fish and invertebrates such as corals, filling stomachs without providing nutrition (Reichert et al., 2018). These plastics can also adsorb chemical contaminants such as persistent organic pollutants, providing another avenue for these substances to enter the food chain (Savoca et al., 2021). Further, plastic production has been increasing, currently exceeding 390 tons annually (Hachem, 2023). When plastic trash ends up in mangrove forests, mechanical forces such as weathering from tidal action and exposure to sunlight can cause these larger plastics to fragment and break down to microplastics (Jahnke et al., 2017). While these microplastics may degrade further, potentially into nanoplastics, it is unknown if they will ever truly break down into non-plastic substances and may linger in coastal wetland environments such as mangroves for years (Davranche et al., 2020; Paduani et al., 2020).

Organic contaminants are a broad category of chemical species that come from a variety of origins including agriculture, industry, and personal use products (Girones et al., 2021). Many of these chemicals have long half-lives and possess characteristics such as hydrophobia which together cause them to persist for long periods of time in mangrove sediment and water (Jones et al., 1999; Bayen et al., 2021). Additionally, organic contaminants have the potential to bioaccumulate up the food chain, threatening mangrove fauna and human populations that rely on mangrove forests for food (Bayen et al., 2019).

Sea level rise is another threat to mangrove forests, particularly those associated with urban environments where they may be subject to coastal squeeze (Schleupner, 2008). This occurs when coastal ecosystems are prevented from retreating landward from rising sea levels due to hard urban structure along the landward edge which is a common circumstance for mangroves (Schleupner, 2008; Tam & Wong, 2000). Simultaneously, warming temperatures due to global climate change (GCC) are allowing mangroves to expand poleward along their original ranges leading to the unique circumstance of non-native mangrove invading and modifying other kinds of ecosystems such as salt marshes (Alongi et al., 2015; Saintilan et al., 2014). Currently, there is little research into how the alterations to the mangrove environment from the changing environmental conditions around mangroves or the how mangrove invasion affects the sequestration of pollution. If mangrove invasion increases a coastline's capacity to sequester contamination, it becomes important to understand how that contamination may be released when mangroves are cleared for urban development, harvested for charcoal, or pushed out by rising sea levels.

One area that has been modified by mangroves is Moloka'i, Hawaii where mangroves were introduced in 1902 to address erosion resulting from feral grazing animals and agricultural activities (Allen, 1998). Following introduction, mangrove quickly spread to other islands altering the Hawaiian coastline (Wester et al., 1981). Mangrove removal has been ongoing around Hawai'i. This necessitates an understanding of how mangroves influence the distribution of contamination in the intertidal zone and the risk of contamination release associated with its removal.

A study was designed to investigate how non-native mangroves control microplastics and organic contaminants relative to open coast habitat to address the following research questions:

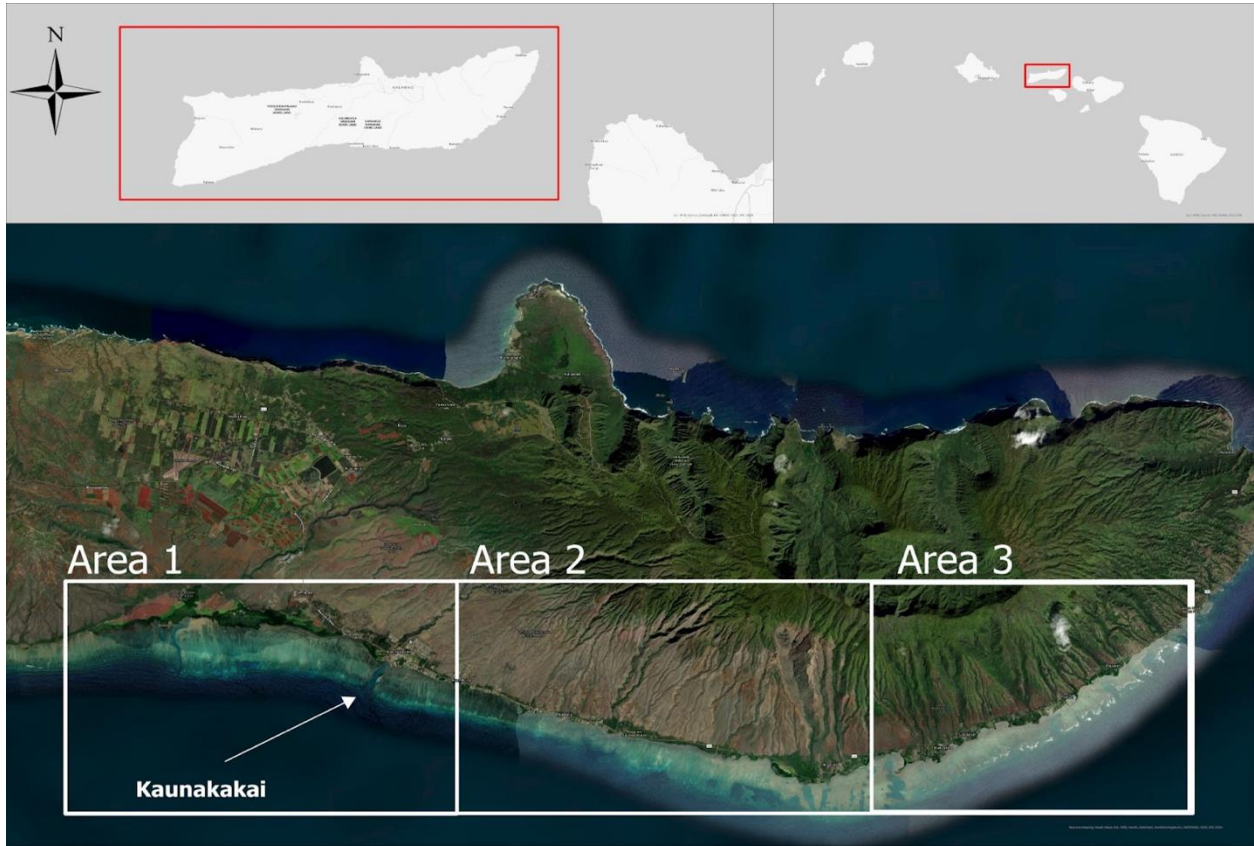
1. Does the presence of non-native mangrove influence the distribution of contamination along the coast?
2. Which ecosystems compartments in non-native mangrove are contaminated and what is the implication for mangrove removal?

2.0 Methods

2.1 Study sites

As mangroves have been removed from many areas in Hawai'i, the island of Moloka'i was selected due to the presence of large mangrove stands remaining on the island. Moloka'i also has recent and agricultural activities that takes place on the island, which creates a reasonable expectation for finding some level of pesticide contamination. The study took place along the southern coastline of Moloka'i Hawaii, which was selected due to the remaining mangrove presence on the island (Figure 2-1). Moloka'i is the fifth largest and fifth most populated Hawai'ian Island with a total size of 673.4 km² and a population of 7,287 at the time of the study (U.S. Census). The population is concentrated onto a relatively small land area along the southern coastline where the largest town on the island, Kaunakakai, had a population of 3,419 when the study took place (U.S. Census). The primary industries on Moloka'i are agriculture and ranching, with tourism comprising only a small part of annual revenue. Agriculture is largely concentrated in the northwestern part of the island. The central and eastern parts of the island are dominated by the Moloka'i forest preserve and are relatively unpopulated. The island's major thoroughway, HW state route 450 traverses the island east to west and is adjacent to many of the sites sampled for this study. Fishponds dot the coastline along the area selected for the study, with some currently in active use for aquaculture and education. Fishponds operate by using stone blocks to create enclosed spaces around a section of intertidal and shallow subtidal zone; entry and exit of water is

controlled through gates which prevents fish from exiting. Several study sites are located within these fishponds.



Area 1				Area 2				Area 3				
1-FMS	2-CCG	3-NPT	4-MSK	5-ALL	6-MI9	7-OLW	8-KWU	9-WCR	10-PLA	11-NIP	12-KPK	13-LEN
Sea	Sea	Sea	Sea	Sea	Sea	Open	Sea	Sea	Sea	Sea	Sea	Sea
	Open	Open	Open	Sea 2	Open		Sea 2	Open			Open	Open
				Open	Land		Open	Land				
							Open 2					

Figure 2-1. Location of sampling site split into three areas, abbreviated site names present in each area, and which transect types were present at each site. Specific sampling locations and names left out at landowner request.

