L-Università ta' Malta

University of Malta, Faculty of Science

A preliminary quantitative and qualitative investigation of microplastics sampled from Maltese nearshore waters

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Malta, 2023

OCEANOGRAPHY

Abstract

This study aims to characterize the microplastic pollution of the Maltese nearshore waters from an innovative perspective by targeting to shed more light on the dynamics of microplastic accumulation within the water column around the Maltese Islands over two year (2022 and 2023). This pioneering survey is in fact examining different sea layers in order to determine whether different plastic polymers accumulate with increasing water depth. We have performed an experimental sampling methodology, with the modification of our Manta trawl which has been used for the sampling of the surface water, We executed horizontal tow sampling by maintaining the Manta net at a stable depth (6 ±1m and 10 ±1m) in a parallel position, by setting up the perfect balance between the weighting of the frame of the Manta net and the speed of the tugboat to keep the net tight. The focus of our research was on the examined lower size (50-1000µm) range as the larger pieces (1000-5000µm) were typically not found in the water column samples but rather, almost exclusively in the surface samples; hence, the comparison was out of scope. Most of the particles were made of 27.9% Acrylic, 27.2% PE (Polyethylene) , 23.9% PP (polypropylene) and 11.6% PS (polystyrene). Average between the two years, 60% of the total number of plastic particles found to be distributed within the 50-350µm range. We have observed that the Microplastic pollution level of the surface waters were 0.25-7.363 particles/m^3, while in the sub-surface water column on 10 meters from the surface we found 1.090-6.60 particles/m^3.

Acknowledgement

This research project has been made possible through a joint collaboration between **the University of Malta - Oceanography Malta Research Group**, **Zibel**, Environmental NGO and **Eurofins Analytical Services Hungary Ltd**.

The same research has been part-supported by the **Elisabeth Mann Borgese Bursary** of the **International Ocean Institute** which has been awarded to Ede Kossari Tarnik for 2022-23.

Special thanks to:

- Prof Alan Deidun - University of Malta, Oceanography Malta Research Group
- Dr Adam Gauci - University of Malta, Oceanography Malta Research Group
- Alessio Marrone - University of Malta, Oceanography Malta Research Group
- Johann Robert Galdies - University of Malta, Oceanography Malta Research Group
- Andrew Schembri - Zibel
- Arkadiusz Srebnik - Zibel
- Dr Gábor Bordós - Eurofins Analytical Services Hungary Ltd.
- Bence Prikler - Eurofins Analytical Services Hungary Ltd.

Research website:

https://ocean.mt/deep-see/

Outreach:

https://timesofmalta.com/articles/view/substantially-higher-concentration-microplastics-maltese-waters.1027925

https://greendeal.mt/insights/collaborators/project-deep-see/

https://www.eurofins.hu/hu/analytical-services-hungary-en/news/clothing-tag-in-the-sea/

Content

Introduction

In the 21st century, plastics became a part of every aspect of our life, their usage has been continuously increasing in the recent decades. Plastics are indeed used in many areas of life that have truly improved our quality of life, however in many instances it would be necessary to reconsider and re-structure its consumption habits. The evident presence of plastic waste in the environment in various forms is the result of the inappropriate and irresponsible industrial and costumer behaviour as well as the underdeveloped waste and wastewater treatment procedures in many countries. Large organizations that considerably have a significant impact on the use of single-use plastic have implemented a developed sustainable attitude, after evaluating the need to use unsafe goods for the environment and have eliminated or replaced them with recyclable resources, as well as putting a large effort on mitigation and elimination of the emission sources in all aspects of their operation. However, the impact due to its irreversible state has become one of the most significant environmental challenges of our time. To further understand the impact of plastic waste in the environment, identifying the sources of emission and regulating the use and emissions will be inevitable contra-action of the future.

In a country, like Malta, where the nation is facing major environmental challenges due to its fast-growing population, sudden climate changes, increase in waste production and a reduction of natural space, we shall put a high effort on studying the changes of our impact on the environment. Malta is struggling to handle and treat the generated waste due to the unstructured and uncontained waste collection system and due to the lack of land space and local treatment facilities.

According to the survey carried out in March 2023 by the Malta National Statistics Office, namely the ‘’Single-Use Plastic Survey” [1], only 49% of the questioned Maltese habitants have heard about the term of Microplastics. Moreover, 69% of the participants were not aware that certain waste streams are containing single-use plastic, and 18% of them has admitted to disposing these waste streams onto the ground. This study perfectly shows the need for changing of the mindset of the Maltese habitants and the need to raise awareness, since these are essential for the mitigation of this crisis.

