Czech University of Life Sciences Prague

Faculty of Environmental Sciences

Master's Thesis

Distribution of microplastic in coastal waters in Guam

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Environmental Sciences

DIPLOMA THESIS ASSIGNMENT

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Nature Conservation

Thesis title

Distribution of microplastic in coastal waters in Guam

Objectives of thesis

Many studies have reported how ordinary marine microplastics are in different marine habitats, particularly in coastal areas. Guam island in Micronesia (Western Pacific) was featured as a place with a growing problem of plastic debris, but there has not yet been any research on marine macro or micro plastic debris.

Objectives of thesis:

To determine differences in concentration, size, colour and number of microplastic particles in coastal water samples of Guam and to determine differences in microplastic contamination.

Methodology

1) Water sampling in coastal waters of Guam using a neuston net with 300µm mesh, speed 2 knots for 20 minutes, transects approx. 1200 meters (in time of student internship in University of Guam)

2) Filtering sampled water and extracting microplastic particles (in the Hydrology Lab, FES Prague)

3) Discovering amount, weight, size and colour of microplastics, using hot needle method

4) Data digitalizing and data analysis

5) Discussion of the results in the context of protection and conservation of coral reefs

The proposed extent of the thesis

65 pages

Keywords

microplastics, coral reefs, Pacific Ocean, Guam, conservation

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Recommended information sources

Akdogan, Z., & Guven, B. (2019). Microplastics in the environment: A critical review of current understanding and identification of future research needs. Environmental pollution, 254, 113011.

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Declaration

I declare that I have worked on my master's thesis titled "Distribution of microplastic in coastal waters in Guam" by myself and I have used only the sources mentioned at the end of the thesis. As the author of the master's thesis, I declare that the thesis does not break any copyrights. In Prague on 31/3/2023

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This work is dedicated to my beloved brother, who left us too sudden for me to share the joy and stories from my adventure. Your memory will always be cherished in my heart.

Abstract
Microplastic pollution is a growing global environmental problem that poses significant risks to marine ecosystems and organisms. This study focused on the distribution of microplastics in the coastal waters of Guam, a small island in the Western Pacific Ocean with a diverse and rich marine environment. As an initial investigation, the study aimed to fill the gap in local plastic pollution data, identify areas most affected by plastic debris and microplastic contamination, and lay the groundwork for future long-term research on plastic debris in Guam and its impact on coral reefs. We used a neuston net with a 300-micron mesh to sample 24 transects covering various locations, including areas with significant human influence, near river mouths, and convergence zones of water currents. The samples were then sent to the Czech Republic for analysis using the hot needle method. Plastic particle concentrations ranged from 18.7 to 152.4 particles per m3 (SD 36.92), with Pago Bay (south and north) showing the highest pollution levels. In total, 1061 particles were found, ranging in size from <0.25 mm to 46 mm. This study provides a first look into the distribution of microplastics in Guam's coastal waters, offering data for monitoring and evaluating marine plastic debris concentrations. The findings can form the basis for future research on the effects of microplastic pollution on Guam's marine ecosystems and help develop effective conservation strategies to protect and restore the island's coral reefs and other marine habitats. Further long-term studies are needed for more comprehensive data, including sampling during wet and dry seasons and considering the specific dynamics of water currents when concluding. This approach will be crucial for creating well-rounded conservation strategies for Guam's marine ecosystems.

Key words: microplastic, coral reef, Pacific Ocean, Guam, conservation

Abstrakt

Mikroplastové znečištění je rostoucím celosvětovým environmentálním problémem, který představuje značná rizika pro mořské ekosystémy a organismy. Tato studie se zaměřila na rozložení mikroplastů v pobřežních vodách Guamu, malého ostrova v západní části Tichého oceánu s rozmanitým a bohatým mořským prostředím. Jako úvodní šetření měla studie za cíl vyplnit mezeru v místních datech o plastovém znečištění, identifikovat oblasti nejvíce zasažené plastovým odpadem a kontaminací mikroplasty a položit základy pro budoucí dlouhodobý výzkum plastového odpadu na Guamu a jeho dopadu na korálové útesy. Pro vzorkování 24 transektů jsme použili neustonovou síť s velikostí ok 300 mikronů, která pokrývala různá místa, včetně oblastí s významným lidským vlivem, blízko ústí řek a v konvergenčních zónách vodních proudů. Vzorky byly poté odeslány do České republiky k analýze pomocí metody horké jehly. Koncentrace plastových částic se pohybovala od 18,7 do 152,4 částic na m3 (SD 36,92), přičemž nejvyšší úrovně znečištění byly zaznamenány v zátoce Pago (jih a sever). Celkem bylo nalezeno 1061 částic o velikosti od <0,25 mm do 46 mm. Tato studie poskytuje první pohled na rozložení mikroplastů v pobřežních vodách Guamu a nabízí data pro sledování a hodnocení koncentrací plastového odpadu v moři. Zjištění mohou tvořit základ pro budoucí výzkum vlivu mikroplastového znečištění na mořské ekosystémy Guamu a přispět k vývoji účinných ochranářských strategií na ochranu a obnovu korálových útesů a dalších mořských biotopů ostrova. Pro získání komplexnějších dat jsou třeba další dlouhodobé studie, včetně vzorkování během období sucha a deště a zohlednění konkrétních dynamik vodních proudů při závěrech. Tento přístup bude zásadní pro vytváření vyvážených ochranářských strategií pro mořské ekosystémy Guamu.

Klíčová slova: mikroplasty, korálový útes, Tichý oceán, Guam, ochrana

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Introduction

Plastic: A Revolutionary Material Transforming Economy and Industries

Plastics have played a crucial role in shaping the modern world with their diverse applications and transformative impact on various industries. Since the development of the first synthetic plastic, Bakelite, in 1907, plastics have revolutionized the economy due to their low production costs, versatility, and durability. The use of plastics in various industries, such as packaging, automotive, and electronics, has driven technological advancements and fueled economic growth. The unique properties of plastics, such as being lightweight, corrosion-resistant, and easily moulded, have created countless innovative products that have improved our daily lives. Furthermore, plastics have contributed to the efficient utilization of resources, as they have replaced traditional materials like glass, metal, and wood in numerous applications, often resulting in reduced energy consumption and decreased production costs. The widespread adoption of plastics has facilitated global trade and spurred economic development, highlighting their essential role in human progress (Thompson et al., 2009; Andrady, 2011; Hopewell et al., 2009).

The emergence of Concerns and Early Efforts to Address Plastic Pollution

The awareness of plastic debris in the environment grew as the pollution's consequences became more apparent. Derraik (2002) notes that plastic debris had been observed in the ocean since the 1960s and the 1970s recognized its impact on marine life. However, it was not until the 1990s that concerns about plastic pollution gained broader public attention. This growing concern led to increased research, monitoring, and efforts to regulate plastic waste. Barnes et al. (2009) discuss the accumulation and fragmentation of plastic debris, highlighting the significance of this issue and its potential long-term consequences on ecosystems. The authors also emphasize the need for better waste management strategies and reducing plastic use to mitigate the problem. Ryan et al. (2009) provide insights into monitoring the abundance of plastic debris in the marine environment and discuss various strategies to address the issue, including beach clean-ups, educational campaigns, and adopting policies such as plastic bag bans or taxes. These efforts have aimed to reduce plastic pollution and raise awareness of its impacts, but the challenge of managing plastic debris remains a pressing concern.

Chemical Impacts of Plastic Pollution on the Environment, Human Health, and Wildlife

Chemicals from plastics can have significant effects on the environment, human health, and animals. The migration and release of chemical additives in plastics during their use, disposal, and recycling can lead to detrimental environmental impacts (Hahladakis et al., 2018). These chemicals, such as phthalates, bisphenol A (BPA), and flame retardants, can accumulate in organisms, causing endocrine disruption, reproductive toxicity, and neurotoxicity (Wright et al., 2013; Galloway, 2015). Marine plastic pollution threatens seafood safety, as the chemicals present in plastics can enter the food chain and ultimately be consumed by humans (Seltenrich, 2015). Micro- and nano-plastics ingested by marine organisms can lead to physical damage and the potential transfer of toxic chemicals (Wright et al., 2013). This diverse range of microplastic contaminants can result in complex toxicological effects on marine biota, affecting ecosystem health and function (Rochman et al., 2019). Flame retardants, added to plastics to reduce flammability, can accumulate in the tissues of animals and humans, potentially leading to

neurodevelopmental delays, thyroid disruption, and other health concerns (Hahladakis et al., 2018). Ingestion of microplastics by filter-feeding organisms, such as mussels and oysters, can accumulate these chemicals in their tissues, posing potential risks to human health when consumed as seafood (Seltenrich, 2015).

Marine Plastic Debris: Categories, Distribution, and Environmental Impacts

Plastic debris in the environment can be categorized into nano-, micro-, meso-, macro-, and mega plastics. Nanoplastics, the smallest particles below 100 nm, pose risks due to their potential to penetrate cell membranes and cause molecular damage (Gigault et al., 2018). Microplastics, ranging from 100 nm to 5 mm, originate from sources such as personal care products, synthetic textiles, or the fragmentation of larg er items (Duis & Coors, 2016; Browne et al., 2011). Meso plastics, sized 5 mm to 2.5 cm, are fragments from larger plastic items. Macro plastics, between 2.5 cm and 1 m, include common litter items like plastic bags and bottles (Barnes et al., 2009). Mega plastics larger than 1 m include abandoned fishing gear and shipping containers (Kühn et al., 2015). Distribution varies based on local sources, ocean currents, and coastal geomorphology. For example, Eriksen et al. (2014) found microplastics abundant in subtropical gyres and high-density human areas, while macro and mega plastics were dispersed throughout oceans. Impacts on the environment differ; micro- and nano-plastics are ingested by organisms, potentially causing physical damage and chemical transfer (Browne et al., 2011), while larger plastics can entangle, suffocate, and injure marine life (Kühn et al., 2015).

As we transition from discussing marine debris in general, it is essential to focus on the specific context of Guam, our study location. The Pacific Ocean has drawn significant concern regarding the accumulation of marine debris, primarily due to garbage patches, such as the Great Pacific Garbage Patch, the closest to Guam (Lebreton et al., 2018). Guam, located near the Coral Triangle, has a unique and diverse marine biome, including vibrant coral reefs, many fish species (Burke et al., 2011), and critical mangrove forests (Donato et al., 2012).

Guam

Guam, a U.S. territory in Micronesia, is the largest and southernmost of the Mariana Islands, located in the Western Pacific Ocean. The island is approximately 50 kilometres long and 14 kilometres wide, covering an area of about 550 square kilometres. (Amesbury & Hunter-Anderson, 2008). The island's population is around 170,000, with its capital, Hagåtña, situated on the west coast. Guam has a tropical climate with an average temperature of around 86°F (30°C)high humidity (Cuetos-Bueno et al.,2015), and is prone to typhoons. Its rainy season typically lasts from July to November, while the dry season runs from December to June. (Lander, 2004). Guam's marine environment, situated within the broader Pacific Ocean, is of great concern due to the accumulation of plastic debris. The North Pacific Garbage Patch, a collection of floating plastic debris, is the closest to Guam and can have significant ecological impacts (Kaiser et al., 2017). Guam's marine ecosystems are home to various precious coral species, fish, and mangrove forests. For example, coral reefs in Guam host many endemic species and are essential for coastal protection, food provision, and supporting tourism and recreation (Burke et al., 2011).

Conserving Guam's Coral Reefs: Understanding Microplastic Pollution and Environmental Stressors

Guam, located right outside the Coral Triangle, is uniquely positioned as a small island in the Western Pacific Ocean with a rich and diverse marine environment. The island's coral reefs are home to an array of marine species, including numerous fish species and various types of corals (Burke et al., 2011). Guam's coral reefs provide essential ecosystem services, such as coastal protection, food provision, and supporting tourism and recreation. However, Guam's marine ecosystems are under increasing pressure from various threats. Similar to other Pacific subtropical ecosystems, Guam has experienced severe coral bleaching events over the past ten years, devastatingly impacting the island's coral reefs. Rising ocean temperatures primarily cause these bleaching events due to climate change. (Burdick et al.,2008).The unusually warm waters stress the corals, causing them to expel the symbiotic algae (zooxanthellae) that provide them with nutrients and give them their vibrant colours (Stat et al., 2008). As a result, the corals turn white and become more susceptible to diseases, leading to a decline in their health and, in many cases, death. Researchers who have been monitoring the coral reefs in Guam are deeply concerned about the rapid degradation of these vital ecosystems. They are alarmed by the frequency and intensity of the bleaching events, which are happening at a rate that leaves little time for the reefs to recover (Raymundo et al.,2019). The loss of healthy coral reefs has significant implications for the marine environment and the human communities that rely on them for sustenance, coastal protection, and tourism. The study of the microplastic distribution and the assessment of water pollution is crucial for Guam's coral reefs, especially considering recent research findings. According to studies by Hall et al. (2015), Axworthy and Padilla-Gamiño (2019), Rotjan et al. (2019), and Reichert et al. (2018), coral reefs may switch to heterotrophy for survival when experiencing environmental stress, such as the loss of symbiotic algae due to elevated water temperatures. Floating microplastic particles, often covered in algae or ingested by zooplankton, can be mistakenly consumed by corals attempting to feed on plankton. This microplastic ingestion poses multiple threats to the already vulnerable corals. For instance, microplastics adhering to coral polyps can limit their access to food, further stressing the organisms. Additionally, microplastics can carry alien biota, which may introduce harmful bacteria that cause diseases in corals. The energy expended by corals to combat these diseases weakens their immunity, leaving them even more susceptible to the detrimental effects of environmental stressors.

Understanding the distribution of microplastics and the extent of water pollution in Guam is essential for implementing effective conservation strategies to protect and restore the island's coral reefs. By mitigating the risks associated with microplastic pollution, we can help preserve these vital ecosystems, which are crucial for marine biodiversity, coastal protection, and the livelihoods of local communities.

Assessing Microplastic Distribution and Impacts in Guam's Coastal Waters: Aims, Objectives, and Hypotheses

This study aims to assess and understand the concentration, distribution, and impact of marine plastic debris, particularly microplastics, in the coastal waters of Guam. To achieve this goal, the study aims to:

1. Obtain the necessary data to monitor and evaluate the concentration and size distribution of marine plastic debris in Guam's coastal waters, which has not been previously done.
2. Fill the data gap regarding local plastic pollution and identify the areas most affected by plastic debris and microplastic contamination in Guam.
3. Initiate long-term research focusing on plastic debris in Guam and its effects on marine organisms.

The objectives of this study are to test the following hypotheses:

4. Coastal waters in Guam exhibit similar pollution levels compared to other Pacific Ocean locations but are generally less polluted than shelf seas.
5. Microplastic concentrations are elevated in areas with significant anthropogenic influence (e.g., high population density), near river mouths, and in convergence zones of water currents.
6. South Pago Bay is more heavily impacted by microplastic pollution than North Pago Bay.
7. Northern Guam experiences reduced microplastic pollution due to relatively strong constant water currents and decreased land-based input, while higher concentrations of microplastics are found in the south (including southeast and southwest) due to the greater number of river outlets.

Review of Methods for Microplastic Sampling: Techniques and Approaches

Various methods have been developed to sample microplastics in water, each with advantages and disadvantages. The choice of a particular method depends on the research objectives, the type and size of microplastics being targeted, and the water body under investigation. In this chapter, we discuss the most commonly used sampling methods for microplastics in water and compare their effectiveness, drawing from multiple sources for each sampling type.

Equipment for Microplastic Sampling

Neuston and Manta Nets

Neuston net sampling is a popular method for collecting microplastics floating on the water's surface. Neuston nets with a usual mesh size of 330 µm have been proven effective in capturing microplastics from various locations (Fig. 1a), such as the North Pacific Central Gyre (Hidalgo-Ruz et al., 2012), the North Pacific Subtropical Gyre (Moore et al., 2001), and the Northeast Atlantic (Lusher et al., 2014). They have also successfully sampled microplastics in the North Atlantic subarctic waters (Desforges et al., 2014) and the North Pacific Ocean (Eriksen et al., 2013). One advantage of this method is that it can cover large areas and collect a representative sample of surface microplastics. However, neuston net sampling is limited to surface waters and may not detect microplastics in the water column or at the bottom.

Manta trawl sampling is another technique to collect microplastics from the water surface. Manta trawls with a mesh size of 333 µm have been employed in various studies, such as in the English Channel (Fig.1b) (Kaiser et al., 2017), the North Pacific Subtropical Gyre (Goldstein et al., 2012), the western North Atlantic (Law et al., 2010), the Mediterranean Sea (Doyle et al., 2011), and the European Atlantic coastal waters (Collignon et al., 2012). Manta trawl sampling is efficient for covering larger surface areas, but it is limited to surface waters like neuston net sampling.

The main distinction between neuston and manta nets is their design and sampling focus. Neuston nets are rectangular framed nets designed to skim the very surface of the water, focusing on the surface microlayer. In contrast, manta nets feature wing-like floats that allow them to sample both the surface and the first few meters of the water column, depending on vessel speed (Goldstein et al., 2012; Moore et al., 2001).

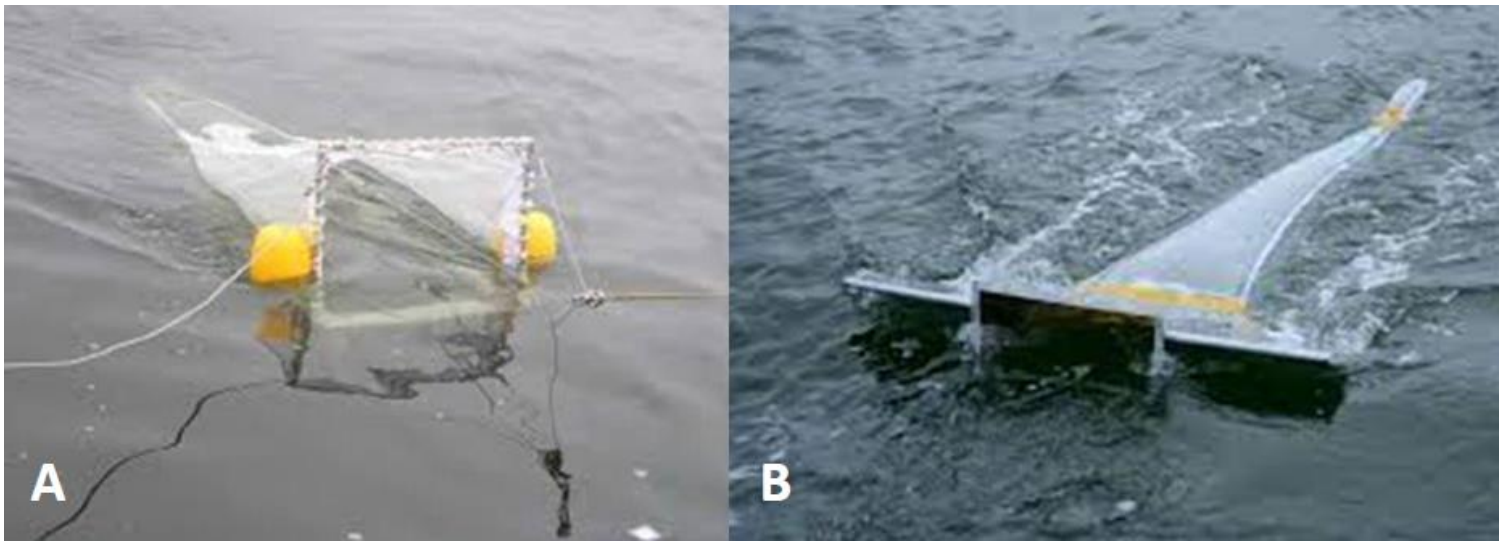

Figure 1: Neuston net (A) and Manta net (B) in use. Source: GESAMP (2019), Pasquier et al. (2022)

Van Veen Grab Sampling: Targeting Microplastics in Sediments

Van Veen grab sampling is a method specifically designed for collecting microplastics in sediments, making it suitable for shallow water bodies such as estuaries, rivers, and coastal areas (Fig. 2). This technique has been successfully applied in various studies, revealing high concentrations of microplastics in some locations, such as Belgian coastal waters (Claessens et al., 2011). It has also been employed to sample microplastics from deep-sea sediments in the Atlantic and Indian Oceans (Woodall et al., 2014), intertidal sediments in the United Kingdom (Thompson et al., 2004), the Venice Lagoon (Vianello et al., 2013), and the sediments of the Irish Sea (Browne et al., 2010).