2.2 QA/QC

Plastic is a ubiquitous environmental contaminant also common in lab settings. To prevent plastic contamination from entering samples while processing, several precautions were taken. Dyed pink clothing was used at all times in the lab and field, additionally all clothing and lab coats worn in the lab were cotton. All deionized (DI)-water used to rinse equipment or used as part of processing, as well as all reagents used for processing were passed through a 20-micron sieve before use. All equipment was triple rinsed before use, and for this methodology rinsing refers to using 20-micron sieved DI water specifically. Airfall controls were prepared using sieved DI water and rinsed glassware. These controls contained a small amount of sieved DI water and were opened each time samples were exposed to the air to account for airborne microplastic particles; this includes glass jars used as environmental airfalls while collecting samples in the field. Finally, procedural controls were used with each round of processing; these controls received the same treatments and reagents as the samples, but did not receive any sample.

For pesticide analysis, all sampling equipment was rinsed with water daily. All glassware was acid washed with 10 percent HCl solution and then rinsed with DI water and dried prior to field work. Foil used to contain sediment and plant tissue samples was triple rinsed with tap water prior to use.

2.3 Field Methods

Samples were collected in March of 2022 from 13 paired open coast and mangrove sites along the habituated southern shoreline of Moloka'i. Sites were selected based on accessibility and included areas of differing urban development. At each site, up to three ~30 meter transects were measured parallel to the shore along open coastline (control), the seaward edge of mangrove stands (sea), and, when accessible, the landward edge of mangrove stands (land). In total, three sites contained all three transects, five sites contained paired mangrove and open coast transects, three sites contained just seaward edge mangrove transects, and two sites contained just open coast transects for a total of 27 transects.

Transects were arranged by placing pink plastic flags 10 meters apart. Sampling points were placed in the root zone along the seaward edge of the mangroves (sea transects), and along the landward edge of the mangrove zone (land transects), where accessible. At open coast sites, sampling points were placed about one meter below the low tide line, roughly equivalent to where the points were placed along seaward edge mangrove transects.

To survey MPs, hydrophobic and hydrophilic pesticides, sediment and porewater were collected at all transect points. Additionally, root, leaf, and propagule tissue samples were collected at transects where mangroves were present. Porewater samples were collected by inserting an MHE stainless steel PPX36 PushPoint sampler ~25 cm into the sediment. A glass syringe which was used to draw out the porewater was attached to the sampler using Tygon® PVC Tubing. Porewater was then stored in 100 mL glass jars with metal screw tops. Aluminum foil rinsed with tap was inserted below the cap to keep out environmental contamination. Sediment samples were collected using a 20-inch Eijkelkamp Threaded Peat Sampler. To collect the sediment cores, the peat sampler was inserted into the surface of the sediment to a depth of about 30 cm. After collection, the core length and hole depth were recorded and the sediment cores were wrapped tightly in rinsed and pre-labeled foil.

To create a representative root sample, root balls were collected from three randomly selected areas within the root zone of a tree at the transect point. To collect the root balls, a four-inch sediment knife was inserted to the hilt and a roughly cube-shaped chunk of sediment was carved out. Then the three root balls from a single plot were combined and homogenized into one sample before being tightly wrapped in rinsed and pre-labeled foil. Leaf and propagule samples were collected by hand by plucking three randomly selected specimens from different branches or trees adjacent to the transect point. Specimens were then tightly wrapped in rinsed and pre-labeled foil. All samples were transferred to a cooler with ice upon collection.

2.4 Lab Methods

Organic contaminant (pesticide) analysis was quantified by a collaborating USGS Organic Chemistry Research Laboratory at the California Water Science Center, Sacramento. The expense of organic contaminant analysis limited the analysis to a subset of sediment, porewater, and plant tissue samples. In all, 37 sediment samples, 11 root samples, 12 porewater samples, 4 leaf samples, and 5 propagule samples were analyzed. Extraction and quantitation methods follow those of Black et al., 2023.

In house processing was done to separate microplastics from sediment and porewater using different density separation methodologies.

2.5 Porewater

Porewater MP extraction was accomplished through a series of three sequential density separations using DI water saturated with NaCl (Fisher Scientific®) to achieve a density of approximately 1.2 g/mL. Saturation was accomplished by slowly adding salt to a 2-liter glass mason jar that was being stirred on a hotplate until the salt no longer fully dissolved into solution. This salt solution was then filtered through a 20-micron sieve prior to use as lab grade salt may be a source of microplastic contamination. Following each addition of salt solution, the samples were allowed to settle overnight. The samples were then filtered through a MilliporeSigma™ Isopore™ Polycarbonate Membrane Filters, 10 µm filter with the aid of a vacuum filtration apparatus. The filter was then transferred to a rinsed petri-slide for drying. The salt water was kept after the sample filtration process and salt was slowly re-added until saturation was achieved, indicated by the inability for salt to fully dissolve into solution. With the first addition of salt solution 1-2 drops of olive oil were added to the sample as prior research indicates this helps prevent MP's from sticking to the surface of the glass beaker (Karlsson et al., 2017). As biofouling may alter the behavior of MPs in a salt solution, 50 mL of filtered 50% hydrogen peroxide solution was added with the third addition of salt solution to oxidize organic matter.

2.6 Sediment

Sediment cores were divided in a clean fume hood for organic contaminant and microplastic analysis prior to processing. Cores were first divided vertically into two segments, before being divided further horizontally into three ~eight cm sections using a clean sediment knife (Figure 2-2). This process resulted in a top, middle and bottom core section for each form of analysis. As some cores were shorter than 25 cm due to compression or an inability to collect larger cores the bottom section was omitted.

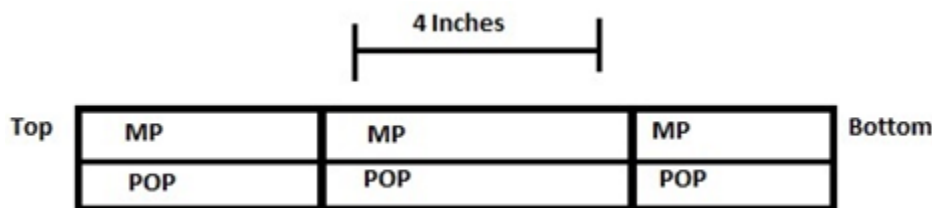


Figure 2-2. Schematic demonstrating how cores were divided prior to analysis

Microplastics were separated from sediment using a protocol described by Silva Paes et al. (2022) with small modifications. Briefly, the samples were transferred to a drying oven set at 70°C to dehydrate to a constant weight over three days. After careful homogenization of the dry sediment, about ~50 g from each sample were divided across four rinsed 50 mL plastic centrifuge tubes. 35 mL of the prepared zinc chloride (ZnCl₂) solution (Nasco®, density 1.6 g/mL) was then added to each centrifuge tube. The tubes were shaken thoroughly for 1 minute to mix the sample and reagent, then centrifuged at 3500 rpm for 15

minutes. After centrifugation, the supernatant from each tube associated with a single sample was poured through a rinsed 20-micron sieve to collect the MPs. The 20-micron sieve with the remaining sample was then thoroughly rinsed using sieved DI water into a clean and rinsed glass beaker. 50 mL of filtered 30% hydrogen peroxide (H₂O₂) was then added to the samples to oxidize any adhered organic matter. After being left overnight in the H₂O₂ solution, the contents of the beakers were filtered onto a 10-micron filter using a rinsed vacuum filtration unit and stored in a Petri slide for drying and microscopy.

The collected ZnCl₂ solution was then filtered through a 10-micron filter with the aid of the vacuum filtration apparatus and re-used up to three times to reduce the waste from and cost of the experiment. Research indicates that the zinc chloride can be reused up to five times without significantly reducing the efficacy of the density separation (Rodrigues et al., 2020). For this study ~75% of the samples utilized zinc chloride that was either fresh or had only been used once before.

Percent organic matter was calculated using the sediment loss on ignition protocol (Heiri et al., 2001). A core dedicated to sediment characteristics was collected from each transect at the middle transect point. To perform percent organic matter analysis, each layer of the core was homogenized, and a portion of the layer was transferred to a tin weight boat of known weight. After drying at 70°C for three days, the dry weight for each sample was recorded. Following this, the samples were subjected to 550°C heat for four hours. The dry weight following this combustion process was recorded for each sample. This process was repeated three times. Percent organic matter was determined by calculating the percent difference between the dry weight of the sediment before and after combustion.

2.7 Microplastic analysis

Microplastics were counted and identified using a Zeiss Primostar 3 microscope with Labscope software under both 10× and 4× magnification. Any particle that was at or less than 5 mm in length, without cellular structure, even throughout its length, and did not easily break under pressure from a metal probe was identified as a possible microfiber. The only other potential type of microplastic found were films, which were characterized as uniform in color, and difficult to break. Suspected plastics were photographed, after which the color, size, and form were recorded. A subset of these plastics was sent to a collaborating lab at Oregon State University for chemical identification using microscope Fourier transform infrared spectroscopy (u-FTIR).