For this reason, we have established this research to further understand what impacts the Maltese islands are facing in terms of Microplastic pollution involving an innovative perspective to not only assess what is floating on the surface of the nearshore waters, but also to understand the transferring phenomenon of the microplastics into a lower sea level without sedimentation. The research results will provide us with a better understanding of the potential deviation from the theoretical total microplastic pollution level in nearshore waters. This will help us gain further insight into the behavior of microplastics in the marine environment. For the sake of a valuable outcome, We have identified 3 different potentials ‘’polluted hot-zones’’ around the Maltese Islands, which are exposed to obvious anthropogenic emissions and we have chosen a reference side along the coast which shouldn`t have any direct human interaction in its pollution level. The monitored sites were 1. **Xgħajra,** located along the south-eastern coast of Malta, subject to a sewage outfall 2. **Għar Lapsi** located along the south-western coast of Malta, subject to the reject (brine) outfall of a reverse osmosis plant 3. **Ħofra ż-Żgħira** located off the south-eastern coast of Malta, subject to the discharge of the cooling water of the local power plant and the 4th control/reference site **Selmun** which located off the north-eastern coast of Malta**. (Figure 1).** The variation in pollution levels between the surface and different depths, which is explained during this research may suggest a phenomenon that warrants further investigation.

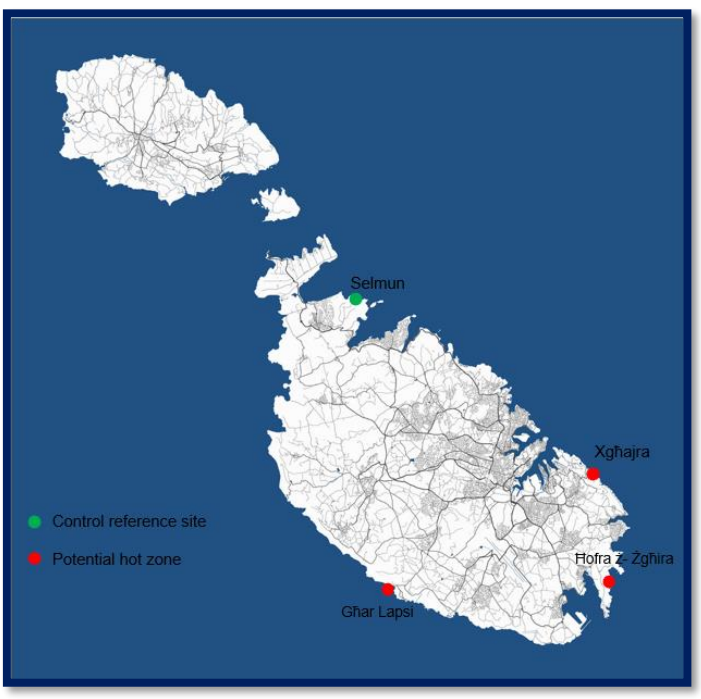


Figure 1 Map of Malta showing the sampling points.

Literature review

Introduction of Microplastics

Tiny pieces of plastic were first observed in the seas in the 1970s [2], however the term microplastics was only used in the 2000s [3] first. Over the past few years, several studies have reported the presence of microplastics in seas, oceans, and unfortunately proved the effectivity of the spread of pollution and the diversity of its sources, that there is a high number of researches proven the presence in wastewater, fresh water, food, air , soil and drinking- water, both bottled and tap water, which raising questions and concerns about the impact that microplastics in drinking-water might have on human health. [4]

The definition of microplastics is not completely straightforward yet, as these represent a diverse range of material types, shapes, colours, and sizes. The term microplastic refers to the size of a fragmentable plastic waste which come from waste that is released into the environment and shredded there. In the absence of a clear definition within current legislation, we are identifying microplastics as solid synthetic-polymer-containing particles of no more than five millimetres in their longest dimension [5] , however microplastics are classified into three different size ranges based on existing research protocols. Those between 5 mm and 1 mm in their longest dimension are called large microplastics (LMP), those between 1 mm and 1µm small microplastics (SMP) and the particles smaller than 1 µm are called nano plastics (NP). [6]

The disintegration of plastics is the result of mechanical, chemical, physical and biological processes. The most significant of these factors are the ultraviolet radiation, namely UV-B (~ 295-315 nm) and UV-A (~ 315-400 nm) [7] range for the incorporation of oxygen atoms into the polymer chain which provides the necessary activation energy. This process is known as chain explosion resulting in further formation of smaller plastic fragments (photodegradation). In petroleum-based plastics, the disintegration of polymer chains occurs, but this is not followed by the same rate of biodegradation as

in the resulting fragments the molecular weight may still be above a value for microbiological availability. [8] This factor may could be one of the main reasons for the appearance and accumulation of microplastics in the environment. In terms of nano plastics, there are several studies on the formation of nano plastics that examine pH, temperature, and salinity [9] as essential formation conditions, however the determination of this problematic size range is still under research.