Van Veen grab sampling enables researchers to assess microplastic pollution in benthic habitats, providing valuable insights into the distribution of these contaminants in the sediment. However, this method is unsuitable for analyzing microplastics in water columns or surface samples, as its primary focus is on benthic environments.

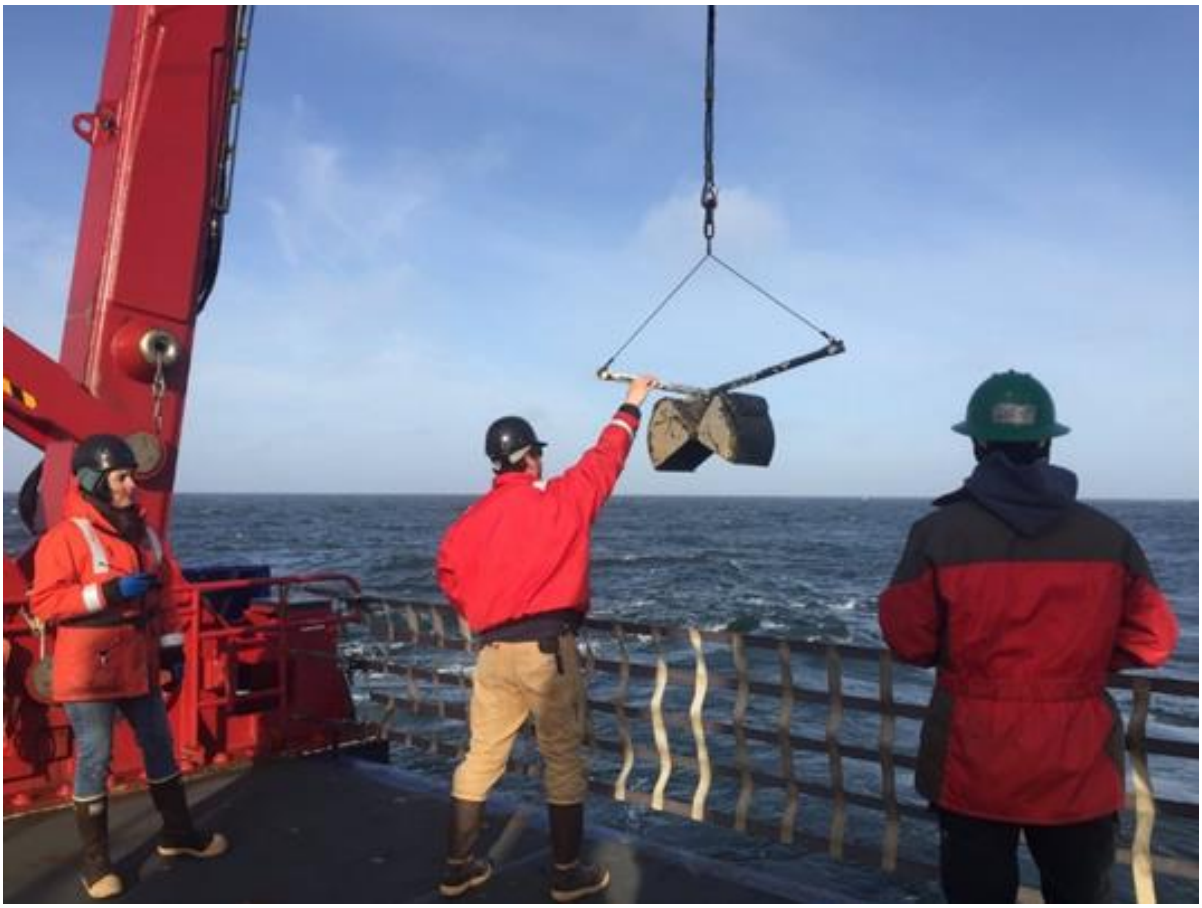

Figure 2: Deploying the Van Veen grab sampler for sediment sampling.Woods Hole Oceanographic Institution. (n.d.).

Niskin bottle sampling: Assessing Microplastics in the Water Column

Niskin bottle sampling is a technique used to collect water samples at various depths in the water column, providing insights into the distribution of microplastics throughout different depths. The Niskin bottle captures water at a specified depth, allowing researchers to take targeted samples (Fig. 3). It operates through a spring-loaded mechanism triggered by a messenger weight sliding down a cable. When the messenger weight reaches the bottle, it activates the mechanism, causing it to snap shut and capture the water sample at the desired depth (Xu et al., 2020). Löder et al. (2014) employed Niskin bottle sampling to assess microplastic distribution at different depths in the Baltic Sea, discovering that microplastics were present throughout the water column.

Similarly, Enders et al. (2015) used this method to study microplastic distribution in the Labrador Sea, finding microplastics in surface waters and at depths of up to 1,000 meters. Chubarenko et al. (2016) applied Niskin bottle sampling to collect microplastics at various depths in the Russian coastal zone of the Baltic Sea, observing the highest concentrations near the surface. Kukulka et al. (2012) demonstrated the effectiveness of this method in assessing microplastic distribution in the North Atlantic subtropical gyre, while Cózar et al. (2014) employed Niskin bottle sampling to collect microplastics at different depths in the Mediterranean Sea.

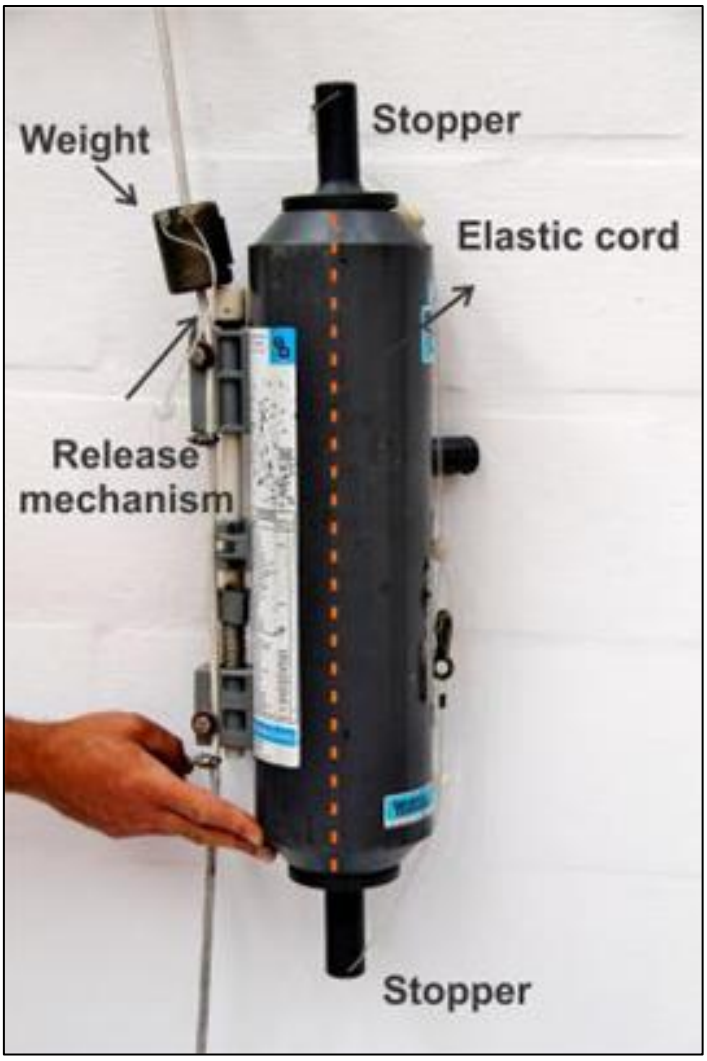

Figure 3: Niskin bottle used for collecting water samples at various depths in the water column. Source: Flanders Marine Institute (VLIZ). (n.d.).

Sample Processing Methods for Extracting Microplastics from Water Samples

Several techniques have been developed to extract microplastics from water samples. These methods aim to efficiently separate microplastics from water and organic matter while minimizing the loss of microplastics during the process. Some common techniques are density separation, filtration, and digestion.

Density separation

This method involves using a high-density solution, such as sodium chloride (NaCl) or zinc chloride (ZnCl2), to separate microplastics from water and other denser materials (Fig. 4). By adding the dense solution to the water sample, microplastics with lower densities will float to the surface, while denser materials will sink to the bottom (Claessens et al., 2013; Imhof et al., 2012). The floating microplastics can then be collected using a sieve or skimming the surface. Density separation methods provide efficient separation of microplastics from organic matter and sediments, improving the accuracy of microplastic identification (Imhof et al., 2012). However, they can be limited by using toxic or expensive high-density solutions, which may pose environmental and safety concerns (Shim et al., 2017).

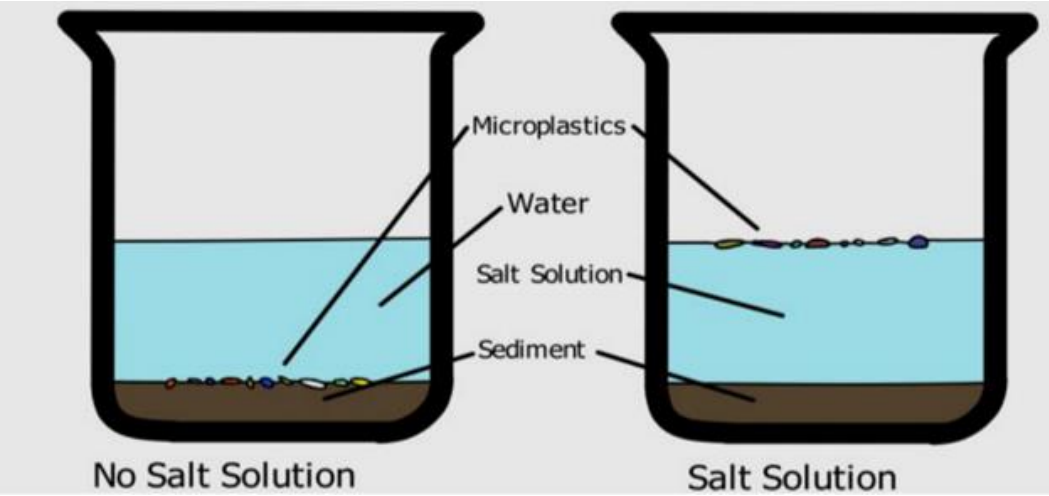

Figure 4:Salt solutions facilitate extraction of microplastics from the sample matrix by altering the sample's density. Adapted from Ang et al., 2021

Vacuum filtration

A vacuum filtration apparatus applies a vacuum to a filter flask, which pulls the liquid through a porous filter, retaining solid particles on the filter surface (Eaton et al., 2005) (Fig. 5). The filtrate is collected in the flask while the residue remains on the filter for further analysis, making it a helpful method for separating microplastics from water samples (Hidalgo-Ruz et al., 2012). The filters can be made of different materials, such as glass fibre, cellulose, or polycarbonate, and can be used in a vacuum filtration system or a manual filtration setup (Desforges et al., 2014; Lusher et al., 2014). The choice of filter material and pore size depends on the size range and type of microplastics targeted in the study.

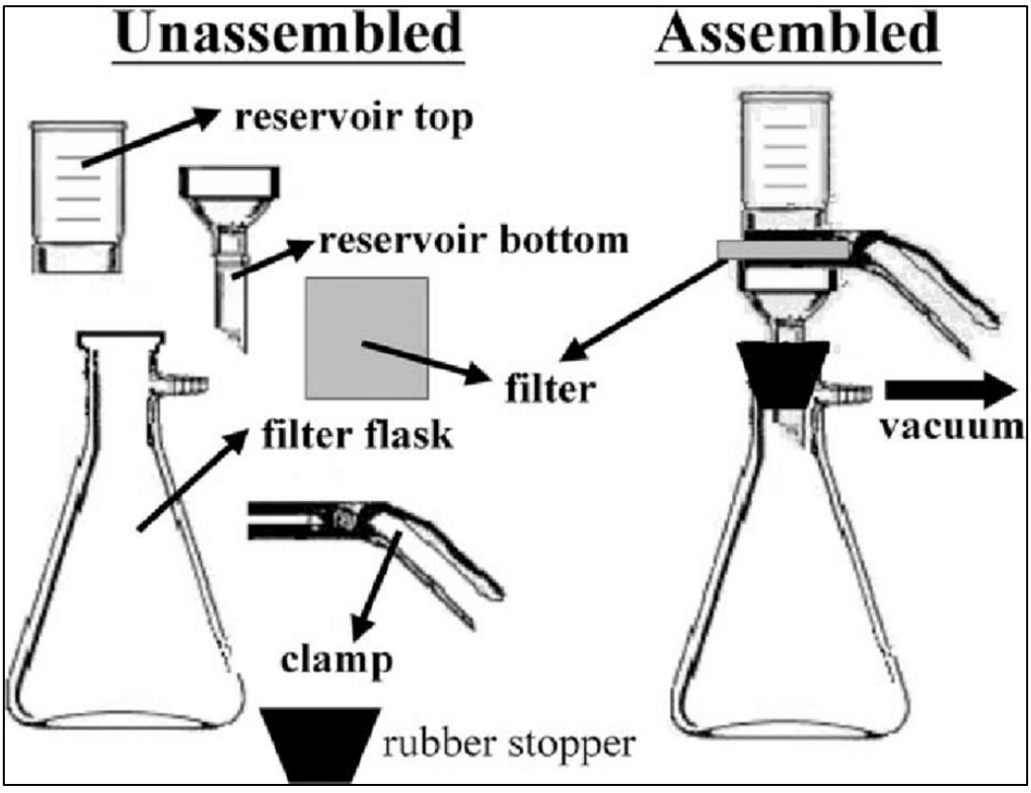

Figure 5: Single-well Millipore filtration apparatus. Adapted from Weeks et al., 2005.

Reducing Organic Matter in Samples

Before analyzing microplastic particles in water samples, it is crucial to effectively remove the organic matter to minimize errors and ensure accurate data extraction. Organic matter, including feathers, leaves, fish, fish larvae, algae, and insects, can interfere with the identification and quantification of microplastics, making the removal step an essential part of the process. Researchers sometimes combine manual and chemical methods to remove organic matter from microplastic samples. Seo et al. (2015) manually removed large organic materials like macroalgae and jellyfish using a 330 μm mesh, ensuring microplastics were not lost. After manual removal, chemical digestion methods can address smaller organic residues. This approach emphasizes the importance of combining effective organic matter removal methods while minimizing microplastic loss.

Chemical digestion

Oxidizing agents such as hydrogen peroxide (H2O2) or potassium persulfate (K2S2O8) can be used to break down the organic matter, while the microplastics remain intact (Nuelle et al., 2014; Shim et al., 2016) (Fig. 6). It is essential to consider the potential impact of the digestion process on the microplastics themselves, as some chemicals may cause them to fragment or degrade.

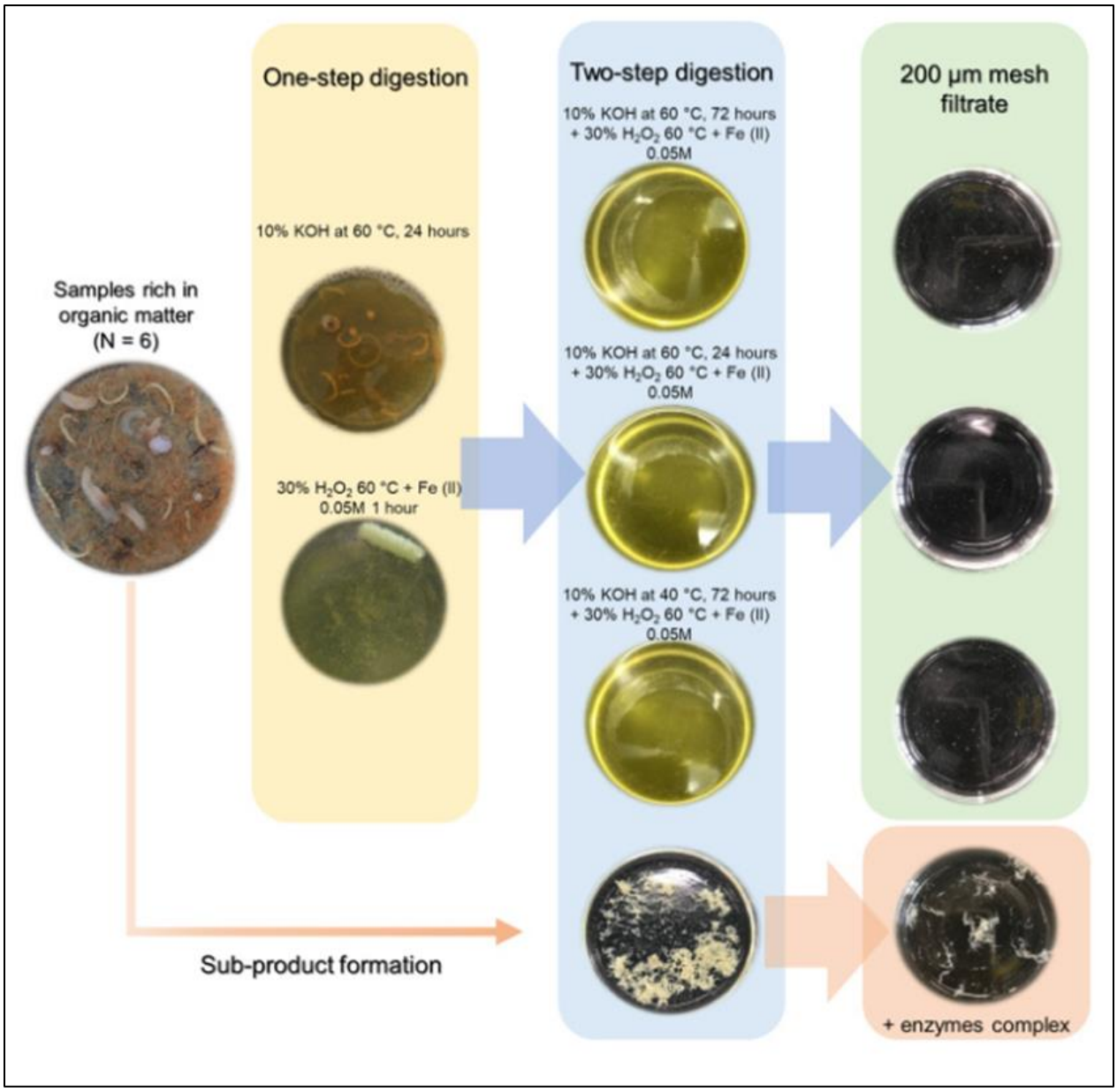

Figure 6: Working flow diagram for the different organic matter digestion procedures with pictures of the results obtained. Adapted from Alfonso et al., 2021.

Enzymatic treatment works by exploiting the specificity of enzymes, which are biological catalysts that accelerate the breakdown of specific substrates, such as proteins or cellulose (Alberts et al., 2002). In microplastic sample processing, enzymes selectively degrade the organic matter without affecting the microplastics, thus simplifying the subsequent isolation and identification of microplastic particles (Song et al., 2015).

Oxidation

As mentioned earlier, oxidation with hydrogen peroxide or potassium persulfate can break down organic matter in water samples (Nuelle et al., 2014; Shim et al., 2016). Hydrogen peroxide (H2O2) is commonly used to dissolve organic matter in microplastic samples. Studies employed various concentrations and durations tailored to research goals and sample

characteristics. Jang et al. (2020) used 30% H2O2 at 60°C for 48 hours on sediment samples, while Cole et al. (2014) used the same concentration at 40°C for 24 hours on seawater samples. Nuelle et al. (2014) applied 10% H2O2 for 24 hours at room temperature, followed by 30% H2O2 for another 24 hours. Imhof et al. (2012) treated sediment samples with 10% H2O2 for 24 hours, followed by 30% H2O2 for 24 hours, and heated them at 60°C for several hours. Foekema et al. (2013) used 35% H2O2 for a week on sediment samples, followed by filtration and visual examination. Hidalgo-Ruz et al. (2012) employed 30% H2O2 for 48 hours on water samples, followed by filtration and stereomicroscope examination. When selecting an appropriate method for specific samples and research goals, these variations should be considered.

Sedimentation: Allowing water samples to settle for a period can help separate microplastics from denser organic matter, which will accumulate at the bottom of the container. This method may require a long settling time and may not be suitable for all microplastics (Cole et al., 2014).