2.8 Standardization

The amount and length of the sediment cores varied due to compaction, which is a natural result of core collection. Due to this, some cores were missing the bottom sections, and the amount of sediment processed for microplastic analysis varied by weight. To account for this, a standardized total of items per kilogram for each transect was calculated through the following formula:

$$(1000/\text{combined weight (g) from all samples in transect}) \times (\text{Total MP (items) from all samples in transect})$$

The porewater was similarly standardized to items/liter with the following formula:

$$(1000 \text{ mL}/120 \text{ ml, which is the sum of the 40 ml collected from each sample in the transect}) \times (\text{total MPs from transect})$$

2.9 Statistical Analysis

Average percent organic matter was determined by calculating the difference between the dry weight of the sediment before and after combustion, then averaging the value from all three trials by top, middle, and bottom layers. After determining the average percent OM content for each layer, the layers were averaged together to achieve average transect OM.

USGS Streamstats was used to determine for each site the basin characteristics drainage area, mean basin elevation, percent area with slopes greater than 30 percent, and basin relief divided by basin perimeter. Rstudio v. 2022.12.0 was used to generate boxplots to visually check basin characteristics' significant differences between mangrove and non-mangrove sites. ArcGIS Pro v. 2.8 was used to calculate population and urbanization (percent impervious surface) within one km of the sites. A generalized linear model (GLM) was constructed to investigate how well MP abundance along the coast of Moloka'i was explained by mangrove presence using rStudio, with differences considered significant at or below the 0.05 level. The model included the alternate explanatory variables level of urbanization within one kilometer of the site (percent impervious surface), percent organic matter of the transects, drainage area, average elevation, basin slope, basin relief, population within one kilometer of the site, and position along the coast line (longitudinal site coordinates). Variation inflation factor (VIF) was used to account for multicollinearity between predictor variables by removing variables from the model in a stepwise fashion until all variables had less than three VIF. As there was correlation between population and urbanization, population was dropped from the model. The model was improved in a stepwise fashion with Akaike Information Criterion (AIC) until no further improvement was achieved (Appendices A).

3.0 Results

3.1 Microplastic Totals

Microplastics were found in sediment at 10 of the 13 sites, and 21 of the 27 transects (Figure 2-3) and in porewater at 12 of the 13 sites, and nine of the 27 transects (Figure 2-3). The average microplastics found per transect in sediment and porewater after standardization to items/kg and items/L were 8.49 and 16.36 items respectively (Figure 2-3). Values from a single plot are contributing disproportionately to the higher porewater concentration (Figure 2-3). Sediment microplastics were most abundant at sites 2-CCG and 5-ALL, both of which were near the major population center of Moloka'i (Figure 2-1). In porewater, the highest concentration of MPs was found at site 6-MI9 with all plastics found in the land transect of the site (Figure 2-3). 6-MI9 had the highest concentration overall. For sediment, sea transects had an average of 7.67 microplastics, while open transects had 9.01 and land transects had 10.12 microplastics (Figure 2-4). In porewater there were an average of 10.89 microplastics in the sea transect, 9.85 microplastics in the open transect, and 63.89 microplastics in the land transect (Figure 2-4).

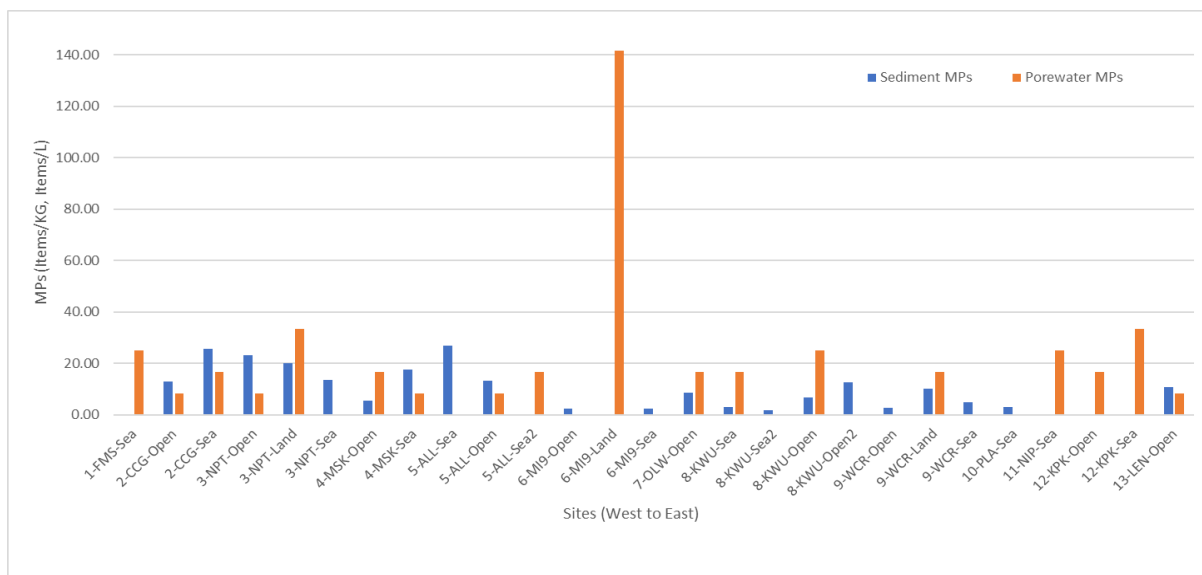


Figure 2-3 Total MPs for porewater and sediment at each site.

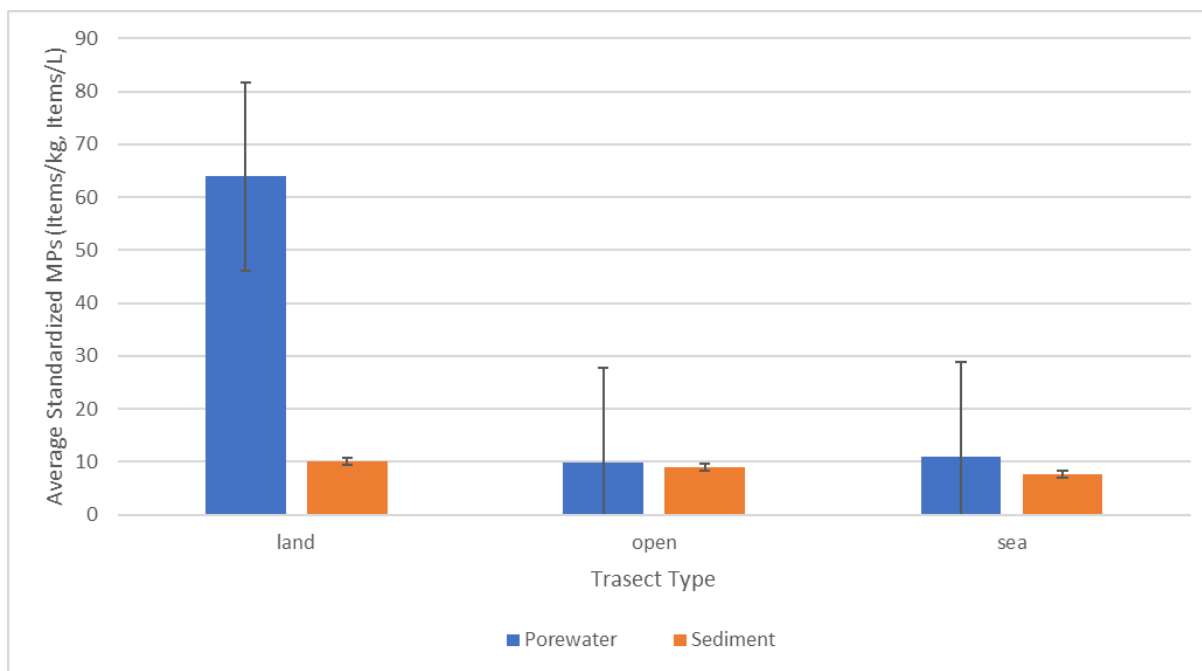


Figure 2-4. Bar plots with standard error representing microplastic abundance in open, sea, and land transects for porewater and sediment.

3.2 Microplastic form and color

Fibers, films, and fragments were the only microplastic forms detected in this study. Microplastic forms were predominantly 2-4 fibers in both sediment (93%) and porewater (96%) (Figure2-5). Films were the second most commonly detected microplastic in sediment (6%) and porewater (3.6%), followed by a solitary fragment found in sediment. Clear was the predominant microplastic color, totaling ~55% in sediment and ~53% in porewater. The next most frequent color was blue for porewater (35%), and tan for sediment (27%). Gray fibers were also detected in low numbers in both compartments.