Microplastics enter the environment in several of ways: primarily from direct disposal, surface run-off and wastewater effluent, but also from combined sewer overflows, industrial effluent, degraded plastic waste and atmospheric deposition.

Plastic production and main types

To acquire knowledge about polymers and plastics and in relation to the topic clarification of the appropriate technical terms, concepts and definitions are essential. This is not always easy, as some concepts are used synonymously (e.g., macromolecule, polymer, plastic), while in other cases the concepts are not clear. When discussing synthetic large-molecule substances, the individual chain is usually understood as a macromolecule. During the polymerization (chemical process for producing polymers) process, a polymer is formed through the combination of macromolecules or polymer molecules, while plastic is basically an engineered processed material, which is generally being used in practice. [10]

The terms polymer and plastic are often used interchangeably; neither does the literature always differentiate between the two terms. However, the definition of plastic contradicts many other ideas in a similar way. The main distinction between the terminologies is the polymer formed during the polymerization process, while it may also be seen in the composition, structure, and characteristics of the material that is utilized. As a result of polymerization, a more or less well-defined molecular weight and chemical composition material are obtained. However, the plastic is not used in this form, as materials, additives or associative substances are added to them as the last step in the material production. [10]

In this part I will describe two general types of plastics available on the market: thermoplastics and thermoset plastics.

Thermoplastics are materials, whose glass transition and/or crystallization temperature is above room temperature. With the temperature increasing, the material melts and goes into a liquid state. Their processing at high temperatures occurs under the influence of an external force. [10] Thermoplastics includes Polyethylene (PE-used in toys, shampoo bottles, pipes, etc.), polypropylene (PP−used in food packaging, snack wrappers, auto parts, etc.), polyethylene terephthalate (PET− often used for water and other beverage bottles), polystyrene (PS−used in foam food containers, eyeglasses, building insulation, etc.), polyvinylchloride (PVC−used in window frames, pipes, cable insulation, etc.), and others including polycarbonates (PC) and polyamides (PA). [11]

Thermoset plastics are those with a glass transition temperature above room temperature. They are generally rigid and have high strength. [10] Thermoset plastics include polyurethane (PUR−used in building insulation, pillows and mattresses, insulating foams, etc.), epoxy resins, some acrylic resins and some polyesters. [12]

All over the world, the production and usage of the plastic materials due to the versatility of these materials has significantly increasing.

According to the statistics published by ‘’Our World in Data’’ in 2019, we have produced globally nearly 460 million tonnes, while compared to 2000 we have more doubled our yearly production **Figure 2**. [13]