Spectrometry Techniques in Microplastic Sample Processing

By using a combination of these spectrometry techniques, researchers can obtain a comprehensive understanding of the composition, structure, and properties of microplastic particles in their samples.

Fourier Transform Infrared (FTIR) spectrometry

In microplastic analysis, various spectrometry techniques are employed. Fourier Transform Infrared (FTIR) spectrometry is a highly sensitive and accurate technique that provides detailed information on the chemical composition of particles, including polymer type, functional groups, and chemical characteristics (Hidalgo-Ruz et al., 2012). However, FTIR requires complex sample preparation, high initial equipment costs, and specialized training for instrument operation and data interpretation (Cole et al., 2014).

Raman spectroscopy I

It is a non-destructive technique that complements FTIR, as it can identify polymers that FTIR may struggle with (Song et al., 2015). It can also be used in conjunction with other imaging techniques, such as microscopy. Nevertheless, Raman spectroscopy has lower sensitivity than FTIR, is susceptible to fluorescence interference, and can be more time-consuming (Käppler et al., 2016).

Pyrolysis Gas Chromatography-Mass Spectrometry (Py-GC-MS)

This technique provides detailed information about polymer composition and additives, can detect lower concentrations of microplastics, and can identify a wide range of polymers (Fries et al., 2013). However, Py-GC-MS is a destructive technique with complex sample preparation and high initial equipment costs (Löder & Gerdts, 2015).

Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX) SEM-DEX offers high-resolution imaging of microplastic particles, and elemental composition data. It is useful for characterizing inorganic additives and contaminants (Mintenig et al., 2017). Its disadvantages include limited surface analysis, specialized training requirements for instrument operation and data interpretation, and high initial equipment costs (Avio et al., 2015).

By using a combination of these spectrometry techniques, researchers can obtain a comprehensive understanding of the composition, structure, and properties of microplastic particles in their samples.

Hot Needle Technique: An Alternative Approach for Microplastic Quantification

The hot needle technique is a simple and cost-effective method for identifying and counting microplastic particles in environmental samples. This method uses a heated needle or probe to touch a suspected microplastic particle's surface. If the particle is a microplastic, it will melt or deform upon contact with the hot needle, indicating its synthetic nature (Löder et al., 2015). This method is beneficial for differentiating microplastics from natural materials, such as plant debris and other organic matter, which do not melt when exposed to the hot needle. The hot needle technique is often employed as a preliminary screening step to identify potential microplastic particles before further analysis using more advanced techniques, such as Fourier-transform infrared (FTIR) or Raman spectroscopy (Löder & Gerdts, 2015). Advantages of the hot needle technique include its simplicity, low cost, and ease of use. However, it does have some limitations. The method may not be suitable for all types of plastics, especially those with high melting points. Additionally, the technique is less accurate than more advanced methods and cannot provide detailed information about the composition or polymer type of the microplastics (Hidalgo-Ruz et al., 2012).

Methods

Location

Guam is a U.S. territory located in the western Pacific Ocean, the largest and southernmost island of the Mariana Islands archipelago. Guam is characterized by a combination of coastal plains, rugged cliffs, and elevated plateaus. The island supports a rich biodiversity, including coral reefs, seagrass beds, and mangrove forests, which provide essential habitats for various marine species. Guam's strategic location in the western Pacific makes it an essential hub for research, trade, and military operations.

Climate and weather

The island's climate is characterized by two distinct seasons: the dry season, which typically lasts from January to June, and the wet season, from July to December. Rainfall is abundant, with an annual average of approximately 2,500 millimetres, and it is predominantly influenced by the Intertropical Convergence Zone and the movement of the Western Pacific Monsoon (Lander & Guard, 2003). The climate of Guam is classified as tropical marine, with relatively stable temperatures throughout the year. With high humidity levels, average monthly temperatures range from around 26.5°C to 28.5°C. Typhoons and tropical storms are common in the region, with Guam being affected by an average of 1-2 typhoons per year.

Table 1: Weather on our sampling days

Area Name	Sample	Date	t, max (°C)	t, min	t, average	Precipitation	Wavelength
Gab Gab, Apra Harbor	AT1	8/7/2022	28.3	23.8	26.11	4.318	0.18
Orote point ERA	AT2	8/7/2022	28.3	23.8	26.11	4.318	0.3
Middle Apra Harbor	AT3	8/7/2022	28.3	23.8	26.11	4.318	0.12
Blue And White	BT1	8/7/2022	28.3	23.8	26.11	4.318	0.25
Family Beach	BT2	8/7/2022	28.3	23.8	26.11	4.318	0.11
Ritidian Poin	CT1	12/7/2022	30.5	25	27.77	1.778	0.47
Coco Palm Garden Beach	CT2	12/7/2022	30.5	25	27.77	1.778	0.38
Haputo ERA	CT3	12/7/2022	30.5	25	27.77	1.778	0.28
Tanguisson	CT4	12/7/2022	30.5	25	27.77	1.778	0.27

Agana Bay	CT5	12/7/2022	30.5	25	27.77	1.778	0.23
Asan	CT6	12/7/2022	30.5	25	27.77	1.778	0.33
Cocos Island	DT1	18/7/2022	30.5	25.6	28.05	0	0.63
Merizo channel	DT2	18/7/2022	30.5	25.6	28.05	0	0.36
Umatac Bay	DT3	18/7/2022	30.5	25.6	28.05	0	0.21
Cetti Bay	DT4	18/7/2022	30.5	25.6	28.05	0	0.08
Ana's Island	DT5	18/7/2022	30.5	25.6	28.05	0	0.18
Agat Bay	DT6	18/7/2022	30.5	25.6	28.05	0	0.09
Haps reef	ET1	25/7/2022	30	23.9	26.94	16.002	0.17
Turtle Rock Island	ET2	25/7/2022	30	23.9	26.94	16.002	0.13
Outhouse beach	ET3	25/7/2022	30	23.9	26.94	16.002	0.14
Pago Bay North	FT1	26/7/2022	28.3	23.3	25.83	41.148	1.4
Pago Bay South	FT2	26/7/2022	28.3	23.3	25.83	41.148	1.34
Talofofo Bay	FT3	26/7/2022	28.3	23.3	25.83	41.148	1.4
Tinago River	FT4	26/7/2022	28.3	23.3	25.83	41.148	1.41

Currents

Guam is situated within the complex oceanographic setting of the western North Pacific. The island is influenced by the North Equatorial Current (NEC) and the North Equatorial Countercurrent (NECC), which play a significant role in shaping the local marine environment. The NEC flows westward to the north of Guam, while the NECC flows eastward to the south. These currents, along with the Mindanao Current and the Kuroshio Current, contribute to a dynamic water circulation system around the island (Fig. 7).

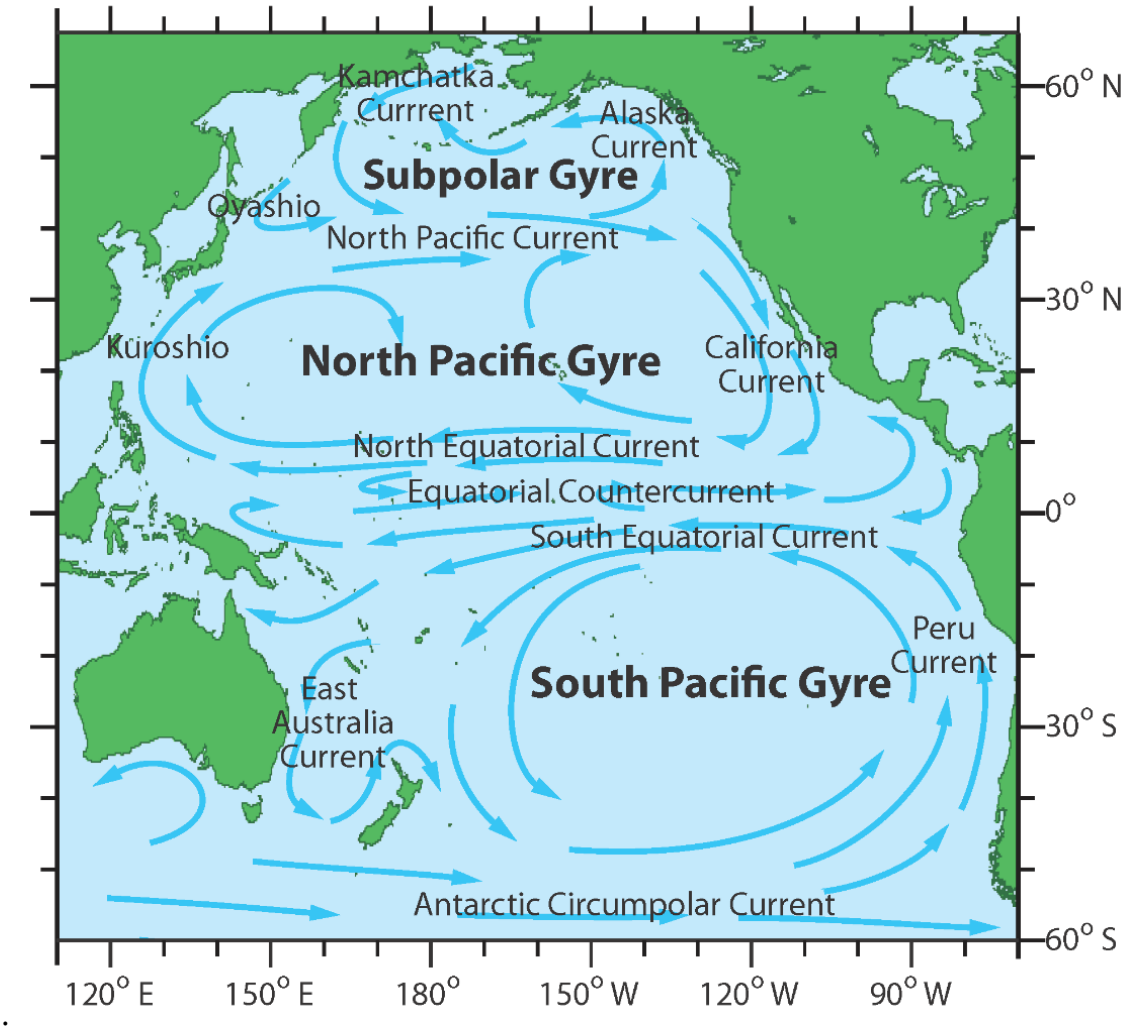

Figure 7: Water currents in the Pacific region

Selection of Sampling Transects and Methodological Considerations

Our study's sampling transects in Guam's coastal waters were selected based on feasibility, safety, local regulations, and resource allocation criteria. We considered accessibility, exclusion of marine protected areas, and avoidance of military zones, among other factors. We used the neuston net method due to its suitability, equipment availability, and team familiarity.

Weather conditions, local forecasts, and safety concerns informed the choice of sampling locations, and the starting points were limited to the western side of Guam. The island's eastern side was challenging to access by vessel, and the northeastern part was excluded due to limited fuel capacity and unfavourable weather conditions.

Chosen locations for sampling

In this chapter, we discuss our selected sampling areas in Guam for studying plastic pollution (Fig. 8). These locations represent diverse human activities and environmental conditions, focusing on potential correlations between human activity and plastic pollution levels. In the

following sections, we briefly overview each location, highlighting factors such as proximity to protected areas, boat activity, and potential contamination sources.

Figure 8: Sampling locations

Apra Harbor Area

Apra Harbor, an important commercial and military port on Guam's central west coast, is known for its sandy and muddy bottom, diverse coral communities, and potential contamination from shipping, military operations, and industrial facilities (Burdick et al., 2008, Schroeder et al., 2001). Selected sampling locations within and around Apra Harbor include Gab Gab (sample AT1), Orote Point (sample AT2), Middle of Apra Harbor (sample AT3), External Apra Harbor (sample BT1), and Family Beach (sample BT2), each with unique features and possible contamination sources, including harbour activities, recreational use, boat traffic, and proximity to harbour operations (Guam Visitors Bureau, n.d.; National Park Service, n.d.).

North Area

The northwest region of Guam is characterized by low human activity, diverse coastal environments, and a focus on recreational use, wildlife conservation, and limited industrial operations. Sampling locations in this region include Ritidian Point (sample CT1), Coco Palm Garden Beach (sample CT2), Haputo Ecological Reserve Area (sample CT3), Tanguisson Beach, Shark Cove (sample CT4), with possible contamination sources such as marine debris, recreational use, and industrial activities (Guam Visitors Bureau, n.d.; U.S. Fish & Wildlife Service, n.d.).

West Central Coast

This region, north of Apra Harbor, features diverse coastal environments and is known for recreational activities, historical sites, and urban influence. Sampling locations include Agana Bay (sample CT5) and Asan (sample CT6), with possible contamination sources being urban runoff and recreational use (Guam Visitors Bureau, n.d.; National Park Service, n.d.).

South Area

Southern Guam is known for its picturesque landscapes, historical significance, and diverse coastal environments. Sampling locations in this region include Cocos Island (sample DT1), Merizo Channel (sample DT2), Umatac Bay (sample DT3), and Cetti Bay (sample DT4), with possible contamination sources such as marine debris, tourism, recreational activities, and boat traffic (Guam Visitors Bureau, n.d.).

Fieldwork

The equipment used for microplastic sampling included:

1. Neuston net (300-micron mesh size, 160 cm long with an opening of 60 cm)
2. Handheld GPS
3. PET plastic 1-litre jar as a detachable net end
4. Two homemade canisters as buoys for the net
5. Hose ring and hose clamp driver
6. Wire to strengthen the hose ring to the net end
7. 1-litre squeeze bottle
8. HDPE 1 litre bottles for sample storing, labelled in advance
9. 3 HDPE 20 litres buckets
10. 100-micron sieve, 25 cm in diameter.

The fieldwork was conducted from a centre console boat, with sampling transects made as close to the shore as possible, given appropriate depth. The neuston net was equipped with two buoys on each side of the net opening, which kept the net at approximately 1-meter depth, allowing us to collect most microplastics that typically float close to the water surface (Fig. 9).

Figure 9: Neuston net with two buoys in use.

At the beginning of each transect, we marked a starting GPS point. We set the neuston net on the side of the boat at a proper distance from the boat engines to minimize water surface disturbance. The boat travelled along the shore at a speed of 2 knots for 20 minutes, covering approximately 1200 meters in the distance. To prepare filtered seawater (FSW), we collected seawater in a bucket and poured it through a 100-micron sieve into another bucket. At the end of each transect, we marked the GPS point for the end of the tow. After removing the net from the water, we thoroughly washed the neuston net with FSW, splashing from the outer side to avoid contamination until no debris or organic matter residues were left. The jar at the end of our net was carefully emptied, ensuring that all contents were thoroughly transferred into a 1-litre HDPE bottle. To guarantee that nothing was left behind in the jar, we used a squeeze bottle filled with FSW to rinse the jar, then poured the contents through a funnel into the HDPE bottle, aided by a small metal spatula to transfer all organic matter that was caught. Primary water samples were stored in a fridge in 1 L PET jars at 4°C to limit the decaying of organic matter.

Sample loss

Challenges arose during sampling in Guam's southern and eastern parts due to inappropriate boat speed at the beginning of one transect or an intense wave exposure. The neuston net and other equipment occasionally struggled in rough conditions, causing some sample loss. We made corrections and always brought extra bottles, hose rings and jars to adapt to unpredictable situations.

Preparing samples for transportation

Equipment:

1. Manual filtration set up
2. 20-micron nylon filters
3. 10 ml plastic vials
4. Petri dishes
5. Squeeze bottle
6. Glass beakers
7. Funnel
8. Distilled water
9. Funnel
10. Metal spatula
11. Tweezers
12. 60 % ethanol

Figure 10: A manual filtration setup used for processing microplastic samples in Guam. The setup includes a mesh filter, a collection container, 20-micron nylon filters, and a tube.

We used a manual filtration setup with 20-micron nylon filters to concentrate samples for microplastic identification. The water sample was poured through the tube into a collection container with a filter inside, allowing gravity to separate microplastics and organic matter from the water. Supplementary tools were employed to wash and remove large organic matter (Fig.

10, 11). Samples from 24 locations were stored in 10 ml plastic vials with 60% ethanol. We obtained the necessary permits from the Guam Department of Agriculture, which inspected our samples prior to transportation to the Czech Republic. This inspection ensured we were not exporting any materials prohibited by CITES regulations.

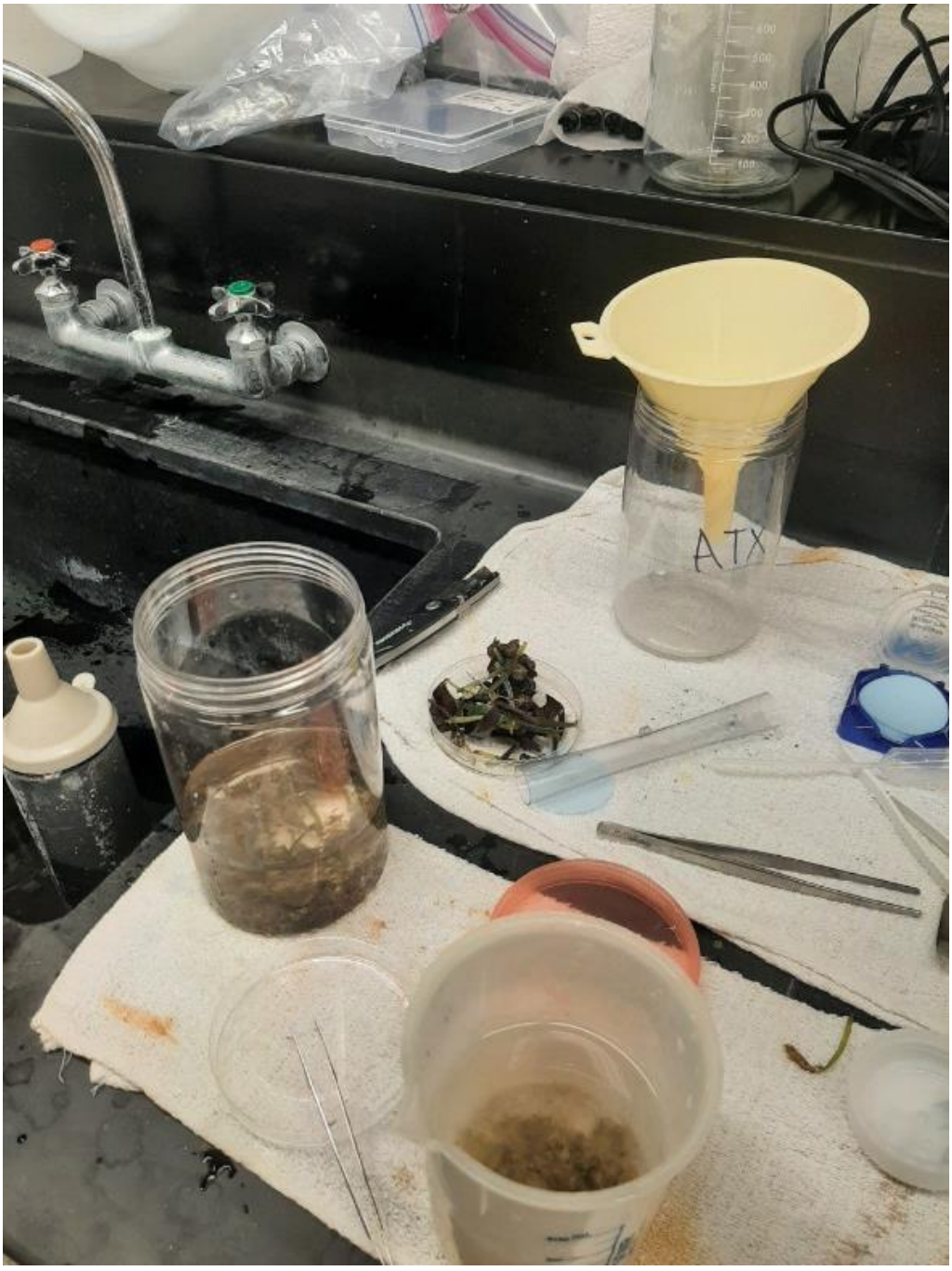

Figure 11: Removing large organic matter from water samples at the Marine laboratory in Guam

Work at the Hydrobiological laboratory in the Czech Republic

Upon transferring our samples to the Hydrobiological lab at the Czech University of Life Sciences in the Czech Republic, we switched to a filtration vacuum apparatus, which provided

increased efficiency, improved consistency, and reduced contamination risk (Fig. 12) (Lusher et al., 2014).