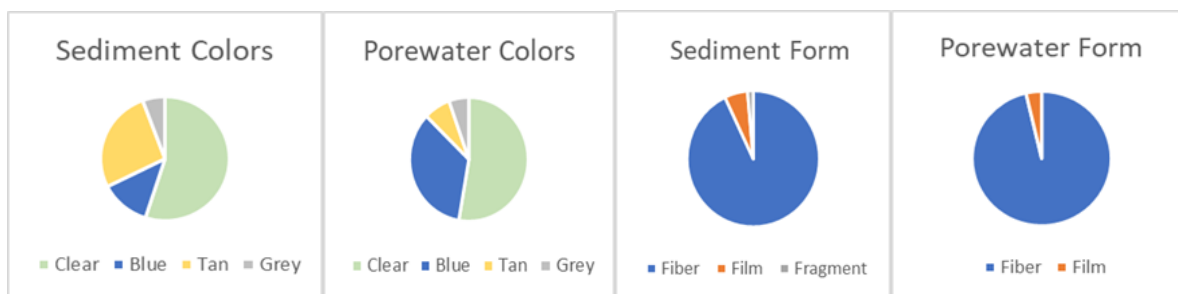


Figure 2-5. Distribution of MPs in sediment and porewater by form and color.

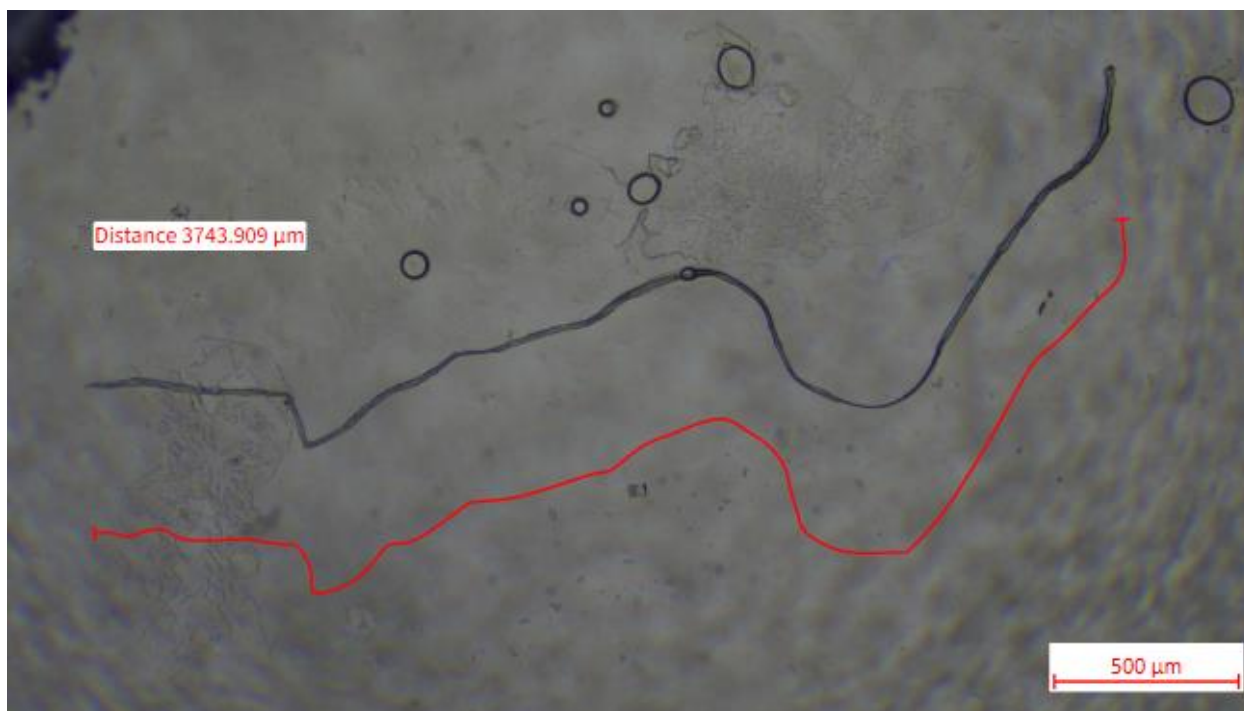
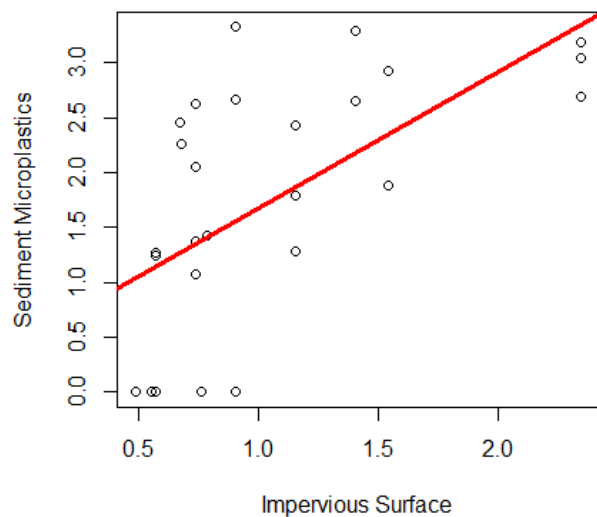


Figure 2-6. Example microfiber at 4x magnification. Sample ID KWU-Sea 2 Porewater 2.

3.3 Relationship between microplastics, mangrove presence, and basing characteristics

No significant differences were observed between mangrove and non-mangrove areas for basin characteristics (Appendices C). The results for percent organic matter, percent impervious surface within one kilometer, and population within one kilometer are presented in Table 2-1. The relationships between sediment and porewater MPs and percent impervious surface, basin characteristics, population within 1 km, and fishpond presence were explored through linear regression (Figure 2-7). There was no significant relationship between the number of microplastics in porewater and any of these parameters ($P > 0.05$) (Appendices D). Through regression analysis via AIC, it is evident that the most appropriate predictor of sediment MP abundance is urbanization. A slight positive linear relationship were found between sediment MPs and percent impervious surface within one kilometer ($R^2 = 0.33$ $P = 0.00096$, $F=14,25$) (Figure 7). The selected model meets the main assumptions of linear regression, i.e.: normality of residuals (Shapiro-Wilks: $P=0.08$, $W=0.93263$), Homoscedasticity (F test for variance: $P=0.2967$, ratio of variances=1.86), no multicollinearity (values with $Vif > 3$ removed from model), and linearity (Figure 2-8).



Statistic	Log % Impervious Surface within 1 km ²
N	27
R ²	0.33
P	0.000959
F	14,25

Figure 2-7 Relationship between Impervious Surface and sediment MPs

Table 2-1 Numbers indicating percent organic matter, impervious surface and human population within one kilometer of each site

Predictor Variable	1- FMS	2- CCG	3- NPT	4- MSK	5- ALL	6- MI9	7- OLW	8- KWU	9- WCR	10- PLA	11- NIP	12- KPK	13- LEN
Percent OM	5.23	6.07	15.05	7.72	7.83	7.54	5.86	5.77	7.92	4.47	5.48	5.47	11.18
percent impervious	0.63	3.09	9.44	3.69	1.47	0.77	0.98	1.09	2.17	1.2	0.74	1.14	0.96
population within 1 km ²	0	242	647	253	30	1	34	13	99	1	14	67	19
Slope (Percent)	144	287	354	374	641	1020	820	924	1170	1160	1000	985	731
Relief (Feet)	1500	1030	4130	437	2270	2840	4950	1580	4600	4470	2140	2850	2010
Drainage Area (Mi ²)	14	0.99	9.04	0.21	0.97	1.59	6.66	0.46	2.02	1.72	0.63	0.54	0.49
Elevation (Feet)	498	388	1780	173	1100	928	2430	531	1920	1730	601	1060	812

3.4 FTIR analysis and Controls

FTIR analyses showed that ~47.5% of analyzed items were synthetic and semi-synthetic, ~29.5% were anthropogenically modified materials, and 23% were natural. The most common types of plastic were polyethylene terephthalate (PET) (31%), polyamide (24%), and polypropylene (13%). Other synthetics include polyvinyl alcohol, polyesterterphthalate, Teflon, and polyester. Analysis of processing and airfall controls demonstrate that on average 1.4 per MPs were present per sample (Table 2-2). Most contamination was airfall from the laboratory.

Table 2-2 Average contamination found in controls per sample in each transect from sample collection, sample processing, and sample partitioning. Control values were added together to calculate the total average MPs contamination for each porewater and sediment sample. *All samples associated to one control bottle which burst in freezer. **Lost in transport.