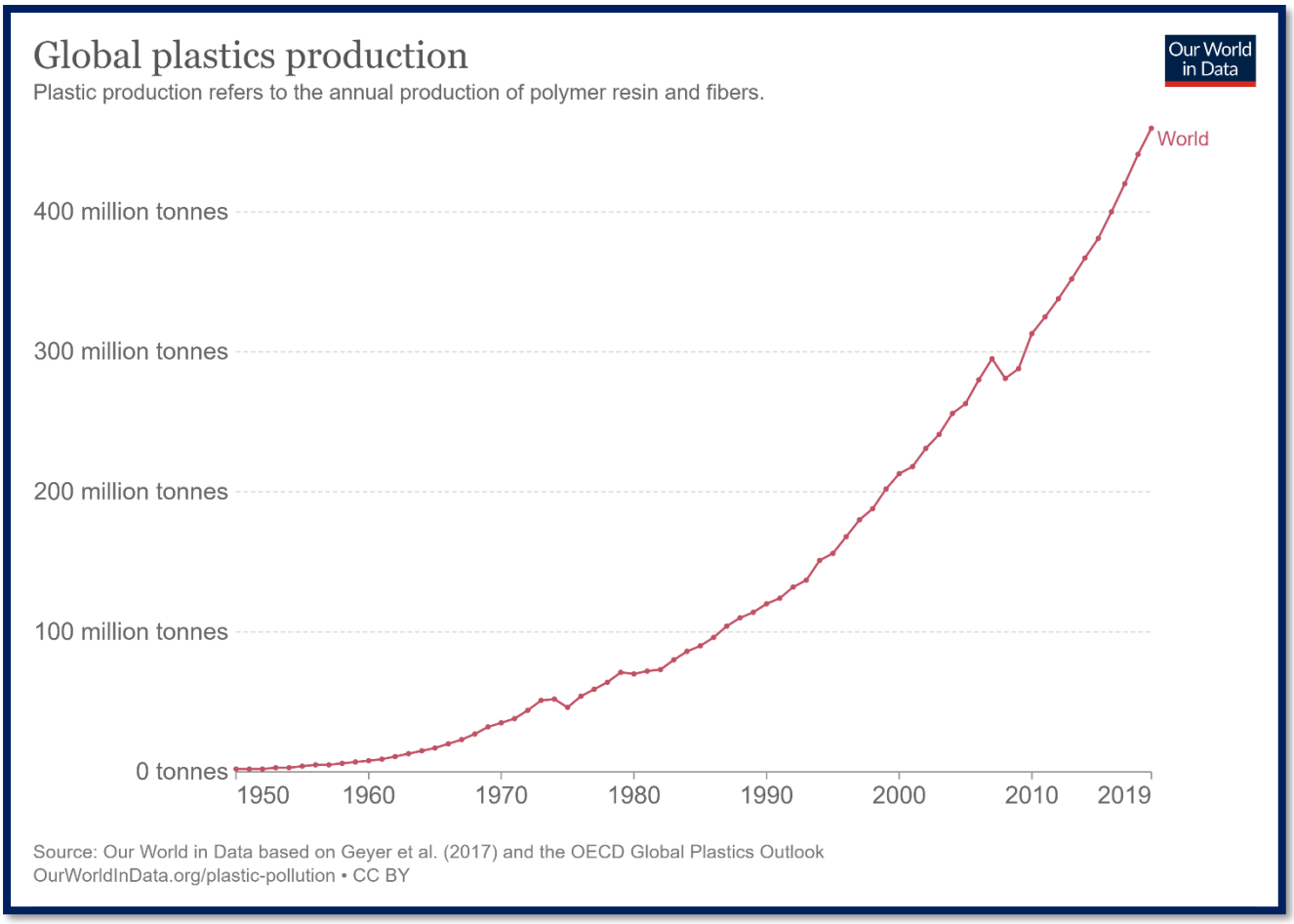


Figure 2 Global plastics production statistics 1950-2019 [13]

According to other statistics (published by Statista Research Department March 2023) in line with the production the distribution of plastic consumption worldwide by plastic class in 2018 are the following. **(Figure 3)** Which later on brings as a really important explanation while we are assessing the microplastic pollution in the marine environment by the type of plastic found.