Figure 12: Vacuum Filtration Apparatus with Water Aspirator Attachment at the Hydrobiological laboratory, Czech Republic.

Preliminary test for contamination

Despite implementing various precautionary measures to minimize contamination, such as cleaning Petri dishes and work surfaces before and after use, wearing clothes made from natural fibres, and donning cotton lab coats, our laboratory was not sterile. To assess the level of contamination in the lab, we conducted a test to determine the presence of potential microplastic-like particles and ensure the accuracy of our study results. We assessed potential microplastic-like contamination in the lab by placing clean Petri dishes in various locations, including inside and outside a fume hood and near the refrigerator. We left two dishes at each location for a week to gauge potential contamination.

Microscopic examination revealed small coloured fibres in the dishes, with the most found near the fridge surface and the least near the ceiling (Figure 13, 14a; Table 2). However, a hot needle test showed no reaction, suggesting they were not microplastics and would not interfere with our study.

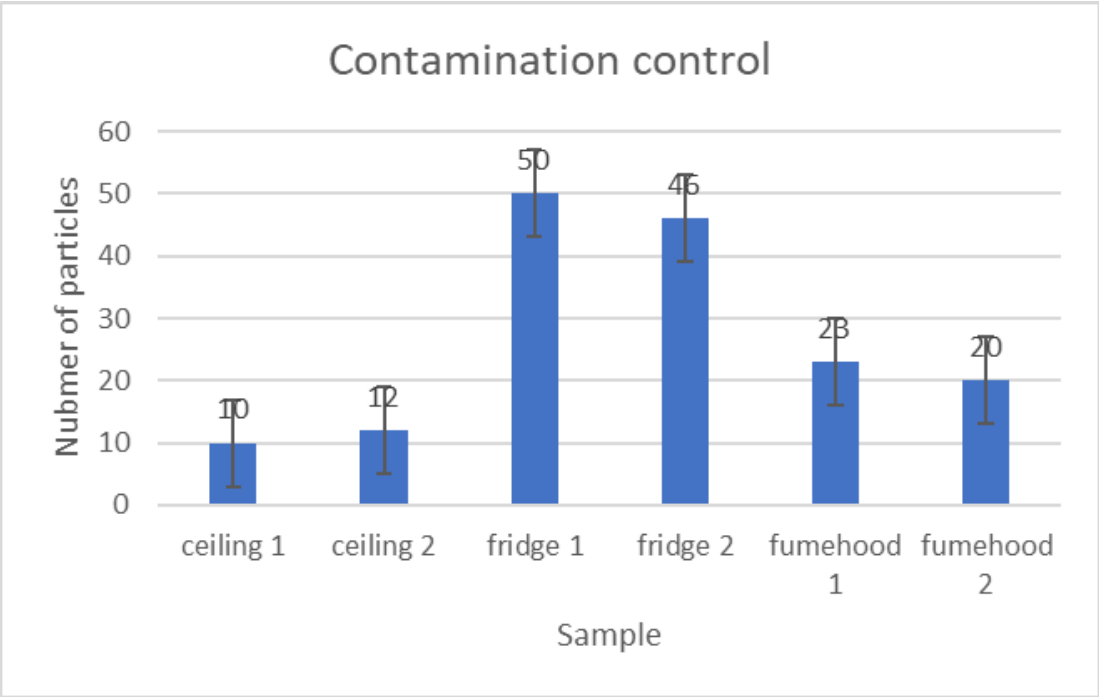

Figure 13: Number of fibres in each sample during a contamination control with a standard deviation

Table 2: Number of fibres in each Petri dish at the contamination test

Sample	**N of fibres**
ceiling 1	10
ceiling 2	12
fridge 1	50
fridge 2	46
fumehood 1	23
fumehood 2	20

The fibres likely originated from paper towels used for drying equipment and resembled cellulose fibres found in the MicroLab Gallery (Fig. 14, a), www.microlabgallery.com)

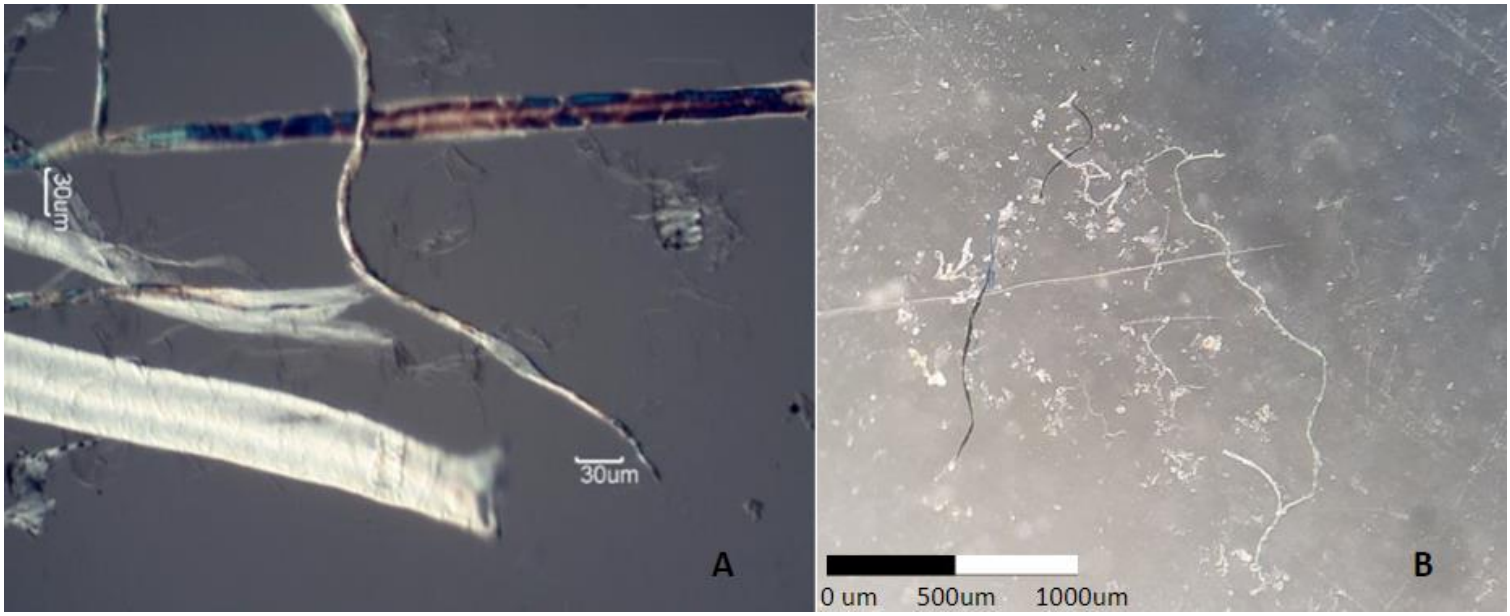

Figure 14: Cellulose fibers depicted in the MicroLab Gallery (A); Fibers found in Petri dishes in Hydrobiological lab (B)

Determining the Optimal Hydrogen Peroxide Treatment Duration for Microplastic Analysis

To identify the ideal H2O2 treatment duration for dissolving organic matter without damaging microplastic particles, we tested hydrogen peroxide treatment times ranging from 15 to 180 minutes (with each subsequent sample increasing by 15-minute intervals) at 40°C using control samples containing filamentous algae, meso- and microplastics that we made in the lab from household items. Based on our findings, we chose a 60-minute hydrogen peroxide treatment for our lab work to effectively dissolve organic matter while minimizing the risk of damaging microplastic particles (Table 3).

Table 3: Duration of H2O2 treatment and its efficiency in dissolving organic matter without damaging plastic particles

Time of H2O2 curing (min)	Deformed MP	Dissolved OM
15	no	no
30	no	no
45	no	no
60	**no**	**yes**
75	no	yes
90	no	yes
105	no	yes
120	no	yes
135	yes	yes
150	yes	yes
165	yes	yes
180	yes	yes

Our experiments showed that durations between 15 and 45 minutes were insufficient for dissolving organic matter (Fig. 15a). From the 60-minute treatment onwards, organic matter dissolved effectively (Fig. 15b). However, starting with the 135-minute treatment, transparent microplastic particles became white, hollow, and fragile, indicating potential damage (Fig. 16).

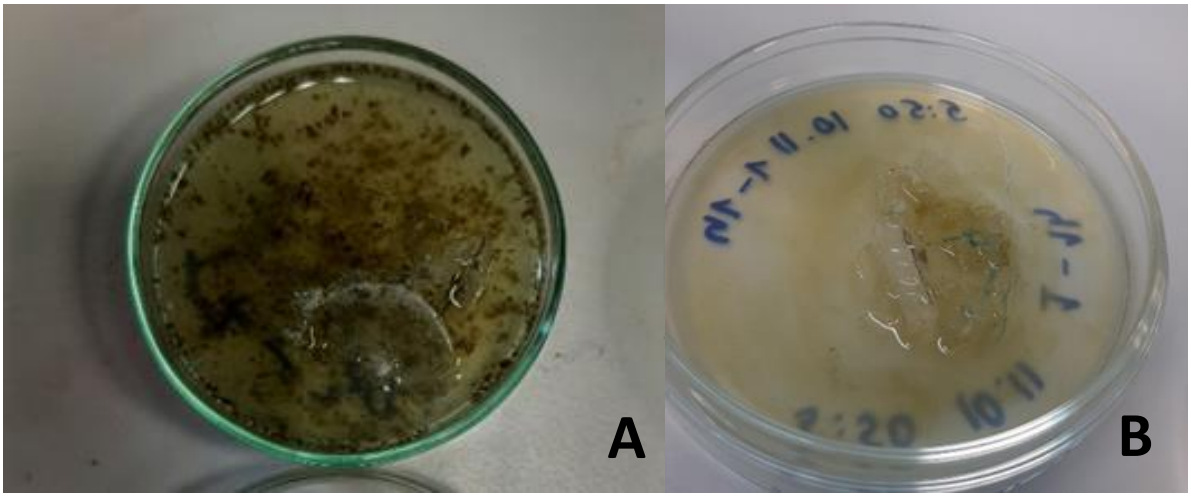

Figure 15: Organic matter after 30 minutes (A) and 60 minutes (B) of H2O2 treatment

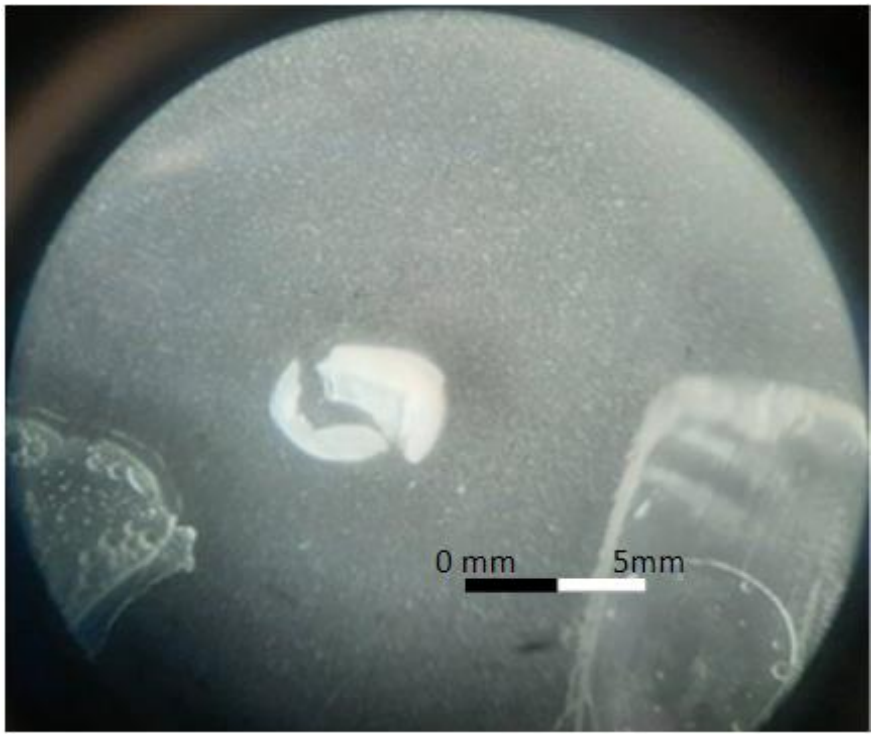

Figure 16: A microplastic particle damaged by H2O2 treatment of 135 minutes

Based on our findings, we chose a 60-minute hydrogen peroxide treatment for our lab work to effectively dissolve organic matter while minimizing the risk of damaging microplastic particles.

Dissolving organic matter in samples and filtration for further analyses

Equipment (Fig. 17):

1. Fume hood
2. Heating bath
3. 35 ml vials
4. 6 cm diameter plastic Petri dishes
5. Squeeze bottle with 30% hydrogen peroxide
6. Squeeze bottle with distilled water
7. Rubber gloves
8. 2 homemade stands for bottles made out of polypropelene food containers
9. Tweezers
10. Metal spatulas
11. Glass beakers

Figure 17: Organic matter dissolution setup

First, we transferred the samples from vials containing 60% ethanol to 6 cm diameter plastic Petri dishes. We added distilled water and left them for 24 hours under a fume hood to allow the ethanol to evaporate before treating it with H2O2. Next, we transferred the dried samples to 35 ml bottles and added 15 ml of 30% hydrogen peroxide, filling each bottle halfway as a safety measure to prevent splashing. For samples with a high amount of organic matter, we divided them between several bottles. The heating bath was preheated to 40°C, and the timer was set for 1 hour. Immediately after removing a stand with samples from the heating bath, we

filtered them using a vacuum filtration apparatus with 20-micron nylon filters (Figure X). We performed H2O2 treatment in small batches to prevent samples from experiencing variable treatment durations due to filtration capacity constraints. During filtration, we also poured distilled water onto the samples to help remove any residual hydrogen peroxide. We then carefully removed the nylon filter with the captured particles and gently transferred the contents to a new Petri dish. We used a squeeze bottle with distilled water and a metal spatula to aid the transfer. Finally, we placed the Petri dishes in the fume hood and let them dry completely.

Stereoscopic Analysis and Hot Needle Test

Figure 18: Set up for quantifying under a stereoscope

Equipment (Fig. 18):

1. Stereoscope
2. Mechanical counter
3. 10 cm diameter plastic Petri dish marked with 2x2 mm grid
4. Probe needle
5. Marker
6. Metal spatula, tweezers, ruler
7. Tea candles, matches or lighter

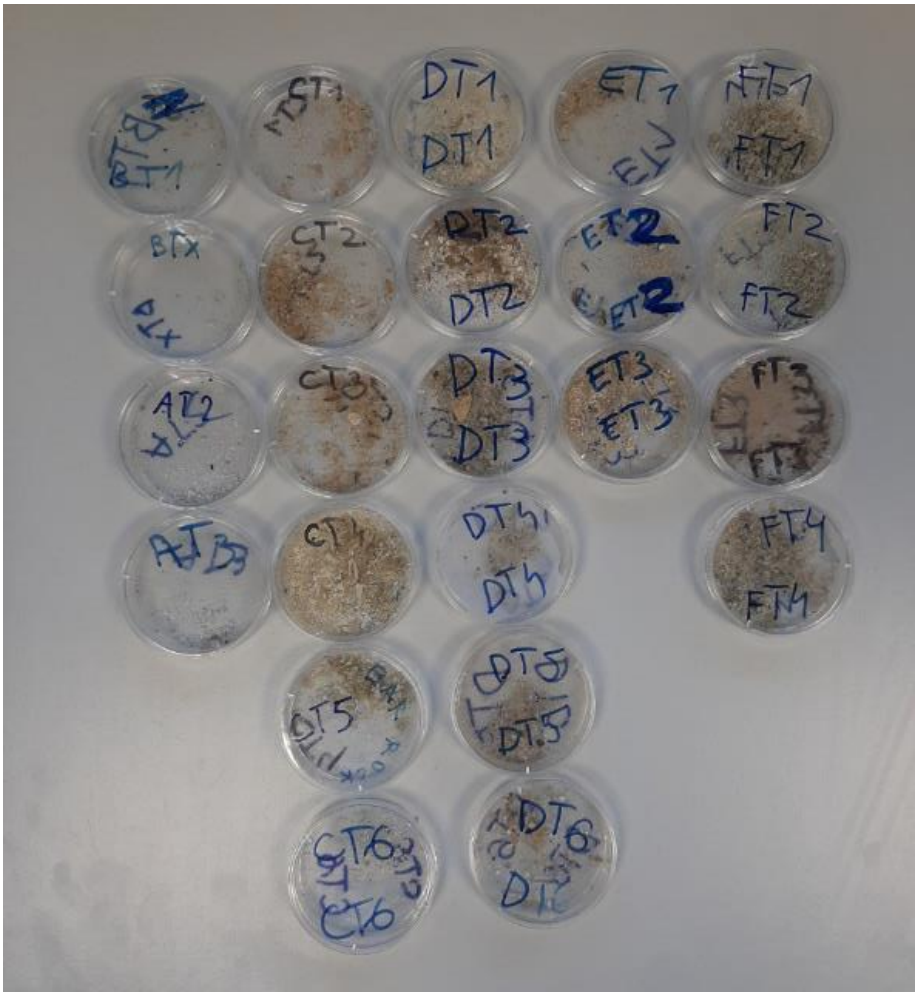

Figure 19: Dried samples ready for stereoscoping

Following the drying process, we began examining the samples under a stereoscope. Some samples still contained traces of organic matter (Fig. 19). To avoid potential damage to the microplastic particles, we opted not to perform an additional treatment round. Instead, we carefully removed the remaining organic matter using available tools. Once the samples were dried, we examined them under a stereoscope. Although some samples still contained traces of organic matter, they were manageable and could be removed under the stereomicroscope using tweezers, a needle, and a spatula. We counted microplastics using a stereoscope by placing the dried sample on a Petri dish that was positioned above another Petri dish which was marked with a 2x2 mm grid. We carefully recorded each microplastic particle's length, type (fibre or fragment), and colour (Fig. 20). All raw data were systematically entered into an Excel spreadsheet for subsequent analysis.

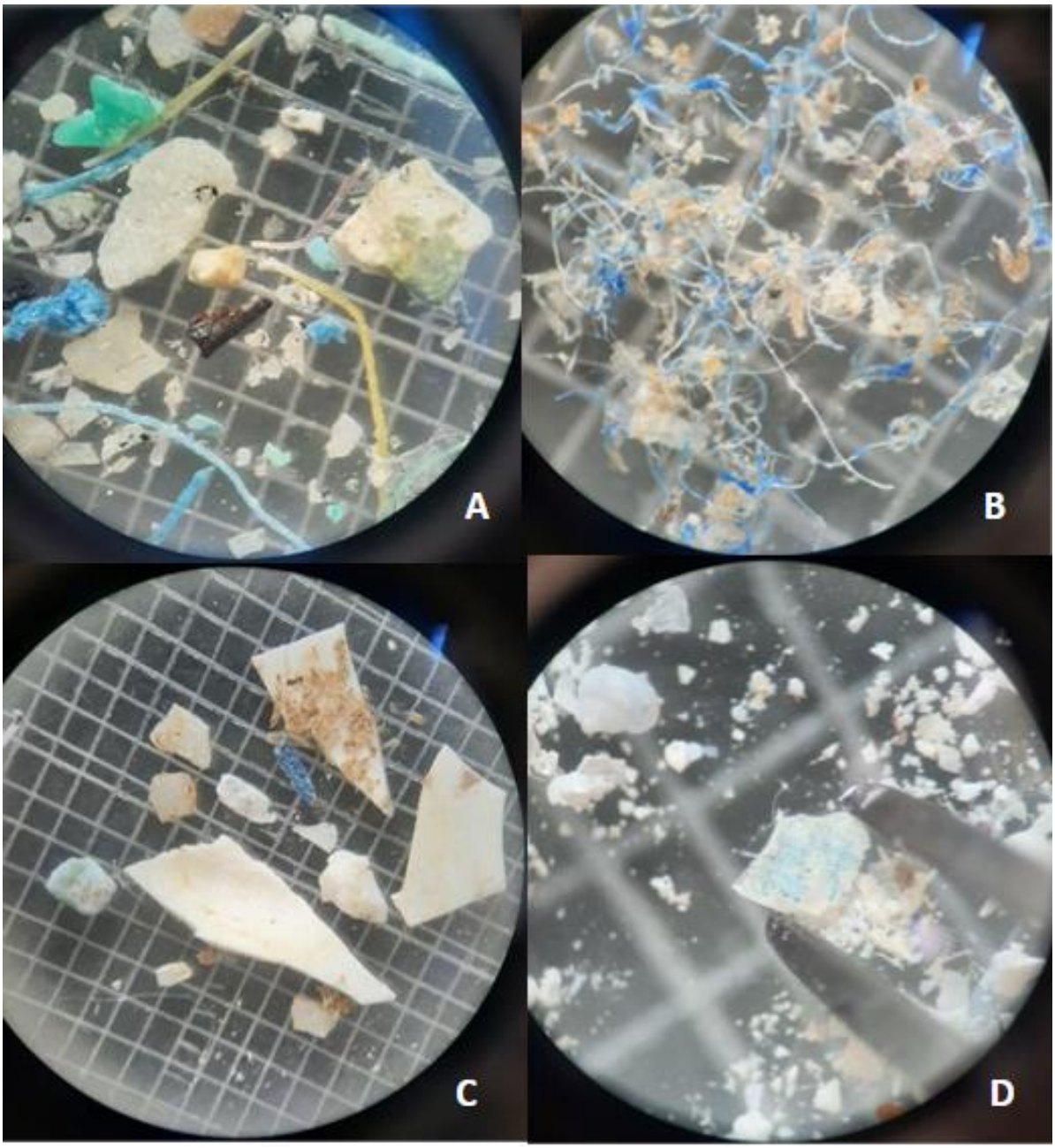

Figure 20: Samples FT1 (A), CT1- (fibres rich sample, B), FT4 (the biggest sampled fragments, C), FT3 under a stereoscope, a marked grid is 2x2 mm (D).