Site	MP Field control: per sample (transect / # of samples) (avg) (# MPs)	MPs Partitioning per Sample (avg) (# MPs)	MPs per sample processing (all steps) (avg) (# MPs)	Final count per sample (# MPs)
1-FMS-Open	0.5	3.47	0.21	4.18
2-CCG-Open	0.17	XXX*	0.05	0.21
2-CCG-Sea	0	XXX*	0.09	0.09
3-NPT-Open	0	0.67	0.12	0.79
3-NPT-Land	0.56	1.33	0.08	1.96
3-NPT-Sea	0.22	1.85	0.09	2.17
4-MSK-Open	0.25	0.04	0.06	0.35
4-MSK-Sea	0.25	0.04	0.06	0.35
5-ALL-Sea	0	0.73	0.13	0.86
5-ALL-Open	0	XXX*	0.14	0.14
5-ALL-Sea2	0.25	0.9375	0.05	1.24
6-MI9-Open	0	1.7	0.03	1.73
6-MI9-Land	0	1.7	0.04	1.74
6-MI9-Sea	0.14	3.88	0.03	4.05
7-OLW-Open	0.14	0.84	0.08	1.06
8-KWU-Sea	0.25	0.83	0.09	1.17
8-KWU-Sea2	0.08	0.291	0.06	0.43
8-KWU-Open	0.17	0.5	0.07	0.74
8-KWU-Open2	0.14	0	0.06	0.2
9-WCR-Open	0	2.13	0.09	2.22
9-WCR-Land	0	1.5	0.06	1.56
9-WCR-Sea	XXX**	2.13	0.07	2.2
10-PLA-Sea	0	2.4	0.08	2.48
11-NIP-Sea	0.29	0	0.13	0.41
12-KPK-Open	0.25	1.31	0.06	1.62
12-KPK-Sea	0	1.25	0.07	1.32
13-LEN-Open	2.29	0.05	0.08	2.41

3.5 Organic Contaminants (Pesticides)

Each sample was analyzed for 178 pesticides as part of a multi-residue pesticide analysis, of these there were detections for six contaminants. The average concentrations and number of detections is summarized in Table 2-3. The most commonly detected contaminant was bifenthrin, found in 96% of sediment samples, 100% of root samples, 20 % of propagule samples, but not found in any leaf or porewater samples. The next most common detection was p,p'-DDE (N=7), followed by three contaminants which had 3 or less detections and average concentrations less than one ng/g. The most notable result was the clear difference between the average concentration of Bifenthrin in roots (243.31 ng/g) and sediment (Open=9.29 ng/g; Sea=11.30 ng/g) (Table 2-3). High concentrations of bifenthrin

were found at multiple sites across the island, and do not seem to be associated with proximity to the population center (Figure 2-8).

Table 2-3. Average pesticide concentrations (by transect type and environmental compartment) detected in the subset of samples analyzed (ng/L for porewater; ng/g dry weight for sediment, roots, propagules and leaves), including sample size (N), and number of detections (following concentrations).

COMPARTMENT (# OF SAMPLES ANALYZED)	BIFENTHRIN AVG (NG/G, NG/L) (RANGE, # OF DETECTIONS))	IMIDACLOPRID AVG (NG/G, NG/L) (RANGE, # OF DETECTIONS)	PERMETHRIN AVG (NG/G, NG/L) (RANGE, # OF DETECTIONS)	P.P'-DDE AVG (NG/G, NG/L) (RANGE, # OF DETECTIONS)	P.P'-DDT AVG (NG/G, NG/L) (RANGE, # OF DETECTIONS)	TRIFLURALIN AVG (NG/G, NG/L) (RANGE, # OF DETECTIONS)
SEDIMENT SEA (N=22)	12.27 (0 - 94.96, 22)	0	0	0.62 (0.20 - 1.03, 2)	0	0.40 (1)
SEDIMENT OPEN (N=13)	9.29 (0.16 - 39.23, 13)	0	0.24 (1)	0.55 (0.32 - 0.95, 3)	0.32 (1)	0.30 (0.20 - 0.39, 2)
SEDIMENT LAND (N=2)	0.08 (0 - 0.16, 1)	0	0	0	0	0
Porewater (N=12)	0	37.1 (30.4 - 43.7, 2)	0	0	0	0
Roots (N=11)	243.31 (34.95- 870.79, 11)	0	0	11.57 (2.82 - 20.3, 2)	0	0
Propagule (N=5)	8.60 (1)	0	0	0	0	0
Leaf (N=4)	0	0	0	0	0	0

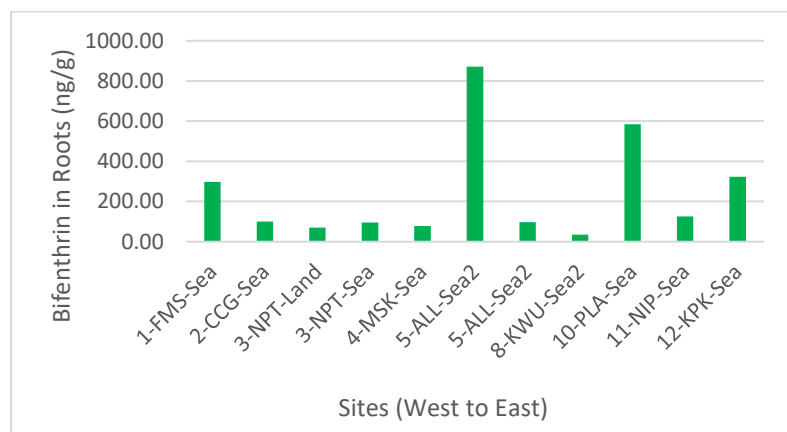


Figure 2-8 Concentrations of Bifenthrin in mangrove roots (ng/g)

4.0 Discussion

Both MPs and organic contaminants are underreported from the Hawaiian Islands, and this study was the first to look at MPs and pesticides on Moloka'i. Microplastic numbers observed in this study were low compared to results reported globally (Deng et al., 2021). Previous work reports MPs in mangrove sediments as low 1.22 items per kilogram to as high as 6390 items per kilogram (Deng et al., 2021). Approximately 5 of the 39 investigations reported fewer average plastics than found in this study (Deng et al., 2021).

The slight linear relationship between impervious surfaces and population within one kilometer and sediment MPs is in line with global trends for mangrove MPs (Figure 2-7). In other studies, the strongest factors determining microplastic distribution in mangrove sediments are land use activities such as proximity to urban development and population dense areas, and outputs from sewage (Deng et al., 2021). Shipping is another source of MPs into the mangrove environment; with the main port of Molokai adjacent to Kaunakakai, shipping may contribute to the Molokai MP contamination along the coast (Deng

et al., 2021). Further, the two most common types of polymers detected, PET (31%) and polypropylene (13%), are a frequent by-product of single use plastics (Chen et al., 2021). The other commonly detected plastic was polyamide (24%). Polyamide is a component of nylon and its source is associated with clothing and fishing nets. Together these plastics account for 68% of plastics found in the sediment and porewater samples. As the MPs in this study likely originated from single use plastics and clothing, the association between MPs and impervious surfaces is logical. However, the analysis for MPs was limited by the small number of sites.

MPs enter mangroves from both seaward and landward directions which contributes to the high levels of MPs seen in mangroves broadly (Deng et al., 2021). The lower levels of contamination found in this study for both MPs and organic contaminants could be reflective of the small population on Moloka'i, which may indicate lower contamination from the landward direction. The sheltered location of Moloka'i within the center of the Hawaiian Island chain may also contribute to the smaller number of MPs seen in this study if it is reducing MP contamination from the seaward direction. However, the lack of previous work investigating MPs across the islands makes it difficult to understand how localized currents may affect the distribution of MPs along the coast. Further research should be done to understand if contamination is low on Moloka'i only or Hawaii broadly.

Bifenthrin was the most commonly detected pesticide and was found at low concentrations in all root samples and nearly all sediment samples. Further, the concentration of bifenthrin was found to be much higher in roots (average=243.31 ng/g) than sediment (average = 11.17 ng/g) (table 2-4). This suggests that bifenthrin is concentrated at mangrove roots. Bifenthrin is a pyrethroid insecticide that sees widespread use and is commonly found in the environment (Delgado-Moreno et al., 2011).