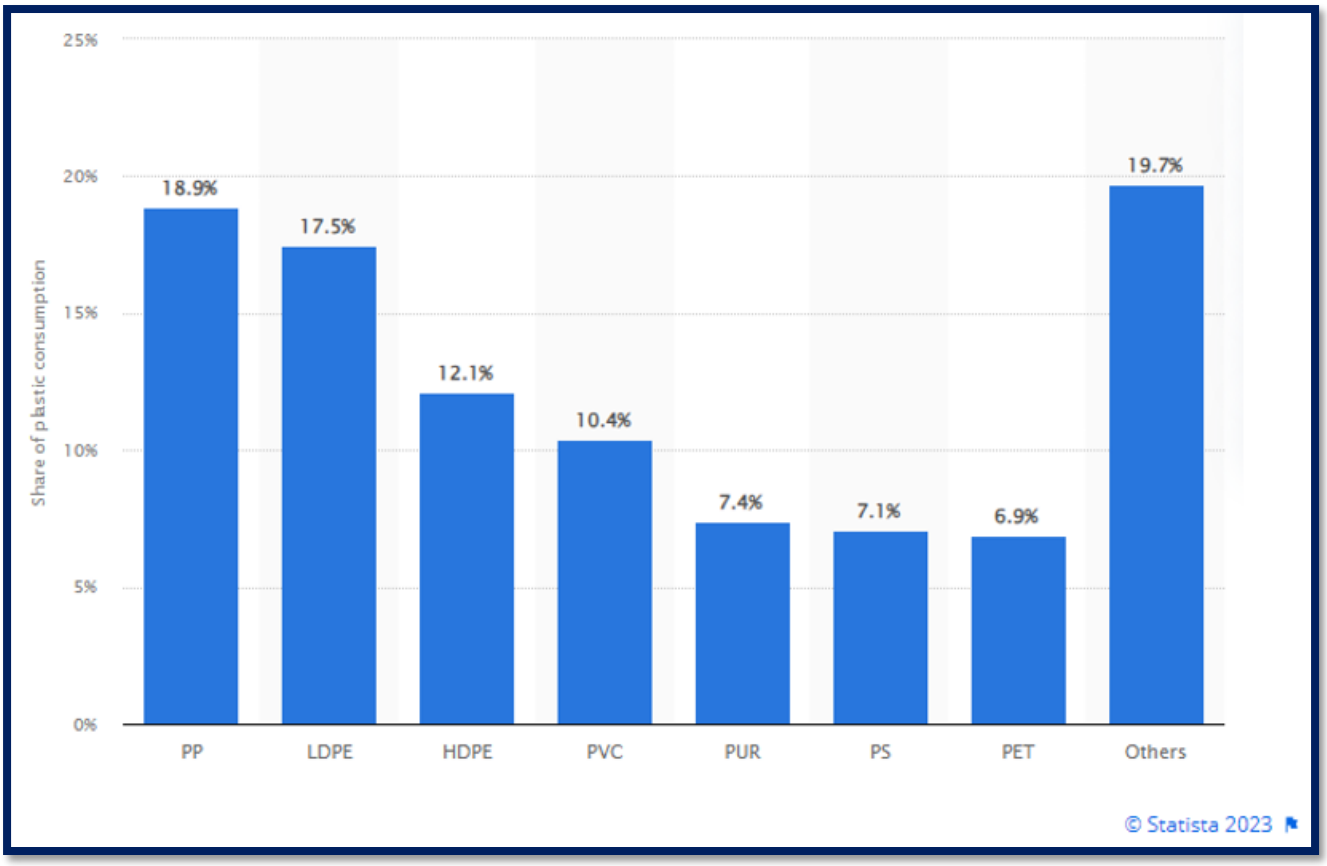


Figure 3 Distribution of plastic consumption worldwide in 2018, by plastic class [44]

External effects that initiate degradation

Degradation can be defined in many ways. Some consider any physical and chemical changes during the lifetime of the polymer. However, some of them are not necessarily results in a drastic deterioration or destruction of the properties of the polymer. Degradation can be classified in many ways. Degradation can be analysed depending on the individual polymers, since different chemical reactions occur as a result of their different chemical structures and their consequences are also different. However, generally we identify the following external events:

Thermal degradation: The cause of chemical changes is thermal energy, temperature rise. This is mainly during the processing of thermoplastics at high temperatures occurs. [10]

Photodegradation: Reactions are initiated by light. The double bonds, aromatic and molecules containing other groups absorb visible and UV light (UV-B (~ 295-315 nm) and UV-A (~ 315-400 nm)). High-energy UV light as a result, chemical, usually radical, reactions are initiated, which usually result in a significant change in the properties of the polymer in a chain reaction involving oxidation. The double bond containing polymers, rubbers and impact-resistant polystyrene are particularly sensitive to UV radiation. [10]

Chemical degradation: Small molecule compounds, acids, bases, solvents, reactive gases degradation occurring as a result belongs to this group. Major changes due to degradation can take place in the polymer, but this often occurs only at high temps., as such the activation energy of reactions is usually high. [10]

High energy radiation: Unlike photodegradation, the effect of high-energy radiation is not selective, its energy is sufficient to break practically all bonds. Due to radiation chain twisting and a decrease in molecular weight usually occur. [10]

Mechanochemical degradation: High external stress causes the breaking of chemical bonds. This occurs during the breaking of polymers, but often during processing or application too. Bond breakage usually results in free un-bond polymers, which are used in further reactions part. The importance of mechanochemical degradation is smaller compared to the others. [10]

Biological degradation: In essence, this is also chemical degradation. Microorganisms have many enzymes produce which can react with certain polymers to decrease the molecular weight and then a resulting in complete polymer degradation. The likelihood of this degradation is quite low as the majority of plastics don't interact with microorganisms. [10]

Microplastic pollution in the Mediterranean Sea

Among plastic waste, Microplastics are receiving the greatest attention as they have been found in all marine environmental matrices and their ingestion by trophic fauna has been widely evidenced because of hazard consequences on organisms and marine ecosystems. [14] The Mediterranean area is highly impacted by the non-European plastic waste emitted into the sea (classified as as primary source). [17]

Figure 4 illustrates the estimated value of plastic pollution which is directly influence the current state of the marine environment in the Mediterranean area published by ‘’Our World in Data” in 2021.