Microplastic Size Categorization: Methodology and Limitations in Preliminary Study

In our preliminary study, we chose 2 mm size intervals for categorizing microplastics due to practical limitations in our sampling and measurement methods. We collected samples and analyzed them using a stereomicroscope. To measure microplastics, we marked 2 mm squares on a Petri dish lid using a needle, which allowed for accurate and consistent measurements.

Given the constraints in resolution and precision, we established size categories based on 2 mm intervals, such as particles larger than 4 mm but smaller than 6 mm. We could also measure smaller particles, as it was easy to see if they were half or a quarter of the 2 mm squares, resulting in additional categories for 0.5 mm and 1 mm particles.

We recognize that our size classification approach may differ from other studies, but we believe it was appropriate for our preliminary research considering the available equipment and resources. Future studies could use more advanced techniques, like image analysis software, to refine size categorization and enable more precise measurements of microplastics across different size ranges.

Data Processing and Statistical Analyses

Our initial data analysis step involved descriptive statistics in Microsoft Excel, providing an overview of our dataset, including measures of central tendency, dispersion, and frequency distributions. This information helped us identify dependencies and trends, guiding our decisions regarding suitable statistical tests for examining specific factors of interest. Further statistical analyses were performed in R version 4.1.2. Our data exhibited a non-parametric distribution (Shapiro, p-value = 7.073e-09), which led us to select the following tests: Anderson-Darling test, sensitive for smaller sample sizes, was used to assess the goodness-of-fit of our data to different distributions; Kruskal-Wallis chi-squared test was employed to compare multiple groups for significant differences; and the pairwise Wilcoxon post hoc test was utilized to identify significant differences between pairs of groups.

Calculating Particle Concentration

To determine the concentration of particles, we divided the number of particles by the volume of water filtered by our neuston net. We calculated the volume using the formula for a cylinder: volume = $\pi \times \text{radius}^2 \times \text{length}$, where the radius is half the inner diameter.

The length was determined using Google Earth Pro, which calculated the distance between each transect's start and end GPS points. Our neuston net had an opening diameter of 60 cm. After obtaining the volume of water filtered for each transect, we divided the number of particles by the volume in litres and converted the result to particles per cubic meter. This unit was chosen because it is commonly used in studies on this topic.

Estimating the Weight of Microplastic Particles

Estimating the weight of microplastic particles was challenging due to their low individual weights, which often fell below the sensitivity of our scale. However, we were able to devise an approximation method:

11. For fibres: In sample CT5, which contained only fibres, we weighed the entire sample and divided the weight by the number of fibres present. This gave us an estimated weight for a single fibre, which we then multiplied by the number of fibres in each sample to estimate their total weight.
12. For fragments: We randomly selected 100 plastic fragments from various samples to obtain a detectable weight on our scale. We then divided the total weight of these fragments by 100 to estimate the weight of a single fragment. This estimate was used to calculate the total weight of fragments in each sample by multiplying the single fragment weight by the number of fragments in the respective samples.

The total weight of each sample was calculated by summing the estimated weights of fibres and fragments within the sample. The total weight of each sample was divided by the volume of water the net passed through in a particular transect, resulting in a weight measurement in grams per cubic meter (g/m^3).

Results

Descriptive statistics

The Pearson chi-squared test indicated a significant difference between the observed and expected average number of particles (Fig. 21) (p value< 2.2e-16).

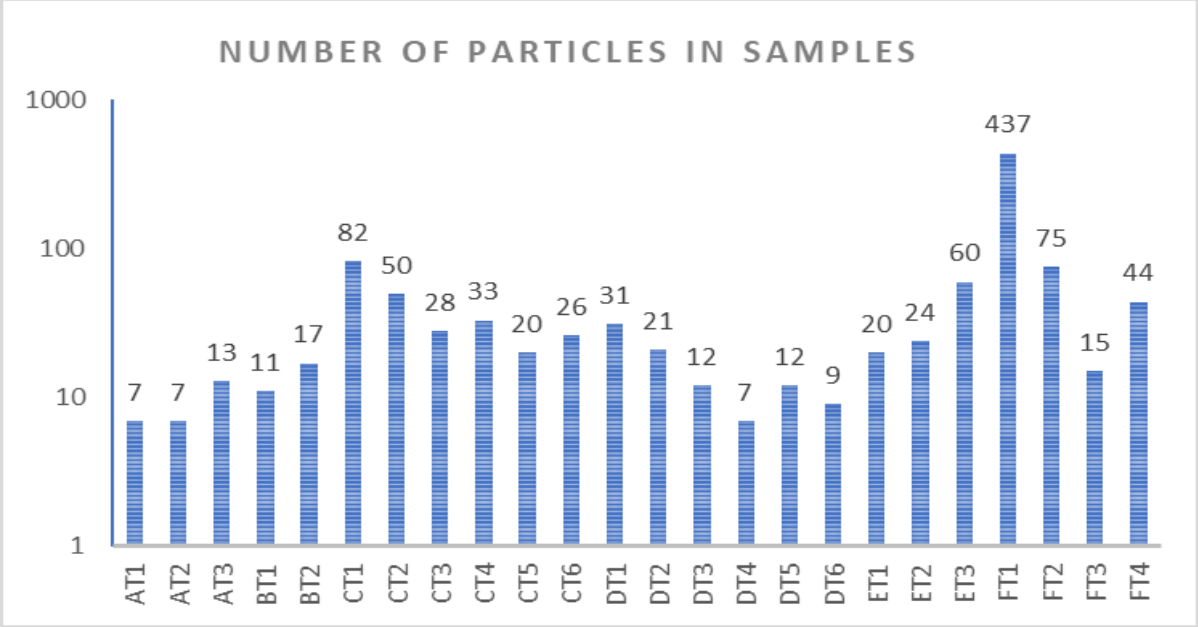

Figure 21: Number of particles per sample

The median number of particles is 20, while the average number for all samples is 44.2. The number of particles' standard deviation is 86.24, suggesting relatively high levels of data variability (Table 4)

Table 4: Descriptive statistics of particle number

Total number of collected particles	1061
Max in sample	437
Min in sample	7
Average	44.2
Median	20
Standard deviation	86.24

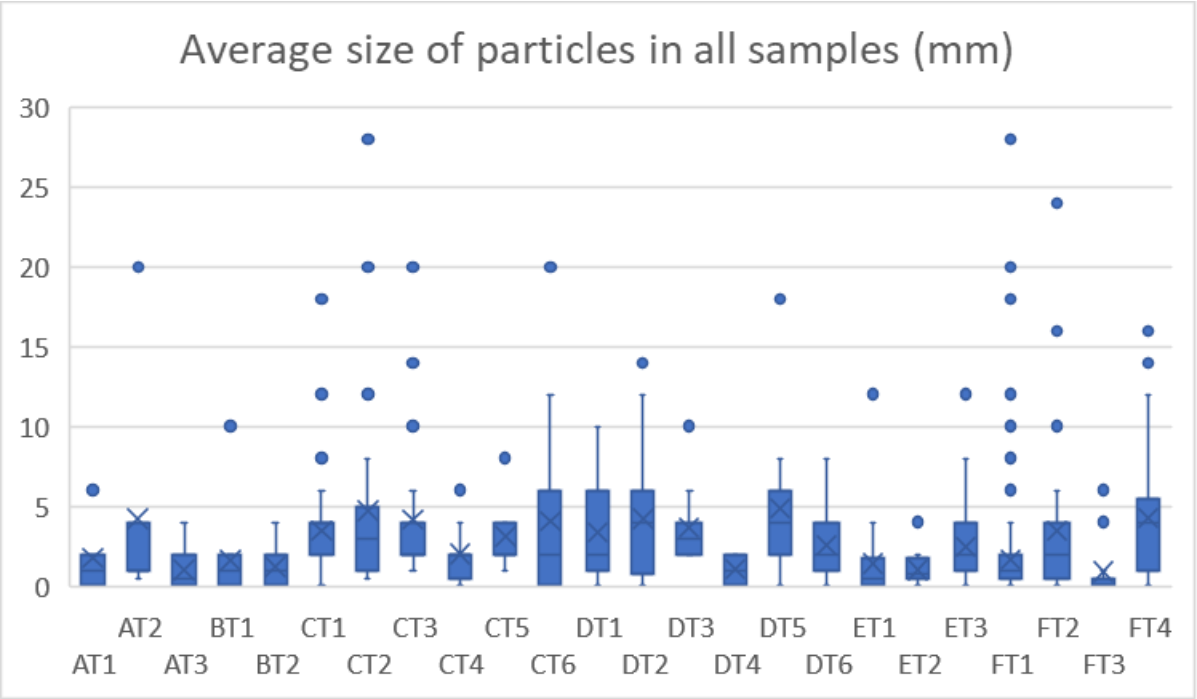

Figure 22: Average size of particles in samples, not including one extreme particle of 46 mm from a sample FT1.

The largest and smallest particle sizes observed were 46 mm and 0.10 mm, respectively (Fig. 22). The median particle size was 1 mm, suggesting that the distribution may be skewed, while the average particle size was 2.51 mm (Table 5).

Table 5: Descriptive statistics of particles size

Max	46
Min	0.10
Average	2.51
Median	1
Standard deviation	3.61

The study separated plastic particles into two groups: fibres and fragments. All samples included fibres, but CT5 was the only sample without fragments. There were 411 fibres found, and 650 total pieces (Figure 24). Transparent was the most prevalent colour in the sample, accounting for 551 particles, followed by blue (252) and white (151) particles. Grey and pink were unique colours with only 3 and 2 particles (Figure 23).

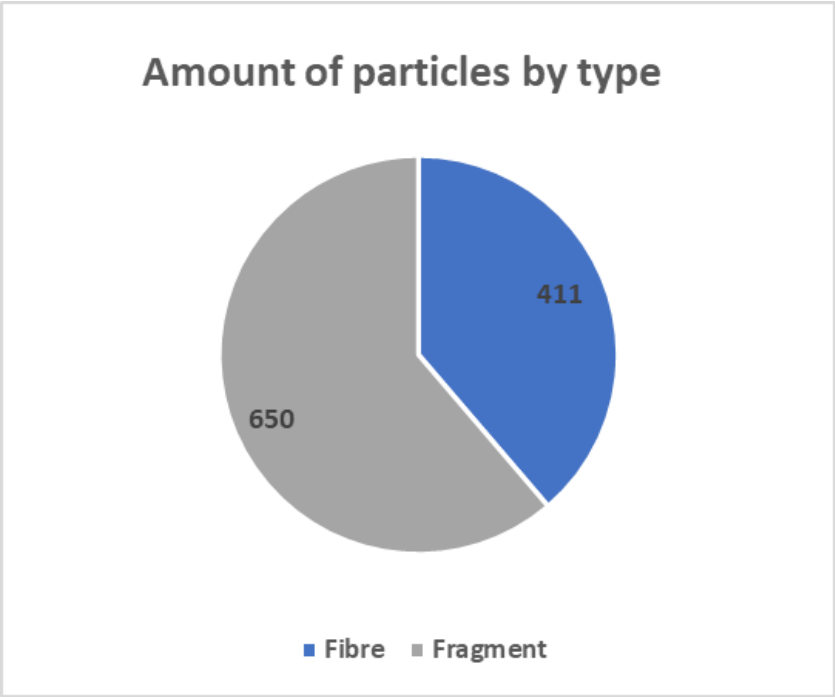

Figure 24: Number of particles found by type

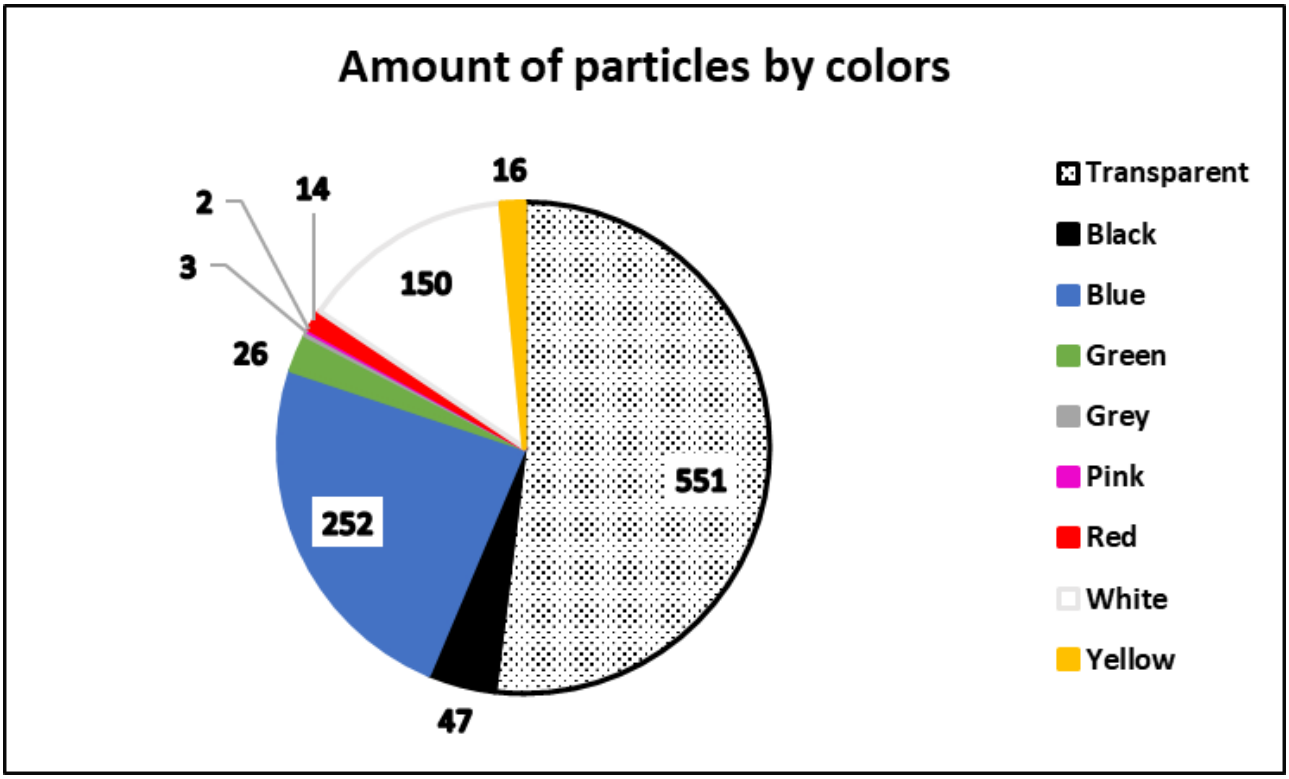

Figure 23: Number of particles found in samples by colours

We used a chi Pearson's squared test and Pairwise proportion tests with Holm's adjustment method test to detect a significant difference in the number of fibres and fragments across all samples and between particular samples. The results indicated significant differences between ratios of fragments and fibres in many samples (Figure 25; Tab S1, Figure S1, Supplements).

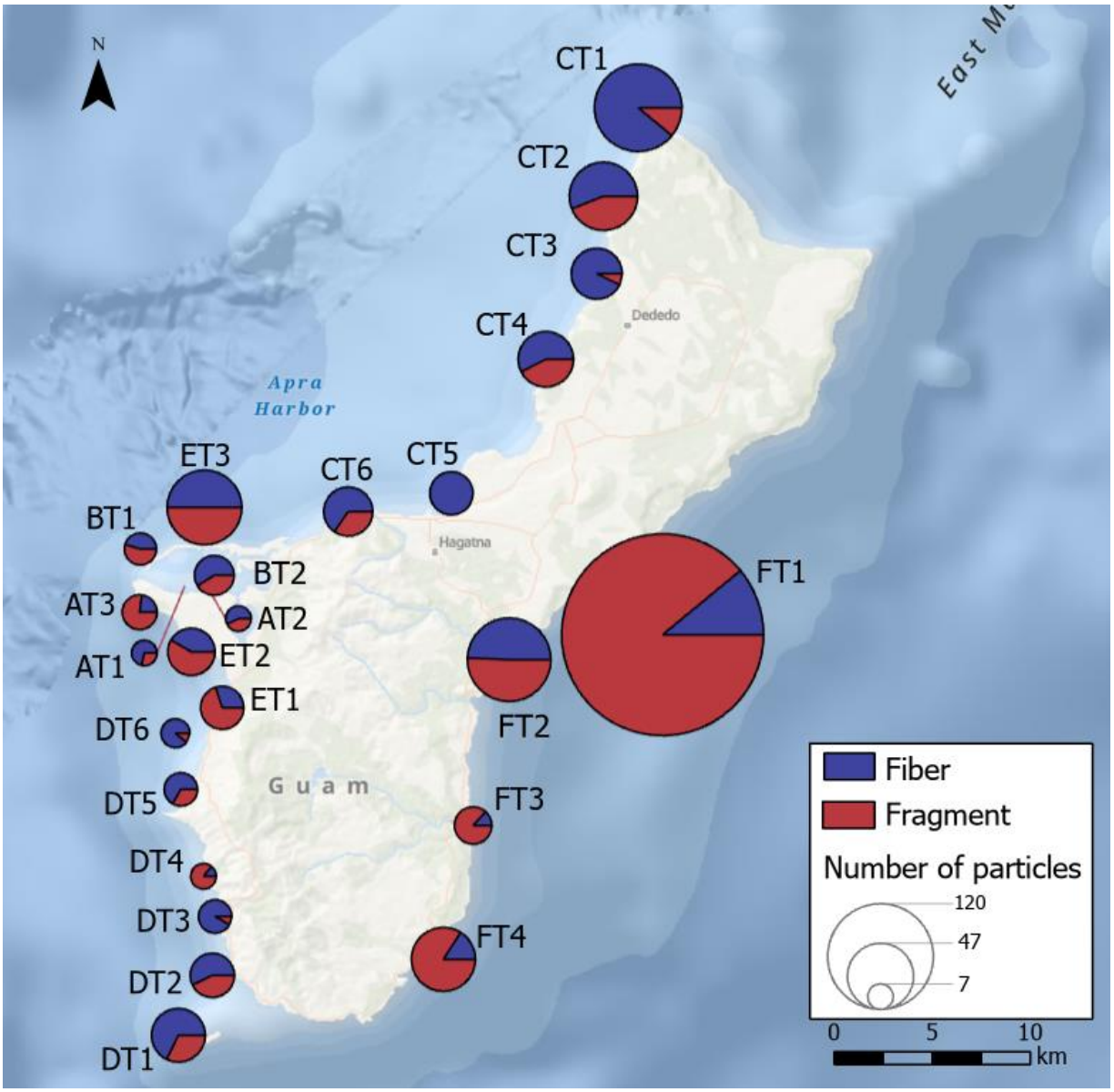

Figure 25: Distribution of plastic particles and their fibre/fragment ratios

Testing for errors in sample data

In the Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean GESAMP 2019, weather and environmental factors such as location depth, wavelength, precipitation, and amount of sand and organic matter might affect the composition of samples. We ran a series of tests to indicate an error. While sampling, the depth was fluctuating, and our neuston net went through a couple of shallow locations, which affected the amount of sand in the samples.