Bifenthrin is toxic to aquatic ecosystems, with lethal concentrations reported from *ex-situ* studies to be as low as 0.10 ng/g for some species of fish and 8 ng/g reported for aquatic invertebrates, suggesting that the concentrations seen in this study (0.16 – 870.79 ng/g) may be at environmentally toxic levels (Yang et al., 2018; Bifenthrin Pesticide Fact Sheet, U.S. EPA 1998; Anderson et al., 2008). Vascular plants can remove POPs via up taking them through the roots, where they may be translocated to above ground tissues (Girones et al., 2021) However, the lack of Bifenthrin detections in leaves and propagules suggests that Bifenthrin may translocate poorly from roots to other plant parts (Girones et al., 2021).

Pyrethroid insecticides including bifenthrin are hydrophobic and insoluble in water so tend to bind to organic matter in sediments, higher OM content has been found to increase the retention of POPs while reducing its bioavailability (Gammon et al. 2012; Maul et al., 2008); Zhu et al., 2014). Further, Hawaiian mangroves may lack the specialized communities of detritivores that have co-evolved to consume and break down the tannin- and lignin- rich detritus that mangroves produce (Kristensen et al., 2008; Demopoulos & Smith, 2007; Demopoulos & Smith, 2010). Understanding how the unique faunal assemblages interact with the accumulation and consumption of sediment OM will be important for understanding the bioavailability of Bifenthrin and other organic contaminants in the future.

Two other compounds were found at concentrations in excess of 1.0 ng/L or 1.0 ng/g, imidacloprid and p,p'-DDE. Imidacloprid, a hydrophilic compound, which was detected in porewater at KWU Sea and KPK Open transects. These sites are located away from Kaunakakai, but near a facility that is suspected to utilize imidacloprid. Imidacloprid is a neonicotinoid insecticide that is thought to have lower toxicity to fish and mammals, but is highly toxic to insects (Pietrzak et al., 2020). How imidacloprid cycles through the environment, including its relationship to sediment organic matter, is poorly understood (Pietrzak et al., 2020). p,p'-DDE, a breakdown product of p,p'-DDT, was detected in a number of locations (N=7), and was detected at concentrations of 2.82 and 20.3 ng/g in the root compartment. Use of DDT was banned in the United States in 1972, however DDT and DDE are highly persistent in the environment and adsorb strongly to sediment (Pereira et al., 1995).

The scope of the organic contaminant analysis was conducted on a limited number of samples, so follow up studies could confirm how widespread contamination is along the coast of Moloka'i. However, bifenthrin was found in 100% of the roots analyzed, but was retained poorly in other ecosystem compartments in this study. This result matches other investigations that have confirmed the tendency of the mangrove root layer to bind organic contaminants through various mechanisms such as the Fe plaque formed on roots through natural processes (Robin & Marchand, 2022). In light of these findings, future research can be refined to determine the bioavailability and long-term fate of Bifenthrin and other pyrethroid pesticides bound to mangrove roots. Determining these factors about pesticides will better inform ongoing and future mangrove removal. For example, if Bifenthrin remains adhered to roots while breaking down to less toxic substances over time, cutting mangrove trees flush to the sediment may help to maintain the benefits of mangroves (pesticide sequestration, erosion control) while eliminating a portion of its detriments (overgrowing fishponds, harboring invasive species). If Bifenthrin remains bioavailable while adhered to roots then complete removal of mangrove plants could be considered in order to prevent the contaminants from entering the food web. Further, as mangrove removal generally may release contaminants into the environment, the ongoing mangrove removal on Hawaii provides an opportunity to study how non-native mangrove systems store and then possibly release contaminants.

A limiting factor of this study was the lack of access to transects along the landward edge of the mangrove sites. Mangrove are known to help filter and sequester contaminants moving across the intertidal zone from the land towards the sea (Kulkarni et al., 2018). The highest microplastics reported in the study were found within porewater at the land transect 5-MI9 porewater (141.67 items/L) and overall land transects had higher average MPs in sediment and much higher MPs in porewater despite the limited sample size (2-3). However, the high concentrations seen in porewater are partially the result of a single high detection (Figure 2-3). With access to only three land transects, the sample is too small to contribute significantly to the results. Greater access to these transects could have further cemented a trend towards higher microplastics in these areas while allowing a deeper exploration of the role mangroves play in filtering contaminated runoff.

5.0 Conclusions and Recommendations

microplastic contamination is overall lower on Moloka'i than other locations around the world for (Degn et al., 2021; Maghsodian et al., 2022), and proximity to urban areas may be more important for the distribution of MPs along the coast than mangrove presence. Bifenthrin is the most commonly seen pesticide, and was bound to mangrove roots. Future research may determine if decaying mangrove roots become a source of bifenthrin contamination, which will, in turn, inform managers in Hawaii about the role of mangroves as a sink or source for contamination. Further research with larger sample sizes may elucidate the relationship between mangrove trees and coastal contamination on Moloka'i. This includes more samples taken from behind mangrove stands, as well as more research around the rest of the Hawaiian Islands to contextualize the Moloka'i results. Bifenthrin concentrations should be assessed in mangrove species and adjacent ecosystems as well to determine its cycling through coastal ecosystems.

Chapter 3: Mangrove Contamination in Hawaii and Globally

The main focus of this thesis is the ecotoxicology of native and non-native mangrove ecosystems. Both chapters together explore the role of mangroves in sequestering contaminants globally and in the context of non-native mangrove on Hawaii.

Chapter one of this thesis combined the results of recent reviews on contamination in mangrove ecosystems for each of the major categories of contaminants present in the literature. These contaminants are trace heavy metals (TMs), persistent organic pollutants (POPs), microplastics (MPs), polycyclic aromatic hydrocarbons (PAHs), and pharmaceuticals personal care products (PPCPs). The objective of this research was to synthesize the conclusions and suggestions from each of these analyses in order to find lessons that can be applied to contamination research broadly. The literature review specifically sought to answer the following questions:

1. What common lessons from the research into different environmental contaminants can be applied broadly to improve ecotoxicology in mangrove ecosystems?
2. Where are the gaps in contamination research in mangrove that need to be prioritized?
3. How can managers combine knowledge from differing contaminant categories to more holistically manage mangrove ecosystems?

This review of the literature found the contamination research in mangroves is concentrated in Asia, and less work is being done in Africa and the Americas. PPCPs are the most under researched contaminants and represent a major data gap in mangrove ecotoxicology. PPCP research is particularly important as this review highlights the association between PPCPS and proximity to urban areas and wastewater treatment plants, which mangroves are often adjacent too. While there are hotspots for every class of contaminant, of the reviewed contaminants MPs and POPs were found at concentrations that were potentially environmentally harmful with more frequency than TMs and PAHs. There is a lack of research that considers multiple contaminants. Multiple contaminant research offers opportunities, like working to identify bio-indicator species that are appropriate for multiple classes of contaminants which would simplify contaminant monitoring. Finally, public outreach and participation should be considered more frequently to improve contamination research.

Chapter two of this thesis investigated the concentration of key contaminants on Moloka'i, Hawaii with the goal of determining how the presence of non-native mangroves affects the distribution of MPs and organic contaminants along the coastline. Sediment, porewater, and plant tissues (root, leaf, propagule) were collected in March 2022. Samples were collected from paired open coast and mangrove sites so that the abundances of contamination at each site could be compared. MP abundances were determined in porewater and sediment. A broad-spectrum analysis of organic contaminants was done for a limited number of samples from each of the ecological compartments collected. MPs were not investigated in most plant tissues due to technical issues that will be explained below. The objective of these comparisons was to answer the following questions:

1. Does the presence of non-native mangrove control the distribution of contamination along the coast?
2. Which ecosystems compartments in non-native mangrove are contaminated and what is the implication for mangrove removal?

There were two primary results from the Hawaii project. First, in answer to one of the main questions of the study, no statistical relationship was detected between mangrove presence and sediment MPs ($P > 0.05$). However, there was a potential statistically significant relationship detected between the

variable percent impervious surface and sediment MPs ($P=.00096$). Statistical analysis indicates that ~33 percent of variation in sediment MP along the coastline was explained by this relationship ($R^2\approx.33$, $F=14.25$). Additional variables that were analyzed include population within one kilometer, fishpond presence, and percent organic matter of the transects. The basin characteristics drainage area, slope, relief, and average elevation were also used as predictor variables; however, no relationship was detected between these characteristics and coastal MPs. No statistically significant relationships were determined between any variables and porewater MPs. In this analysis the common pyrethroid insecticide bifenthrin was present in most ($n=35$) of the sediment ($N=37$), one of the propagule samples ($N=1$), and all of the root ($N=11$) samples. With a low sample size, it was determined that there was no statistical relationship between Bifenthrin and mangrove presence ($P>.05$). The root bifenthrin values were on average higher (Average=243.31 ng/g) than sediment concentrations (10.57 ng/g). Imidacloprid was detected in porewater at average concentrations 37.1 ng/L and p,p'-DDE was detected in roots at average concentration 11.57 ng/g. All other pesticide detections were below 1.0 ng/Lg. As pyrethroid pesticides are concentrated at the mangrove root layer, leaving the roots behind when engaging in mangrove removal activities may prevent those contaminants from reentering the environment. However, decaying mangrove roots may turn into a source of bifenthrin back to the environment, so more research needs to be done to determine the long-term fate of bifenthrin in mangroves on Moloka'i.