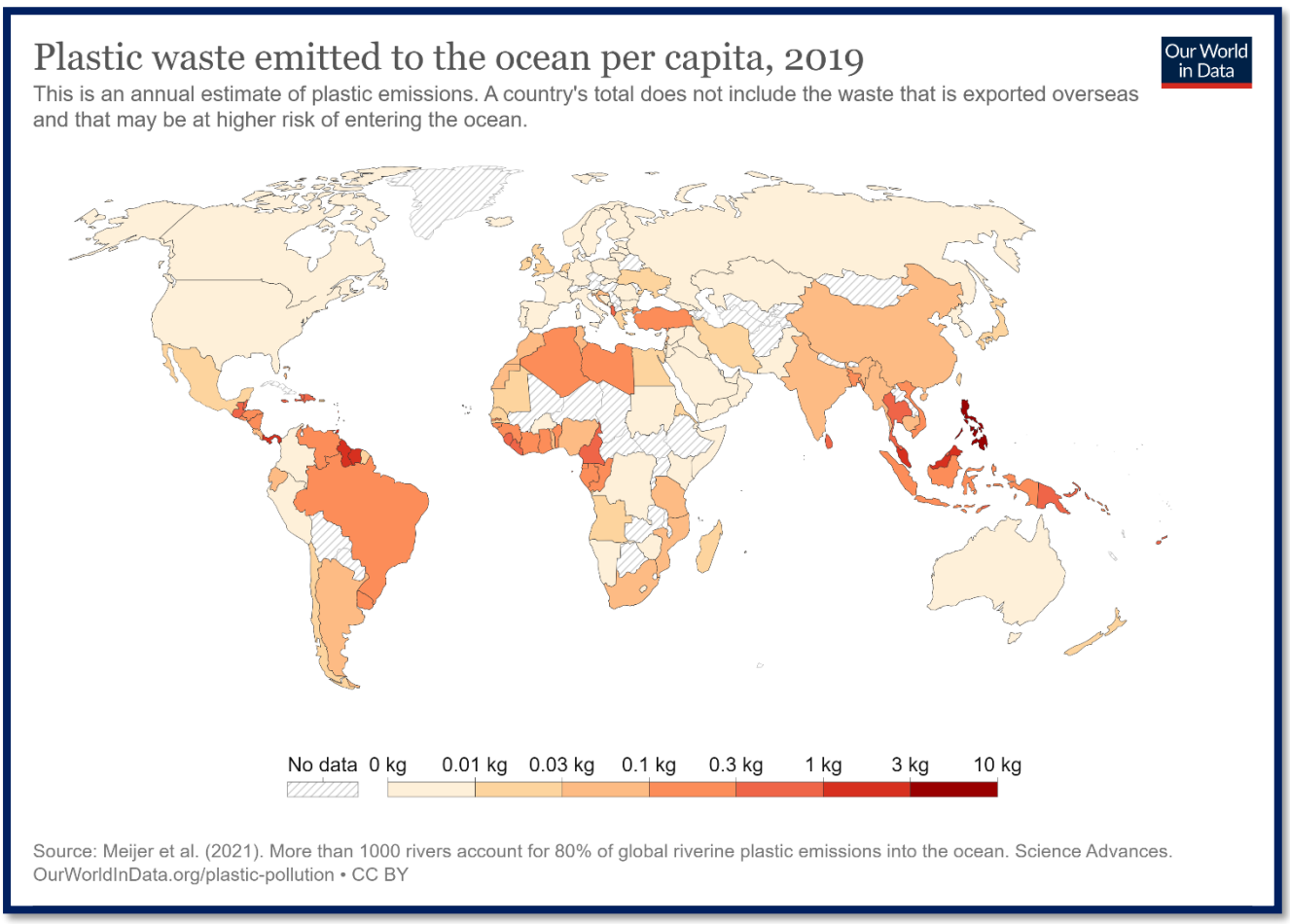


Figure 4 Plastic waste emitted to the ocean per capita, 2019 [45]