Sand and organic matter

In general, samples of sand and surface water from the exact location might dramatically differ in numbers and proportions of meso- and microplastic particles (Erni-Cassola, Gabriel et al., 2019; Minor, Elizabeth C., et al., 2020). Therefore, to avoid possible errors, we tested if the amount of sand in our samples had an impact, and we collected suspended solids from the bottom of the sampling sites.

We indicated a considerable amount of sand in our samples taken from transects with the shallowest minimal depth, which suggested a possibility of a mistake (Kruskal-Wallis, p-value = 0.01771) (Figure S3, Supplements). However, we did not indicate any significant difference in the number of particles in our samples with a high amount of sand (Kruskal-Wallis, p-value = 0.5701, 0.5026 and 0.6122 for fragments and fibres, respectively). Furthermore, just like that, we found that an amount of organic matter also did not affect the total number of particles in samples, nor the composition of fragments or fibres (Kruskal-Wallis, p-value = 0.05888, also 0.07732 and 0.0951 for fragments and fibres respectively). Therefore, the amount of sand and organic matter did not introduce any error in our samples.

Precipitation and wavelength

We found out that the highest number of particles occurred in samples "F.T." that we took in a day with the highest amount of precipitation (Kruskal- Wallis, a p-value of 0.009246) (Figure 26); however, we did not find medians of precipitation groups different. Even though we coul d see some positive trends (Figure S6, Supplements), the Spearman test did not prove this corr elation as statistically significant R=0.35. The p-value of 0.13 suggests that the correlation ma y be due to chance. Further analysis may be needed to determine the nature of the relationship between the variables.

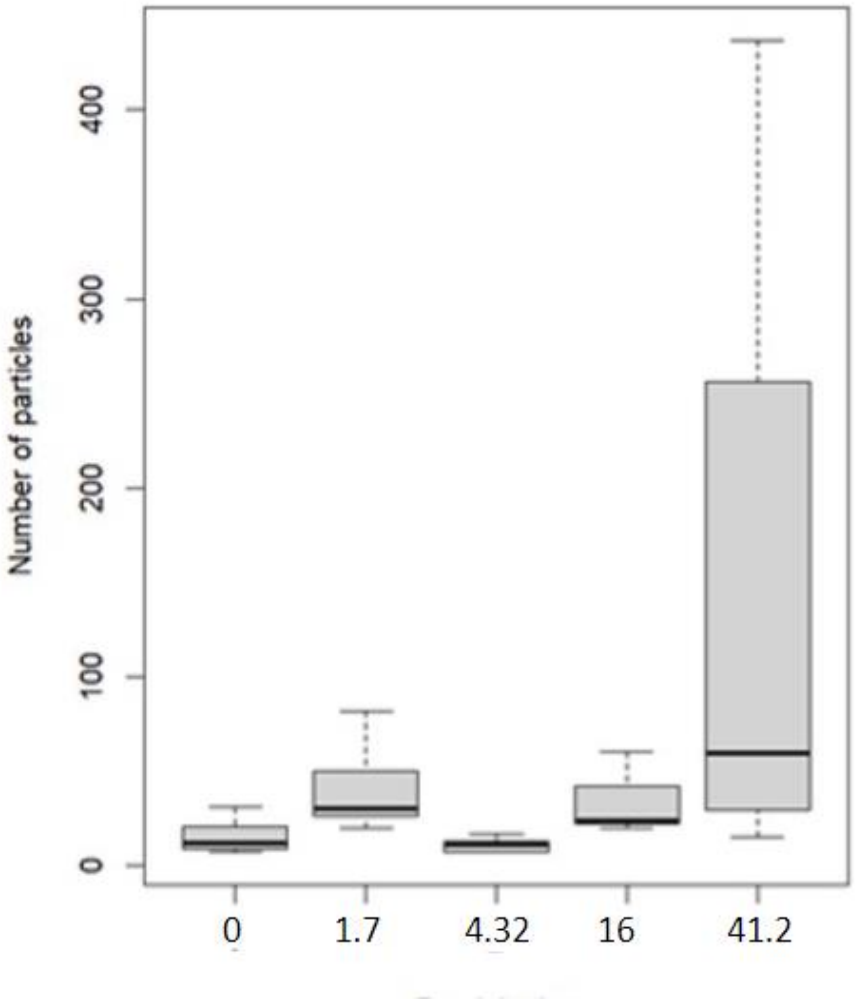

Figure 26: Average number of sampled particles in different levels of precipitation

Number by wavelength

In contrast to the precipitation factor, we found a moderate positive correlation between the number of particles and the wavelength (Spearman, R=0.58, p = 0.0028) (Fig. 27). As it is clear from the graph, the confidence interval pictured as a grey zone is vast, which indicates more uncertainty in the correlation estimate, signalling that it might have been affected by several factors, such as sample size or variability in the data. Therefore, we need to be cautious before claiming anything of relationships between wavelength and precipitation to the number of particles.

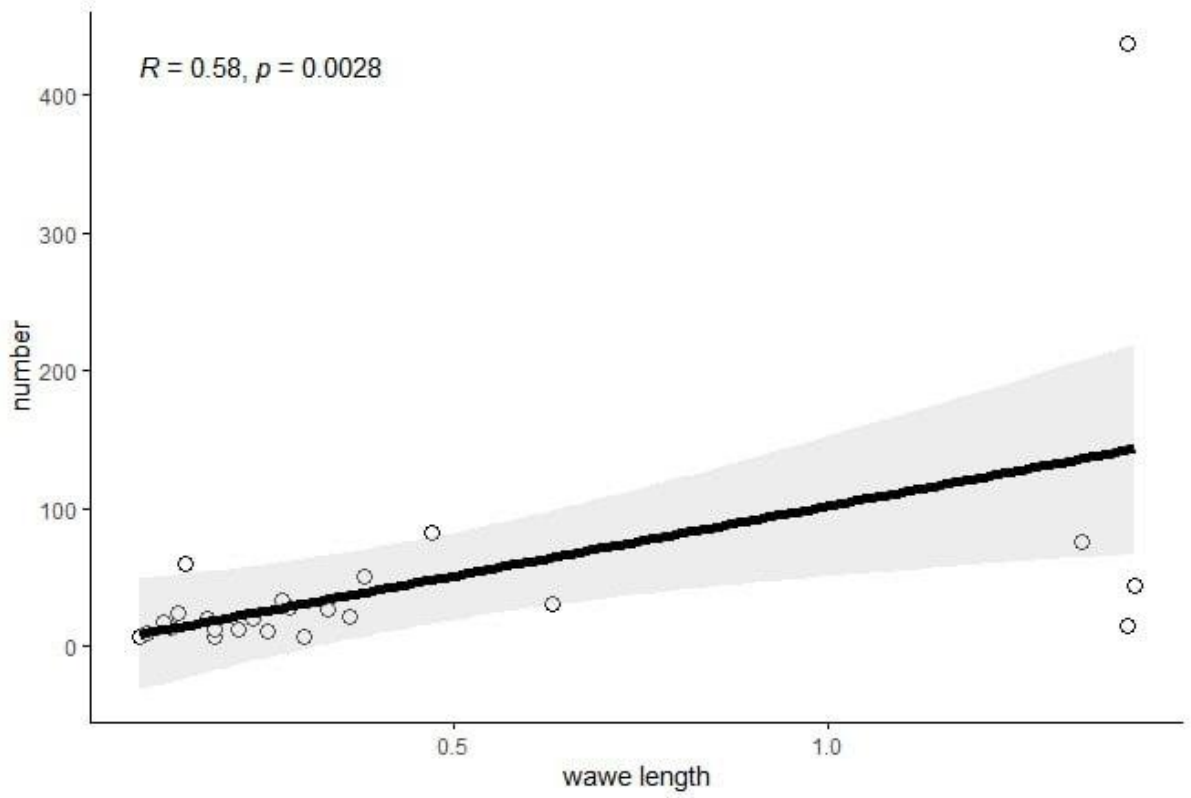

Figure 27: Relationship of the number of particles and wavelength in transects.

SIZE

Generally, relatively "fresh" mesoplastic concentration must increase in areas closer to their sources (Isobe, Atsuhiko, et al., 2015). Therefore, we tested if the size of the plastic was affected by potential contamination sites. We had factors such as River mouth, Wastewater Treatment Plant (WWTP), and Shore Fishing Areas (Fig. 28, S5, S6, Supplements). We found no significant difference in particle size in samples taken in the shore fishing areas (Wilcoxon test p-value = 0.6725).

Wilcoxon test with a p-value = 2.745e-05 have shown a statistically significant difference in microplastic sizes with proximity to a river mouth; however, the boxplot bodies and medians are pretty similar, and both exhibit a substantial number of outliers (Figure 28). Therefore, based on these boxplots alone, since they both contain micro- and mesoplastic particles, it would not be easy to make a strong claim of an ecologically significant difference between the groups. Further research is warranted to understand and interpret these findings fully.

Similarly, proximity to a WWTP has significantly affected sample particle size, showing that smaller particles tend to occur there (Wilcoxon test p-value < 2.2e-16). However, many outliers and similar medians stop us from making a solid conclusion. This question must be studied further.

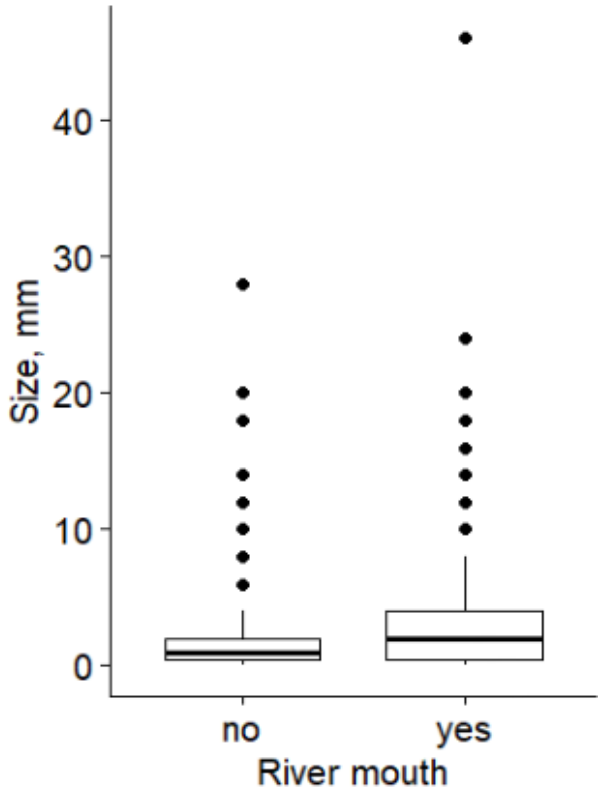

Figure 28: Average size of particles found far and near a river mouth.

The concentration of particles in water

The graph 29 illustrates the concentration of plastic particles in different samples, with the y-axis representing the concentration in numbers and the x-axis representing the different sites. The bars in the graph represent the concentration of plastic particles in each site, with the highest concentration observed in sample FT1, with 1283.7 particles per cubic meter, and the lowest concentration in sites AT1 and DT4, 18,7 and 18,8 particles.

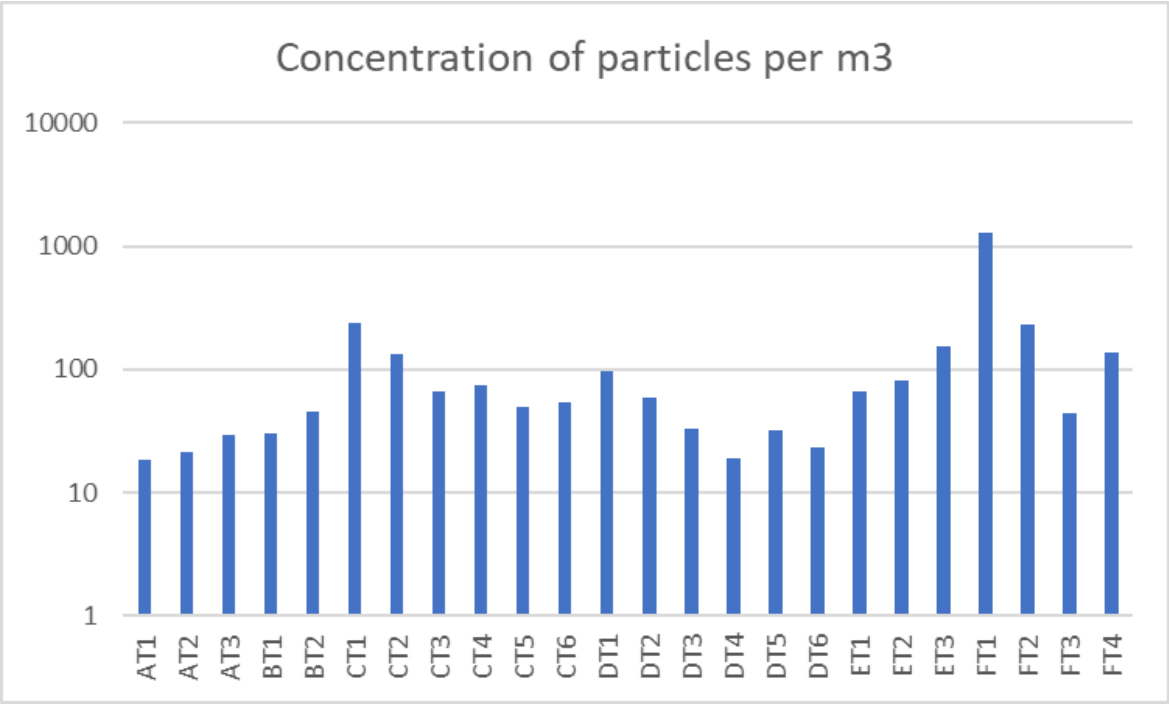

Figure 29: Concentration of plastic particles per cubic meter

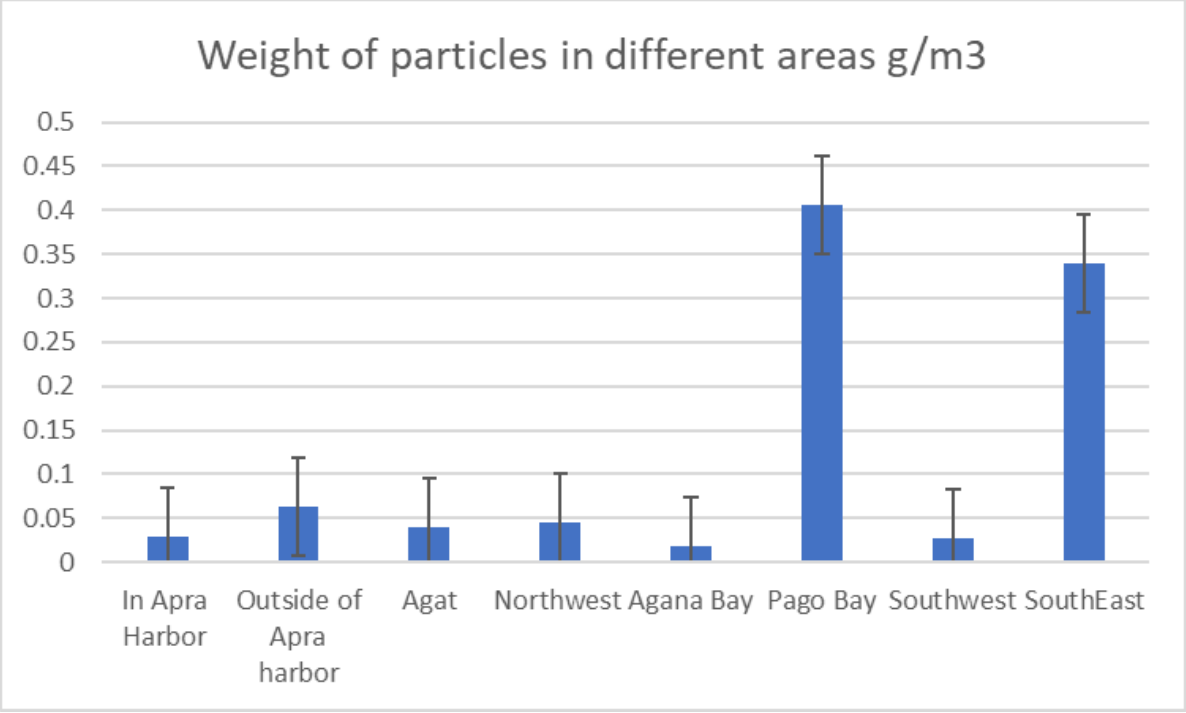

Figure 30: Average weight of particles in different areas

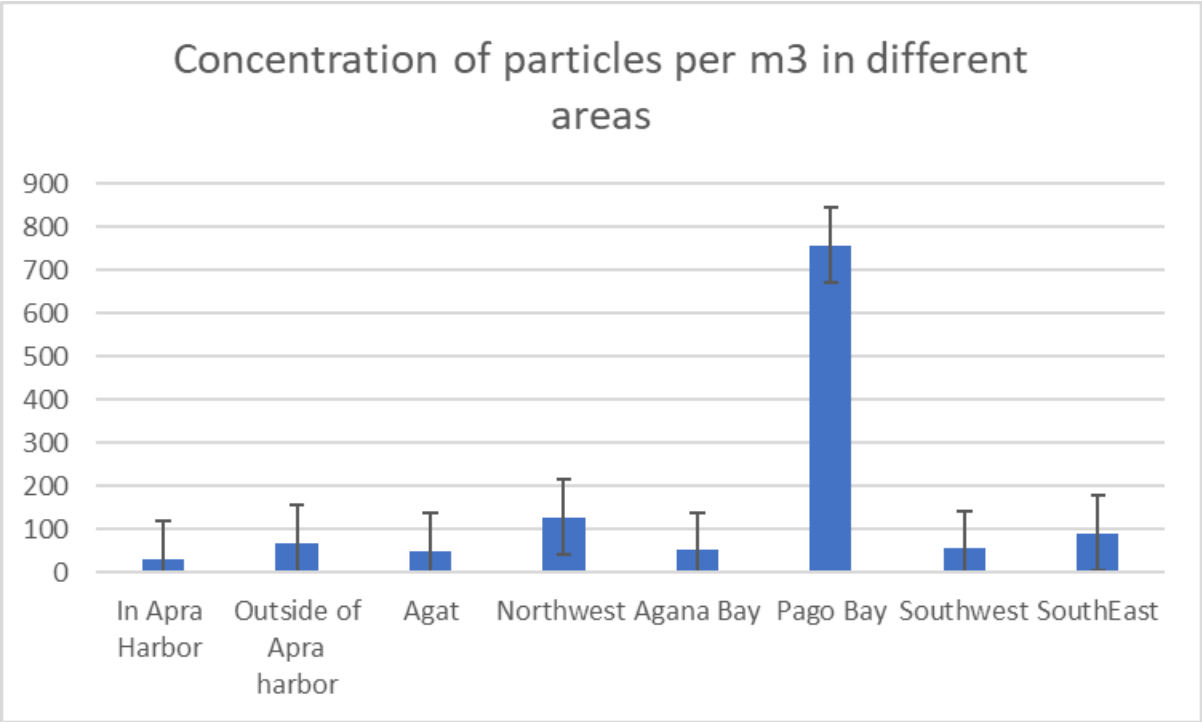

Figure 31: Average concentration of particles per m^3

Relationship of particle concentration and weight

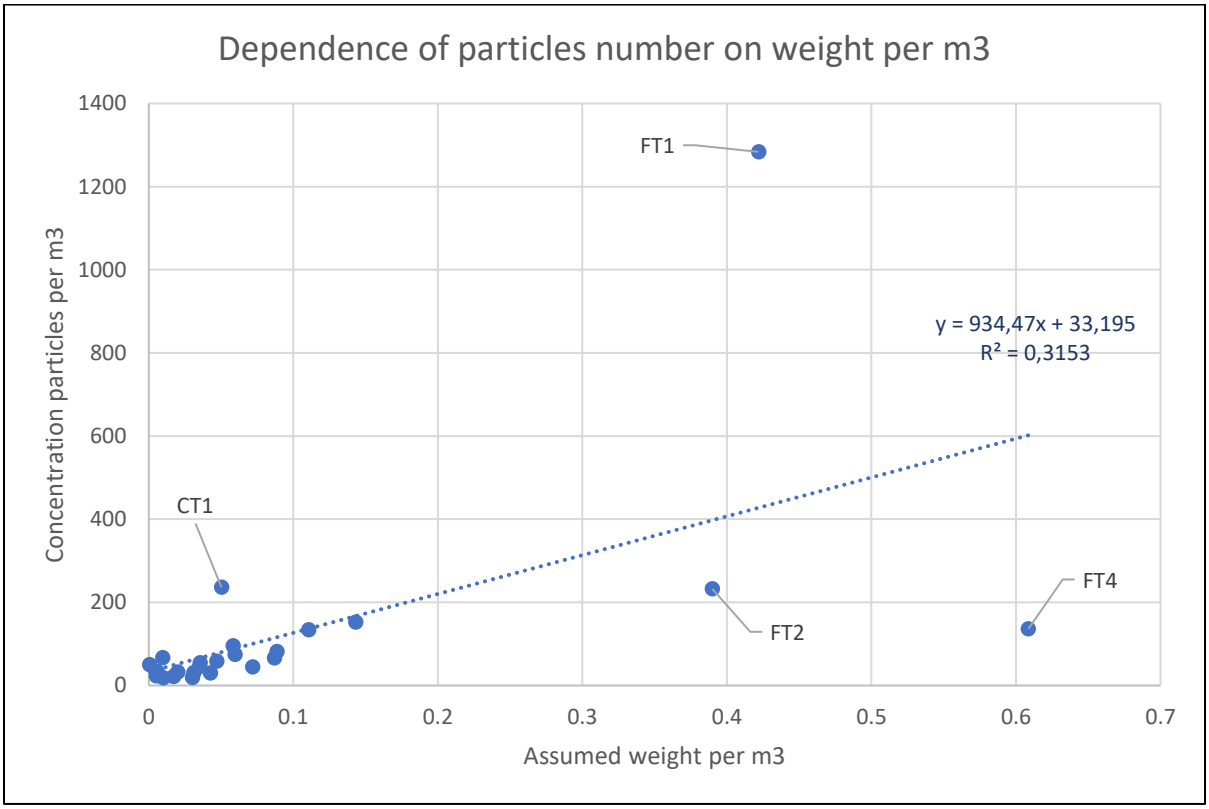

Figure 32: Dependence of particles concentration on weight g m^{-3}.