The results in the Hawaii investigation match several trends identified from the first chapter of this project. First, MPs were associated with proximity to Kaunakaka'i, the main settlement on Moloka'i, in line with the demonstrated trend between proximity to human population and MP concentrations. Second, bifenthrin concentrating at mangrove roots further expands a trend discussed in the reviews where mangroves develop iron plaques that immobilizes organic contaminants. However, mangroves are suggested to be sinks for environmental contaminants in the reviews, and while they do seem to be concentrating pyrethroid pesticides mangrove presence did not seem to have a relationship to coastal MPs. This result conflicts slightly with previous work. This disagreement might be due to the relative lack of MPs compared to other mangrove studies which may be the result of the small population on Moloka'i, the sheltered position of the island in the greater Hawaiian Island chain, the lack of maritime activity in the proximity of Moloka'i, and/or the localized currents around the islands.

Originally one of the goals of this project was to analyze MP concentration in mangrove leaf and propagule tissues. Unfortunately, these tissues proved too difficult to digest with traditional protocols and developing novel protocols was beyond the scope of this project. Future research both in Hawaii and outside of it should consider developing methodology to extract MPs from mangrove aboveground plant tissues as currently there has been little research in this area. The sediment collected from Moloka'i was extremely difficult to work with due to high root and above average OM content, and most traditional digest protocols failed to effectively remove MPs. The protocol that was selected had the downside of using toxic and expensive reagents. Projects expanding on this work should collect excess sediment for the purpose of refining MP extraction techniques and making them less environmentally costly. Future investigations should expand the MP surveys to other islands, which would help contextualize the results found on Moloka'i. Further, more samples should be collected from the landward edges of mangrove stands to better identify if mangroves are filtering contaminants from the landward direction.

Closing Thoughts

This project provided valuable insights on the status of contamination in mangrove ecosystems. One of the primary goals of this project was to determine if mangrove presence increases the coastal concentrations of contaminants in areas where it is not native. Here, it was demonstrated that other variables such as urbanization may be more important. Though this result may partly be due to the unique

circumstance of Moloka'i as a relatively unpopulated island in an isolated island chain. This project also deepens the understanding of the complex relationships between sediment OM, plant presence, and sediment contamination concentrations. Finally, the results provide information to the people of Moloka'i, who will hopefully be able to make more informed management decisions based on the results of this project.

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Appendices

Appendix A. AIC values for sediment and porewater from backwards stepwise modeling

Variables in Sediment Model	AIC
Transect + Fishpond Presence + Longitude + Impervious Surface + OM	-51.6
Transect + Longitude + Impervious Surface + OM	-53.57
Transect + Impervious Surface + OM	-54.61
Transect + Impervious Surface	-54.84
Impervious Surface	-55.54
Variables in Sediment Model	AIC
Transect + Fishpond Presence + Longitude + Impervious Surface + OM	33.21
Mangrove Presence + Fishpond + Longitude + Impervious Surface	31.22
Mangrove Presence + Longitude + Impervious Surface	29.23
Longitude + Impervious Surface	27.25

Appendix B. RStudio Code demonstrating linear model

```
mod.aic5<-lm(logsed1k~coast+mang+fishpond+Long+logperv+percent.om, data=proj)

rst.l5<-summary(mod.aic5)

round(rst.l5$coefficients,5)

summary(mod.aic5)

vif(mod.aic5)

mod.finalaic<-step(mod.aic5)

summary(mod.finalaic)

final<-median(predict(mod.finalaic))

(final1<-residuals(mod.finalaic)[predict(mod.finalaic)>final])

(final2<-residuals(mod.finalaic)[predict(mod.finalaic)<final])

var.test(final1,final2)

shapiro.test(residuals(mod.finalaic))

anova(mod.finalaic,mod.aic5)

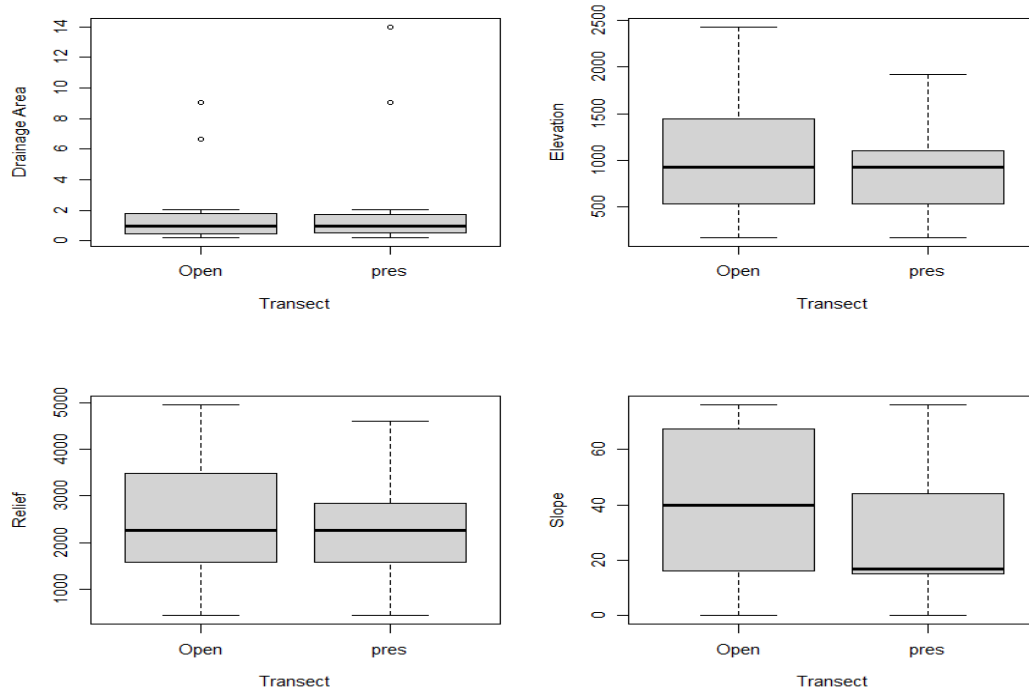
vif(mod.finalaic)

par(mfrow=c(1,1))

plot(logsed1k~logperv, data=proj, xlab="Impervious Surface", ylab="Sediment Microplastics")

abline(mod.finalaic, lwd=3, col='red')
```

Appendix C. Box plots demonstrating differences between basin characteristics for open (non-mangrove) and mangrove (present) sites



Appendix D. Non-significant relationships for porewater

P-Values, degrees of freedom, and F-Statistics for non-significant linear relationships in sediment and porewater

Porewater	P-value	F-Statistic
Percent Impervious Surface	0.93	.0072, 25
Population	0.819	0.0534, 25
Percent OM	0.724	0.127, 25
Fishpond presence	0.963	0.00218, 25

Appendix E. Table of Raw Data for Transect Physical Characteristics

Sites	Fishpond Presence	Percent Impervious Surface	Population Within 1 km ²	Percent OM	Drainage Area (Mi ²)	Elevation (Feet)	Slope (Percent)	Relief (Feet)
1-FMS-Open	No	0.63	0	5.23	14	498	144	1500
2-CCG-Open	No	3.09	242	6.83	0.99	388	287	1030
2-CCG-Sea	No	3.09	242	5.31	0.99	388	287	1030
3-NPT-Open	No	9.44	647	28.6	9.04	1780	354	4130
3-NPT-Land	No	9.44	647	10.37	9.04	1780	354	4130
3-NPT-Sea	No	9.44	647	6.18	9.04	1780	354	4130
4-MSK-Open	No	3.69	253	9.66	0.21	173	374	437
4-MSK-Sea	Yes	3.69	253	5.79	0.21	173	374	437
5-ALL-Sea	Yes	1.47	30	11.42	0.97	1100	641	2270
5-ALL-Open	Yes	1.47	30	7.16	0.97	1100	641	2270
5-ALL-Sea2	Yes	1.47	30	4.9	0.97	1100	641	2270
6-MI9-Open	No	0.77	1	9.22	1.59	928	1020	2840
6-MI9-Land	No	0.77	1	7.04	1.59	928	1020	2840
6-MI9-Sea	No	0.77	1	6.37	1.59	928	1020	2840
7-OLW-Open	No	0.98	34	5.86	6.66	2430	820	4950
8-KWU-Sea	Yes	1.09	13	5.05	0.46	531	924	1580
8-KWU-Sea2	Yes	1.09	13	6.49	0.46	531	924	1580
8-KWU-Open	Yes	1.09	13	4.1	0.46	531	924	1580
8-KWU-Open2	Yes	1.09	13	7.44	0.46	531	924	1580
9-WCR-Open	No	2.17	99	5.65	2.02	1920	1170	4600
9-WCR-Land	No	2.17	99	10.72	2.02	1920	1170	4600
9-WCR-Sea	No	2.17	99	7.39	2.02	1920	1170	4600
10-PLA-Sea	Yes	1.2	1	4.47	1.72	1730	1160	4470
11-NIP-Sea	Yes	0.74	14	5.48	0.63	601	1000	2140
12-KPK-Open	Yes	1.14	67	5.54	0.54	1060	985	2850
12-KPK-Sea	Yes	1.14	67	5.4	0.54	1060	985	2850
13-LEN-Open	Yes	0.96	19	11.18	0.49	812	731	2010