The origin of the marine litter is classified generally into two groups: land-based and sea-based, depending on the entry point of marine litter [24] According to a study, the annual calculated total plastic litter input in the Mediterranean basin is close to 100 000 tons out of these, 50% in generally coming from various land-based sources, while 30% is transmitted through the river channels and 20% is identified as maritime pollution. [24] In a recent study on the whole Mediterranean, the western area of the basin was observed to be less contaminated than the eastern one, while the Adriatic Sea, in the northern most arm of the Mediterranean Sea, has been estimated to be affected by one of the most severe litter pollutions (both sediment and surface water) among Mediterranean regions. [15] [16] In view of the water samples the University of Barcelona performed a survey to figure out which type of polymers are the most common in the Mediterranean area. In 2500 water samples, the most abundant materials are fragments of polyethylene (54.5 %), polypropylene (16.5 %) and polyester (9.7 %) -the most produced thermoplastic polymer worldwide- which float in marine waters and are likely to come from the continent. [18] In the Maltese Islands, an investigation of microplastics (1-5mm) has been conducted on the 5 most popular sandy beach (Għadira Bay, Golden Bay, St. George's Bay, Għajn Tuffieħa Bay and Pretty Bay), which proved that the coastal area of the Island is effected by considerable Microplastic type pollution. The highest levels of Microplastics were reported in Pretty Bay, at 10.81 items/1000cm^3 [19], which is only containing data only between the size of 5-1mm.

In 2021, a project called "Identifying microplastic hotspots in the Maltese waters" was performed by MCAST, AquaBiotech Group and Zibel, where the main aim was to discover pollution hotspots of Microplastics in Maltese waters and to identify them by shape, size, polymer type etc, whereas the most polluted area of Malta has been identified as the Great Harbour, which is a highly exposed location receiving extensive surface runoff after heavy rains. [20]

Environmental and health risk of microplastic pollution

The public health concerns about plastic littering have been identified since the first time noticed in the marine environment in 1970 [2] however, as was detailed before, until today, the annual volume of plastic production is increasing exponentially, while the impact is becoming more and more visible in reality.

As a result , Microplastics have recently attracted the attention of both the general public and the policy makers as well.

Concluded in the publication of the European Commission in 2019 [21], that annually, approximately 11 million tonnes of microplastics are released into the marine environment. One of the main contributors is the worldwide identified main garbage patches **(Figure 5)** in remote areas of the ocean, which are highly exposed to the degradation factors previously mentioned. The largest ever known garbage patch is the ‘’The Great Pacific Garbage Patch’’ which covers an estimated surface area of 1.6 million km^2, which is equivalent to three times of the size of France. [22]

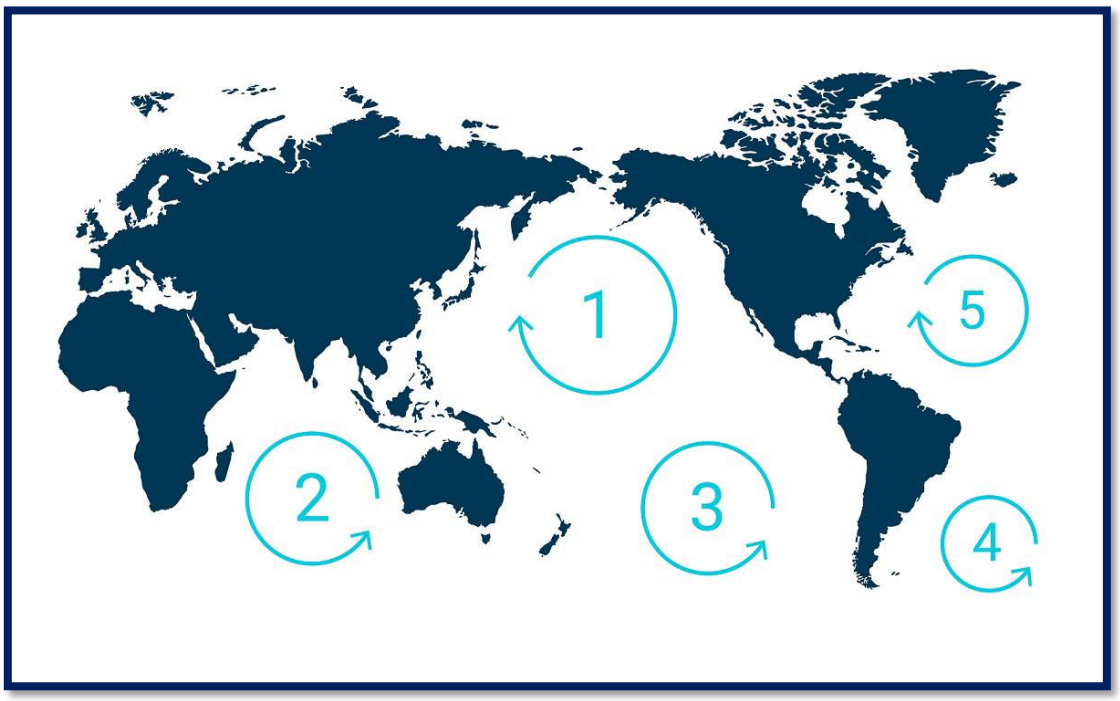


Figure 5 The five offshore plastic accumulation zones in the world’s oceans [23]