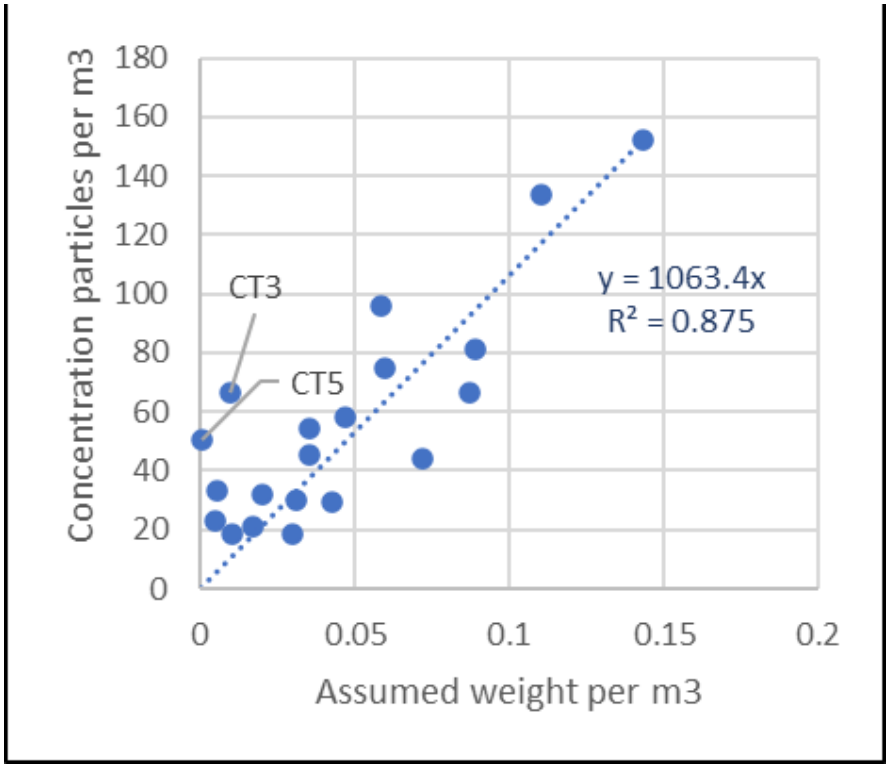

Figure 33: Dependence of particles concentration on weight g m^{-3} (without outliers)

In Figure 32, the concentration of microplastic particles per m³ (Y-axis) is plotted against the assumed weight in grams per m³ (X-axis). A positive trend is observed between the concentration and weight of microplastics, particularly in the X-axis segment from 0 to 0.15 (Figure 13). However, notable data points CT1, CT3, and CT5 display unexpectedly low weights despite their concentrations (Fig. 33). These samples were taken on the same day, under the same weather conditions, and in the northwestern part of the island, suggesting other factors may influence the particles' weight.

Additionally, outliers FT1, FT2, and FT4 exhibit interesting characteristics. FT1 has a low weight (0.421 g/m³) given its high concentration (1283.69 particles/m³), implying smaller particle sizes. FT2, sampled near FT1, is also an outlier but with an unusually high weight (0.389 g/m³) for its concentration (232.2 particles/m³), suggesting fewer but larger particles. Lastly, FT4 has the heaviest weight (0.608 g/m³) with 136 particles/m³.

Discussion

Although our statistical analyses may have limited sensitivity and cannot conclusively establish observed trends, our findings can tentatively address the following hypotheses:

1. Coastal waters in Guam exhibit similar pollution levels compared to other Pacific Ocean locations but are generally less polluted than shelf seas.
2. Microplastic concentrations are elevated in areas with significant anthropogenic influence (e.g., high population density), near river mouths, and in convergence zones of water currents.
3. South Pago Bay is more heavily impacted by microplastic pollution than North Pago Bay.
4. Northern Guam experiences reduced microplastic pollution due to relatively strong constant water currents and decreased land-based input, while higher concentrations of microplastics are found in the south (including southeast and southwest) due to the greater number of river outlets.

Apra Harbour

Kazour et al. (2019) found that 96% of microplastics decreased with increasing distance from the WWTP effluent. This intriguingly contradicts our results from Apra Harbor, where we observed the lowest average amount of microplastic particles. This is surprising considering the high sailing activity from cruise and military ships and four wastewater treatment plants within the harbour. It is important to note that our results do not include the orange flake-like particles, which easily dissolve in water (Fig. S9, S10). Based on observed boat traffic and conversations with local sailors, we hypothesize that these particles could be antifouling paint, as they were the same bright orange colour as the buoys in the harbour. To better understand the distribution of microplastics in the area and the possible contribution of these particles, further investigation is needed, taking into account factors such as boat traffic, wastewater treatment, and other potential sources of pollution.

North of Guam

While we observed low urban activity and no river outlets in North Guam, we found many mesoplastic particle outliers in fibres and higher than average concentrations at sites CT1 and CT2. These results indicate that other factors may influence microplastic distribution, and further research is needed to clarify these relationships. M.P. contamination in these locations suggests that it depends more on seawater currents than on the proximity of anthropogenic activities (Browne et al., 2011).

South central coast and southwest of Guam

The number of plastic particles from the south central coast (samples DT5, DT6, ET1 AND ET2, from 9 to 24) and southwest Guam (samples DT1-DT4, from 7 to 31 particles) was lower than average (44.2 particles), despite Merizo pier (sample DT2) being known for its boat activity, active shore fishing, and targeted annual underwater clean-ups by local volunteer groups (Figure S11, S12 and 13, Supplements). The amount of mega plastic extracted in one day from the Merizo channel highlights the varied distribution of debris in sediments, water column, and the water surface. An ideal study should include samples of sediments and water at different depths to accurately depict the situation in specific locations.

A possibility of Covid-19 lockdown effect on our data

It is important to consider that the absence of a strong correlation between the number of microplastic particles and areas of presumed human activities might be due to the impacts of the COVID-19 pandemic. Tourism, a significant source of marine debris on small islands, influences the input of debris (Hayati et al., 2020; Wilson et al., 2017). Guam, a popular tourist destination, witnessed reduced tourism during the pandemic, leading to decreased human presence on beaches and recreational boat activity. Marine scientists worldwide reported positive changes during this period, such as diminished shipping noise (GeoNoise 2020), which was previously found to lower stress-hormone levels in marine creatures (Rolland et al. 2012). Additionally, in Bora-Bora, a decline in human activity resulted in an increased abundance of reef fish (Lecchini et al., 2021). During our fieldwork in July 2022, restrictions had just begun to ease, so tourists did not fully occupy hotels and beaches. This reduction in human presence and recreational boat activity may have affected microplastic input in the area, underscoring the necessity for long-term studies and more data collection to comprehend better the relationship between human activities and microplastic pollution in Guam's coastal waters. (Hayati et al., 2020). Furthermore, tourists' activity influences the source of debris (Wilson et al., 2017)

South and North Pago Bay

In scientific literature, Pago Bay was once mentioned as a site for sand samples within the study about the developmental impacts of environmental M.P. extracts on the early life stages of Japanese medaka (*Oryzias latipes*). Results of the study showed that the concentration of M.P. particles in the sand on Pago Bay was 5.9 g/m2 (Pannetier et al., 2019).

South Pago Bay was also included in a conference paper saying that there was notably more plastic in sand samples than in the North Pago Bay site (Cacapit & Walsh, 2020). Additionally, a southern part of Pago Bay became an example of plastic as a growing threat, as reported by local news in 2018. In this article, a former marine biology teacher Linda Tatreau warns local society about the staggering number of plastic debris that will turn into microplastic if not removed (The Guam Daily Post, Swartz, 2018).

The paper by Comfort et al. (2019) was instrumental in formulating our hypothesis that South Pago Bay would have a higher concentration of microplastics than North Pago Bay due to the presence of a river mouth. Despite this, our results showed the opposite, with North Pago Bay having a significantly higher number of plastic particles. This finding directs our attention back to the weather factor mentioned in the GESAMP (2019) guidelines and the study by Comfort et al. (2019), which highlighted the complexity of circulation patterns and the significant influence of local wind on the movement of water masses in the area. These factors emphasize the need for a more nuanced understanding of the processes affecting microplastic distribution in Pago Bay.

Comparison with other studies

For instance, microplastic concentrations in the surface waters of the remote, uninhabited coral reefs in the Nansha Islands, located on the western edge of the Coral Triangle, were 0.0556 ±

0.0355 items m-3. These concentrations are lower than those observed in Guam's waters (56.5 ± 36.92 particles per m-3), which could be attributed to the little bit larger mesh size utilized in the Nansha study (333 μm), which may have underestimated the actual pollution there (Tan, Fei, et al., 2020).

A study from Palau, Micronesia (Béraud, Eric, et al.,2022) observed an average microplastic concentration of 50 particles per m3 around reefs, initially appearing similar to the average concentration found in our study. However, the survey in Palau utilized a net with a 100-micron mesh, which raises concerns about the comparison, as we calculated a higher concentration even with less precise equipment, a mesh of 300 microns. Arcadio et al., 2022 conducted a study in the Philippine Sea and reported microplastic concentrations between 17.14 and 24.17 pieces per m3, but based only on ten samples (Fig. 34)

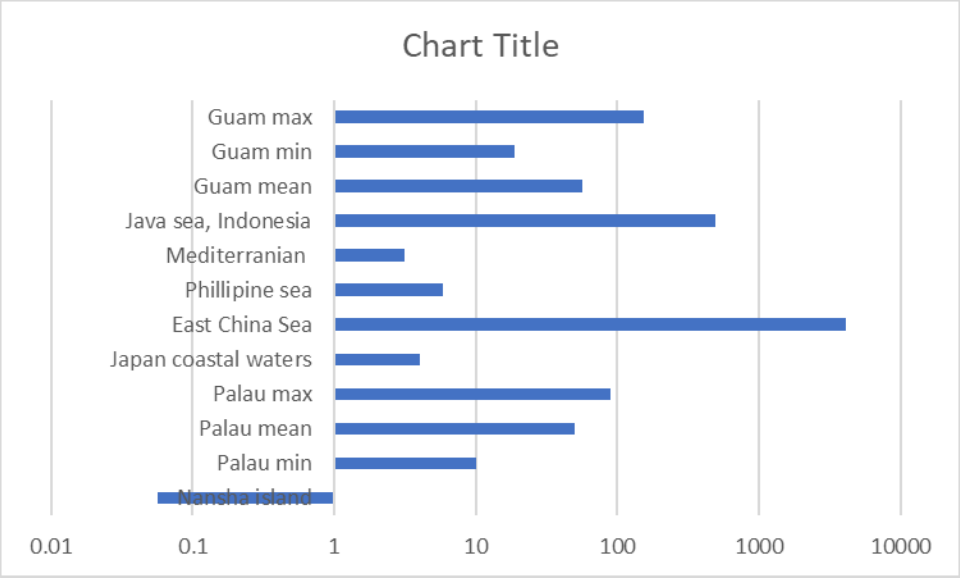

Figure 34: Comparison of concentration from other studies in particles per m^3

Study limitation: more sampling is needed

Our study faced several limitations that may have impacted the accuracy of our findings on microplastic distribution in Guam. These limitations can be categorized into sampling, equipment, and analytical constraints.

1. Sampling Constraints:

- Weather dependency: Unfavorable weather conditions hindered the collection process and potentially led to inconsistencies in the data.
- Single samples per location: This may not capture the full extent of spatial heterogeneity and limit the understanding of microplastic distribution within each site.
- Wet season sampling: Conducting sampling exclusively during the wet season might not accurately reflect microplastic concentrations throughout the entire year, as concentrations can differ considerably between wet and dry seasons.
- Sampling frequency: Our study did not adhere to the recommended sampling frequency of approximately every 25 days, which may limit the strength of our findings.

To address these sampling constraints, future research should consider sampling during wet and dry seasons, collecting multiple samples per location, and adhering to the recommended sampling frequency.

2. Equipment Constraints:

- Absence of a flowmeter: The lack of a flowmeter in our study may have affected the precision of our quantitative measurements.
- Equipment availability: The lengthy process of delivering new equipment to the island prompted us to adapt existing tools for our fieldwork. This resulted in some handmade components that required more attention and increased the risk of losing samples.

Future research should aim to use better resources and planning and attach a flowmeter to the sampling net to obtain more accurate data on the volume of water filtered.

3. Analytical Constraints:

- Hot needle method: This more affordable alternative for quantitative analysis may not be sensitive to plastic particles that melt at higher temperatures, which could have impacted our data in some cases.

Future studies should consider using more sophisticated methods, such as microscopy and Fourier-transform infrared (FTIR) spectrometry, to obtain more accurate and comprehensive results.

The need for a bigger sample size

Increasing the sample size would enhance the sensitivity of our statistical analysis, allowing researchers to derive more robust conclusions from their data. A more comprehensive view of microplastic concentrations and distribution in Guam can be achieved by addressing these limitations in future studies. Although we acknowledge that there are several reasons not to draw definitive conclusions based on our results, we believe that our preliminary study can help address the critical limitation of the current lack of data on microplastic distribution in Guam and its possible effects on the precious reef ecosystems.

How could microplastic pollution be connected to coral reefs?

The reefs of Guam have been significantly impacted by a series of extreme environmental events since 2013. Elevated sea surface temperatures have induced severe island-wide bleaching, while extreme low tides caused additional coral mortality from subaerial exposure on shallow reef flat platforms(Raymundo, L. J., et al., 2019). Given that some reef-building corals respond to thermal stress and bleaching by increasing heterotrophy, this may raise the risk of ingesting microplastics in Guam's coral reefs. Small islands like Guam are likely disproportionately affected by climate change-related stressors due to their high reef-to-land area and reliance on shallow marine ecosystems. Consequently, these islands have experienced gradual declines in their marine ecosystems' health, diversity, and productivity, mainly due to local anthropogenic stressors. Therefore, it is essential to develop effective strategies for mitigating the impacts of microplastic pollution and other stressors on Guam's coral reefs to protect their biodiversity and vital ecosystem services for coastal communities. We hope to stimulate further research and contribute to a more comprehensive understanding of microplastic pollution and its potential impacts on these vital marine habitats by providing an

initial insight into this issue. Axworthy et al., 2019; (Raymundo, L. J. et al., 2019; Hall et al., 2015)

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Supplementary materials

Table of Figures

Table of Tables

Table S 1: Pairs of samples with a significant difference in proportions of fragments and fibres between each other

group1	group2	p	p.adj	p.adj.signif	group1	group2	p	p.adj	p.adj.signif
AT3	CT1	2.61E-07	0.0000668	****	DT1	FT1	6.77E-17	1.85E-14	****
CT1	CT2	0.0000362	0.00877	**	DT2	FT1	6.92E-09	0.00000181	****
AT3	CT3	0.0000266	0.00649	**	DT3	FT1	1.09E-14	2.93E-12	****
AT3	CT5	0.0000163	0.00404	**	DT5	FT1	1.06E-07	0.0000273	****
CT1	DT4	0.00000551	0.00138	**	DT6	FT1	9.57E-11	2.53E-08	****
CT3	DT4	0.0000868	0.0207	*	ET2	FT1	0.0000419	0.0101	*
CT5	DT4	0.0000309	0.00751	**	ET3	FT1	2.93E-14	7.84E-12	****
CT1	ET1	8.09E-08	0.000021	****	CT1	FT2	1.52E-07	0.0000391	****
CT3	ET1	0.000022	0.00538	**	CT3	FT2	0.000142	0.0333	*
CT5	ET1	0.0000164	0.00404	**	CT5	FT2	0.000117	0.0277	*
CT1	ET2	0.00000303	0.000763	***	FT1	FT2	6.58E-16	1.78E-13	****
CT5	ET2	0.000138	0.0326	*	CT1	FT3	1.05E-09	2.77E-07	****
CT1	ET3	7.19E-07	0.000183	***	CT3	FT3	0.00000107	0.00027	***
CT5	ET3	0.000189	0.044	*	CT5	FT3	9.69E-07	0.000246	***
AT1	FT1	0.0000166	0.00409	**	CT1	FT4	2.15E-15	5.8E-13	****
BT2	FT1	5.73E-08	0.0000149	****	CT2	FT4	0.000146	0.0341	*
CT1	FT1	3.88E-52	1.07E-49	****	CT3	FT4	7.96E-10	0.00000021	****
CT2	FT1	5.36E-16	1.46E-13	****	CT5	FT4	1.53E-09	4.02E-07	****
CT3	FT1	3.46E-29	9.5E-27	****	CT6	FT4	0.0000771	0.0184	*
CT4	FT1	1.05E-12	2.8E-10	****	DT1	FT4	0.0000151	0.00375	**
CT5	FT1	2.48E-26	6.8E-24	****	DT3	FT4	0.00000362	0.000908	***
CT6	FT1	8.21E-14	2.19E-11	****	DT6	FT4	0.0000576	0.0138	*