Appendix F. Table of Raw Data for Microplastic Samples Amount and Results for Each Transect

Transect	Grams of Sediment Processed Top Later	Grams of Sediment Processed Middle Later	Grams of Sediment Processed Bottom Later	Total Grams Processed Each Transect	Sediment Plastics (MP/Transect)	Porewater Plastics (MP/Transect)	Porewater Plastics (MPs/L)	Sediment Plastics (MPs/kg)
1-FMS-Open	175	0	0	175	0	3	25	0
2-CCG-Open	136	150	63	349	4	2	8.33	13.11
2-CCG-Sea	150	155	0	305	9	1	16.67	25.79
3-NPT-Open	97	163	90	350	8	1	8.33	23.12
3-NPT-Land	172	136	130	438	7	4	33.33	20
3-NPT-Sea	112	128	106	346	6	0	0	13.7
4-MSK-Open	100	36	42	178	1	4	16.67	5.62
4-MSK-Sea	176	50	0	226	4	1	8.33	17.7
5-ALL-Sea	85	64	0	149	7	0	0	26.82
5-ALL-Open	128	40	0	168	2	1	8.33	13.42
5-ALL-Sea2	139	122	0	261	0	2	16.67	0
6-MI9-Open	165	172	50	387	1	0	0	2.48
6-MI9-Land	115	106	25	246	0	17	141.67	0
6-MI9-Sea	168	180	56	404	1	0	0	2.58
7-OLW-Open	175	125	50	350	3	2	16.67	8.57
8-KWU-Sea	135	141	20	296	1	2	16.67	2.98
8-KWU-Sea2	137	152	103	392	1	0	0	1.92
8-KWU-Open	168	167	186	521	2	3	25	6.76
8-KWU-Open2	136	148	52	336	5	0	0	12.76
9-WCR-Open	163	157	60	380	1	0	0	2.63
9-WCR-Land	174	152	71	397	4	2	16.67	10.34
9-WCR-Sea	129	155	103	387	2	0	0	5.04
10-PLA-Sea	97	135	85	317	1	0	0	3.15
11-NIP-Sea	160	151	71	382	0	3	25	0
12-KPK-Open	132	136	83	351	0	2	16.67	0
12-KPK-Sea	121	71	0	192	0	4	33.33	0
13-LEN-Open	155	160	60	375	4	1	8.33	10.67

Appendix G. Table of Raw Data for Porewater Pesticide Analysis

Site, Transect, Plot	Medium	Mass (g) dry weight	Bifenthrin (ng/L)	Imidacloprid (ng/L)	Permethrin (ng/L)	p,p'-DDE (ng/L)	p,p'-DDT (ng/L)	Trifluralin (ng/L)
NPT-Open2	Pore Water (Dissolved)	0.0322						
NPT-Sea-2	Pore Water (Dissolved)	0.0391						
FMS-Sea-2	Pore Water (Dissolved)	0.0281						
KPK-Open-2	Pore Water (Dissolved)	0.0423		30.4				
ALL-Sea-2	Pore Water (Dissolved)	0.0199						
KWU-Sea-2	Pore Water (Dissolved)	0.0427		43.7				
OLW-Open-2	Pore Water (Dissolved)	0.0317						
WCR-Sea-1	Pore Water (Dissolved)	0.074						
MSK-Sea-1	Pore Water (Dissolved)	0.051						
MI9-Land-2	Pore Water (Dissolved)	0.08						
MI9-Sea-2	Pore Water (Dissolved)	0.072						

Appendix H. Table of Raw Data for Plant Tissue Pesticide Analysis

Site, Transect, Plot	Plant Tissue	Mass (g) dry weight	Bifenthrin (ng/g)	Imidacloprid (ng/g)	Permethrin (ng/g)	p,p'-DDE (ng/g)	p,p'-DDT (ng/g)	Trifluralin (ng/g)
FMS-Sea-2	Roots	0.2154	298.05					
NPT-Sea-2	Roots	0.2131	94.79			2.82		
MSK-Sea-2	Roots	0.19	77.89					
KWU-Sea-2	Roots	0.0515	34.95					
ALL-Sea-2	Roots	0.2136	870.79					
MI9-Sea-2	Roots	0.0882	97.51					
KPK-Sea-2	Roots	0.2148	323.09					
CCG-Sea-2	Roots	0.2065	99.76					
NIP-Sea-3	Roots	0.1493	125.92					
NPT-Land-2	Roots	0.2067	69.67			20.3		
PLA-Sea-2	Roots	0.2161	583.99					
NPT-Sea-2	Leaves	0.2096						
FMS-Sea-2	Leaves	0.2115						
ALL-Sea-2	Leaves	0.2121						
Kwu-Sea-2	Leaves	0.2009						
NPT-Sea-2	Propagules	0.2013						
FMS-Sea-2	Propagules	0.2047						
ALL-Sea-2	Propagules	0.2071						
KWU-Sea-2	Propagules	0.2094	8.6					
KWU-Sea-1	Propagules	0.212						

Appendix I. Table of Raw Data for Sediment Pesticide Analysis

Site, Transect, Plot	Core Segment	Volume (L)	Bifenthrin (ng/g)	Imidacloprid (ng/g)	Permethrin (ng/g)	p,p'-DDE (ng/g)	p,p'-DDT (ng/g)	Trifluralin (ng/g)
ALL-Open-2	Top	5.0322	3.54					
ALL-Open-1	Top	5.0395	20.28					
ALL-Sea-2	Top	5.08	18.94					
ALL-Sea-2	Middle	5.0234	25.2					
ALL-Sea-1	Top	4.985	29.13					
ALL-Sea-3	Top	5.0052	4.68					
CCG-Open-2	Top	5.0604	1.74					
CCG-Open-2	Middle	5.0578	0.16		0.24			
CCG-Sea-2	Top	5.0466	4.04					
CCG-Sea-2	Middle	4.9608	3.14					
CCG-Sea-2	Bottom	4.9522	0.48					
FMS-Sea-1	Top	4.9931	63.37					
KPK-Open-2	Top	5.0064	39.23					0.2
KPK-Open-2	Middle	5.0938	0.27					0.39
KPK-Sea-2	Top	5.0423	1.82					
KPK-Sea-1	Top	5.0407	0.67					
KWU-Sea-2	Top	5.0057	1					0.4
KWU-Sea-2	Middle	5.0117	0.16					
MI9-Land-2	Top	4.9534	0.32					
MI9-Land-2	Middle	5.1487	0.16					
MI9-Land-2	Bottom	5.0495	0					
MI9-Sea-2	Top	5.2787	0					
MI9-Sea-2	Middle	5.2002	0.69					
NPT-Open-2	Top	5.0634	1.5			0.32		
NPT-Open-2	Middle	5.03	0.76			0.38		
NPT-Open-2	Bottom	5.0445	0.71			0.95	0.32	
NPT-Sea-2	Top	5.02	6.14					
NPT-Sea-2	Middle	5.0015	4.32			0.2		
NPT-Sea-2	Bottom	5.0354	1.03			1.03		
OLW-Open-2	Top	5.0762	16.47					
OLW-Open-2	Middle	4.998	9.68					

OLW-Open-2	Bottom	5.0494	22.34					
PLA-Sea-2	Top	4.9345	94.96					
PLA-Sea-2	Middle	5.0388	10.99					
PLA-Sea-2	Bottom	4.9152	0.49					
WCR-Sea-2	Top	4.911	0.77					
WCR-Sea-2	Middle	5.0924	1.85					