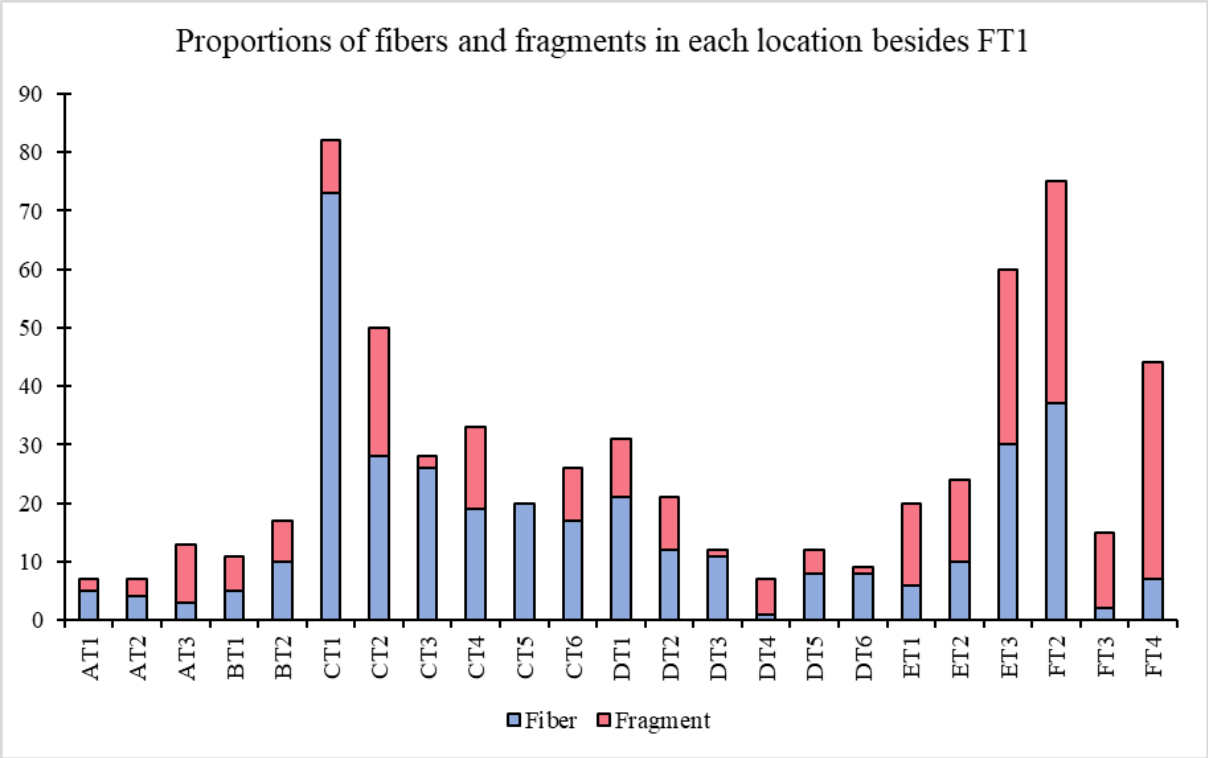

Figure S 1 : Proportion of fibres and fragments in each location besides FT1

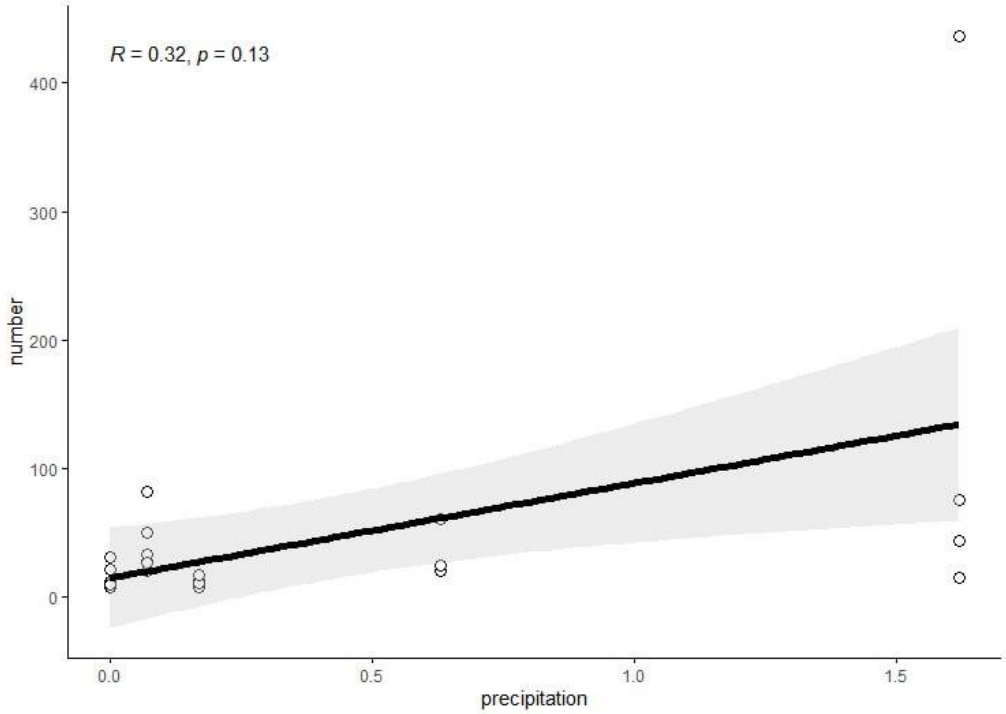

Figure S 2: Relationship of precipitation and number of sampled particles

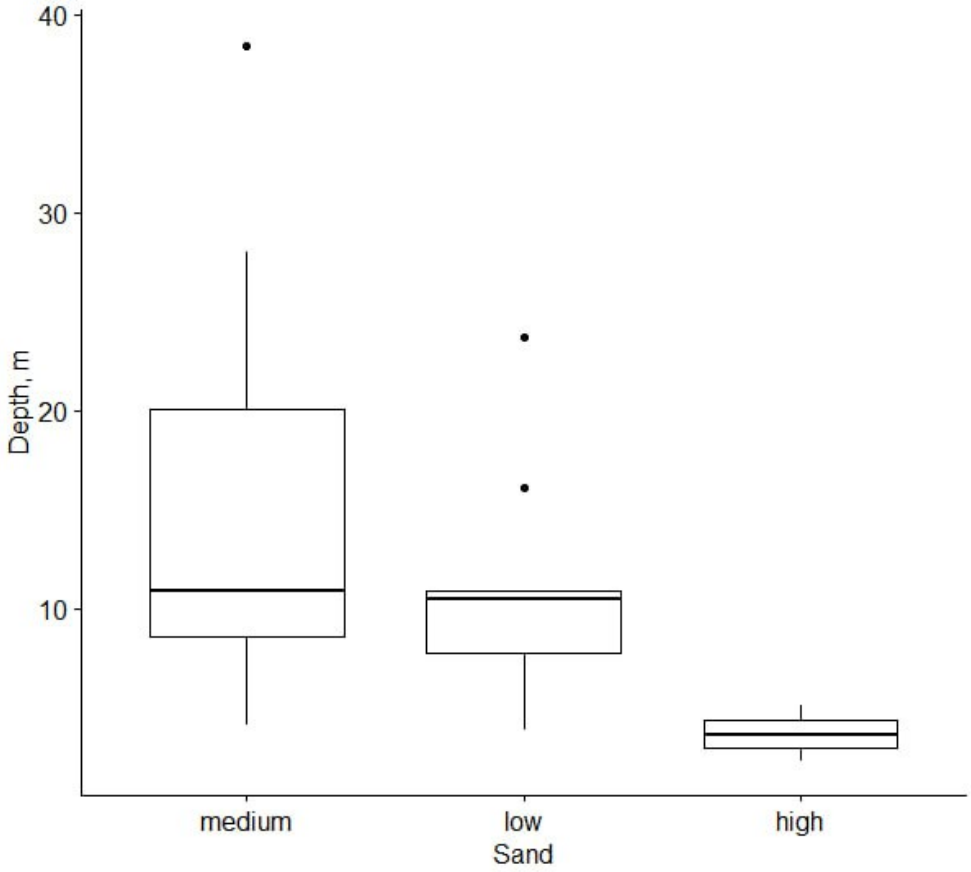

Figure S 3:Amount of sand in samples for transects with different depths

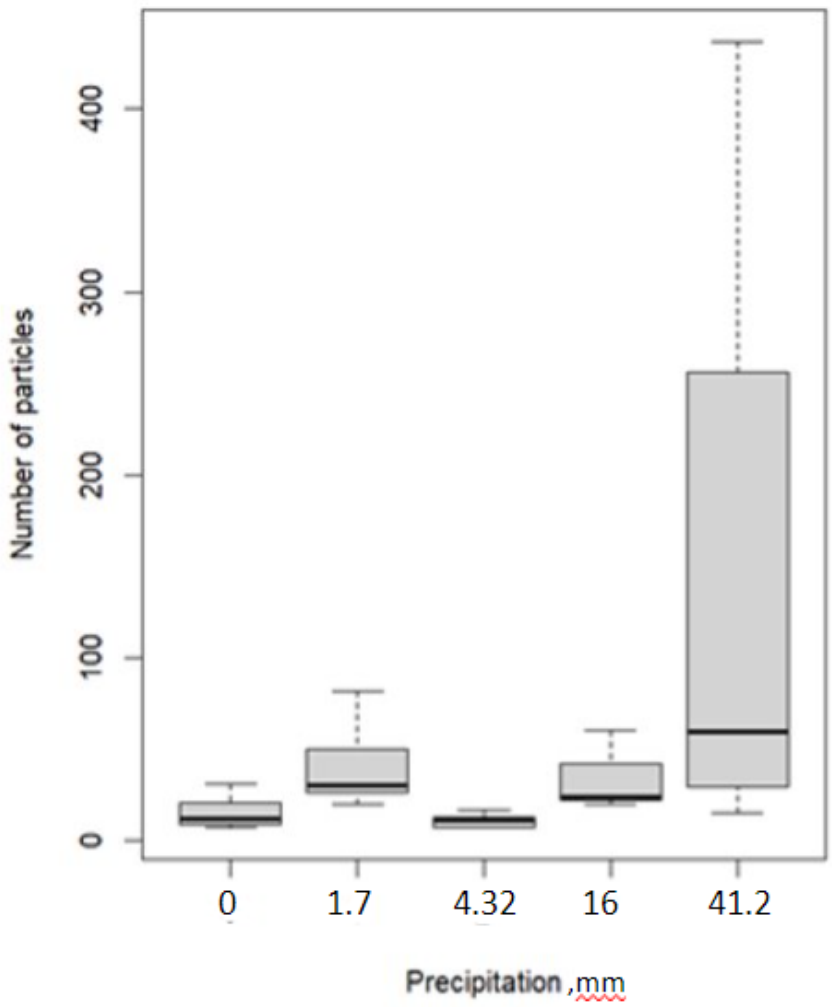

Figure S 4: Average number of sampled per particle during levels of precipitation

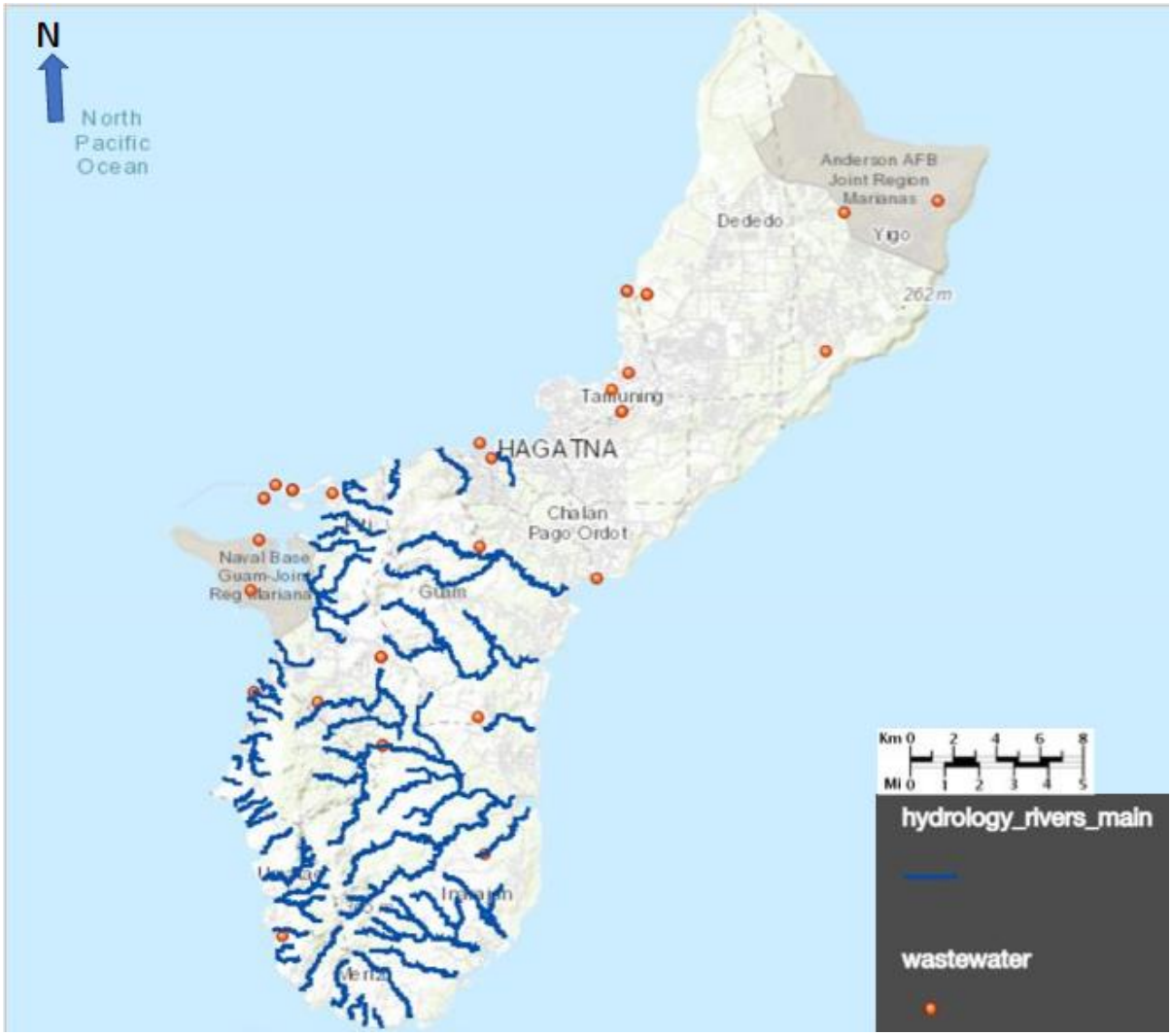

Figure S 5: Wastewater treatment points and central rivers of Guam.

Figure S 6: Shore fishing areas of Guam

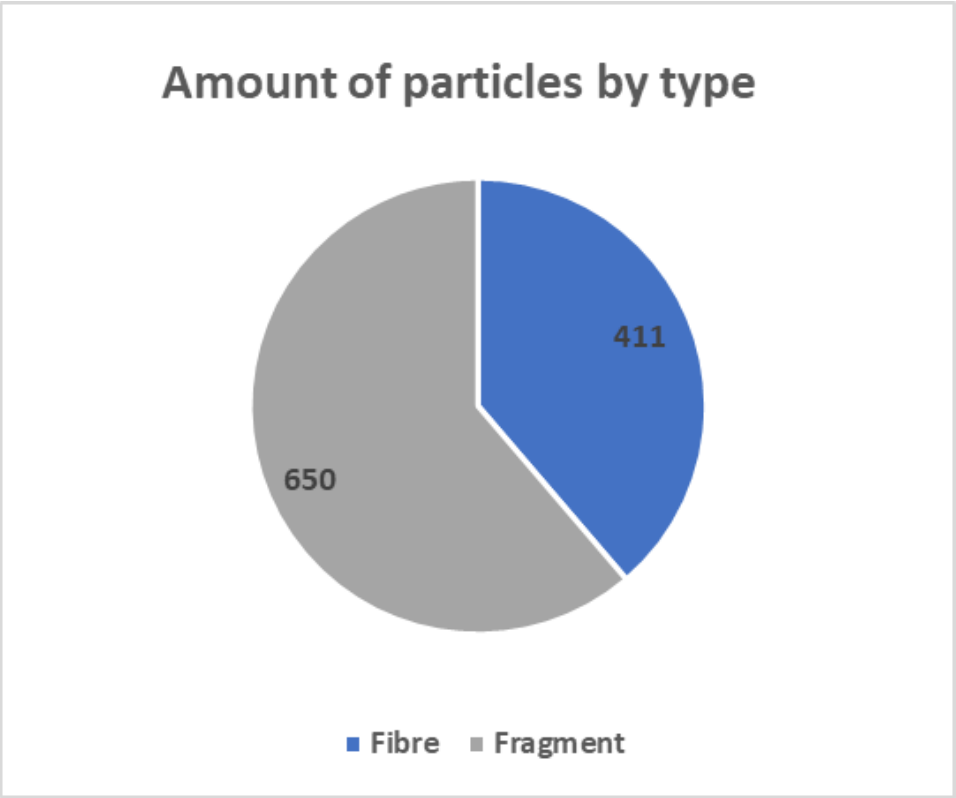

Figure S 7: Number of particles found in samples by type

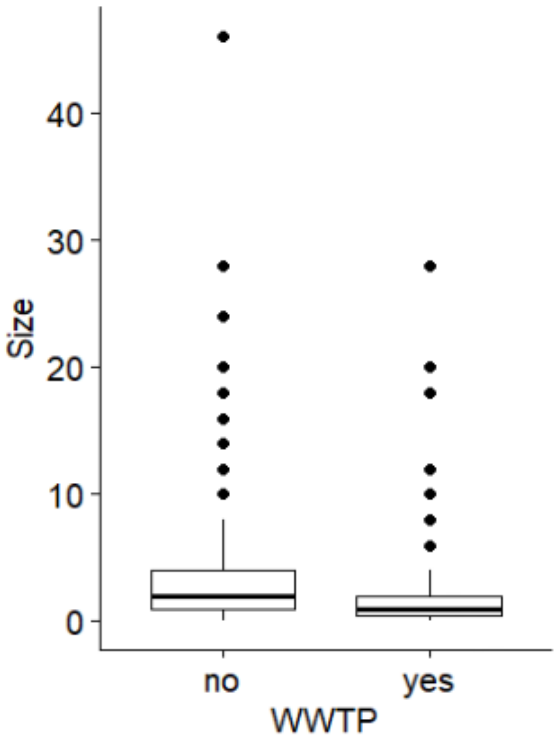

Figure S 8: Caption to ADD

Figure S 9: Neuston net covered in orange flake-like particles of questionable origin, sample AT3

Figure S 10: Filtered AT2 sample with a large amount of orange flake-like particles.

Figure S 11: Flyer for the 5th underwater clean-up event at the Merizzo pier

Figure S 12: Vintage Cans from the 1980s Collected at Merizo Pier during the 5th Annual Clean-up, July 2022.

Figure S 13: Large-Scale Debris Extracted from Merizo Pier, July 2022

Figure S 14: Highly polluted shore of South Pago Bay, southern part, July 2022

Figure S 15: Highly polluted shore of South Pago Bay, Northern part, July 2022

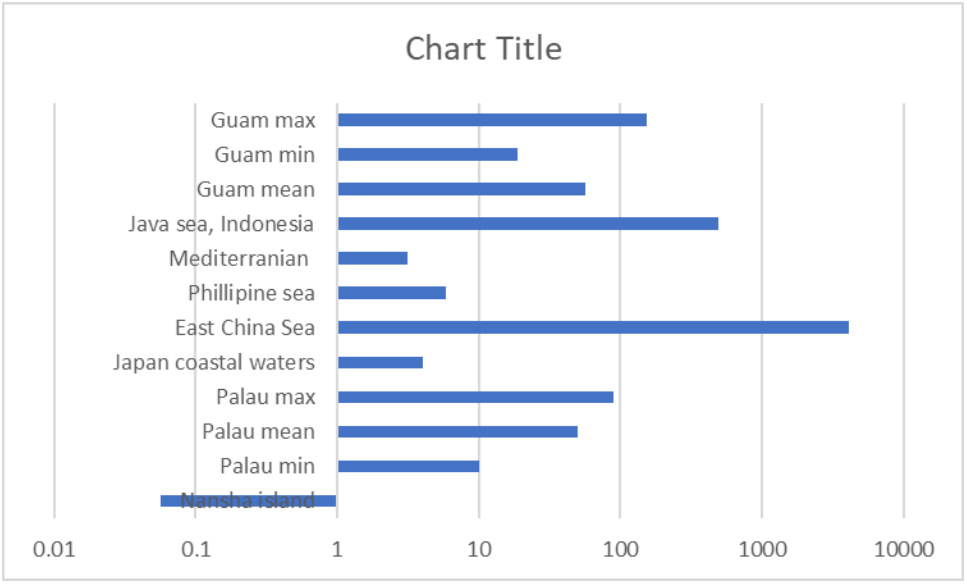

Figure 35: Comparison of concentration from other studies in particles per m^3