The need to address the plastic pollution challenge is fuelled by of many different aspects, such as the discomfort of the general public with visible plastic pollution of water bodies and nearshore areas, as well as concerns about the future negative physiological, behaviour impacts on the flora and fauna of the marine environment (growth, mortality, feeding etc.) [5]

The exact long-term effect of the microplastics in the environment is still not understood completely, due to lack of physical and realistic evidence; however certain studies has examined the direct effects of microplastics in laboratory conditions on lower level of biological organisms. According to these studies, researchers has concluded the potential affect in each biological level. [25] **(Figure 6)**

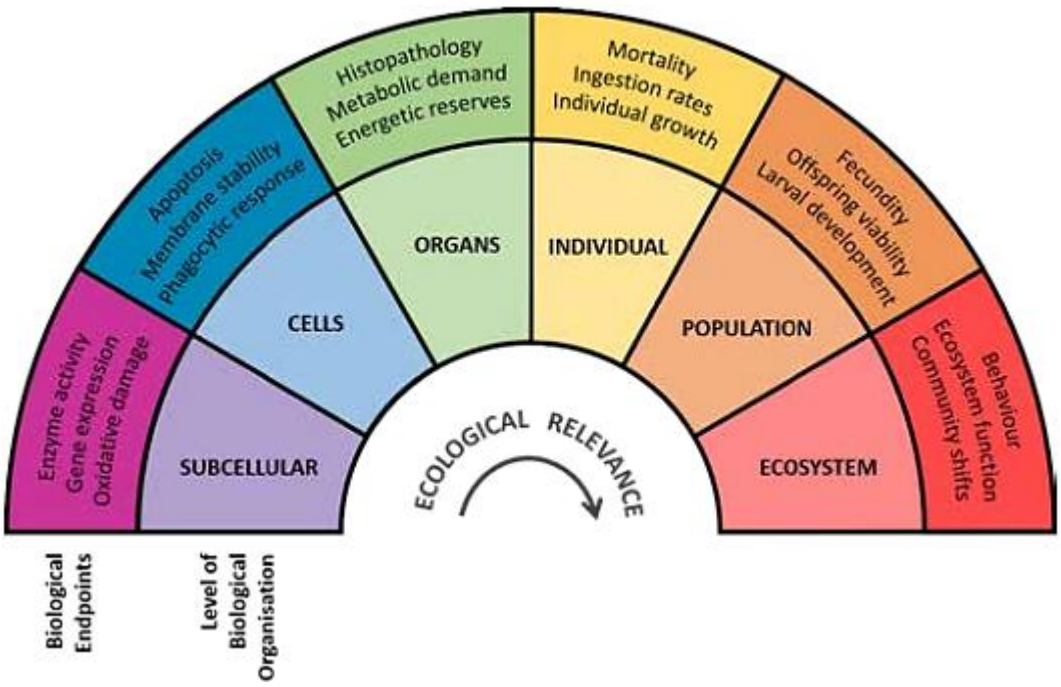


Figure 6 Impacts of Nano and microplastics reported at various levels of biological organisation [25]

The potential hazards associated with microplastics come mainly in three forms: the particles themselves, which present a physical hazard, chemicals (unbound monomers, additives, and sorbed chemicals from the environment), and microorganisms that may attach and colonize on microplastics, known as biofilms. The presence of the microplastics in the environment is posing a high risk on the living elements of the environment, entering to the food chain by being consumed by different

organisms in different sizes. (For example, shells, jellyfishes, fishes, birds) Some studies are examining the effect on wild animals, however, the observed health damages cannot be 100% attributed to the presence of the Microplastics since, in many cases the greater damage was caused by the adsorbed toxic substances, or microorganisms. [27] Some studies which were carried out under laboratory conditions are exposing what potential risks can be occurring focusing on fish and on humans. According to studies, deposit-eating fishes **(Figure 7)** are highly vulnerable to Microplastic ingestion due to their non-selective feeding behaviour. [26] Based on this fact omnivore fish are more exposed to the possibility of direct ingestion of microplastics than carnivore or plant-eating fish. Also studied that the physical property, situation within the water column and appearance are also influencing factors. Microplastics which resemble natural objects seems to be more likely to be ingested, for example white colour can trigger the visual- eaters. [26] Based on many studies which are examining all possible biological and physical effects on the fish, depending on the characteristics of the microplastics, the effect can vary from interruption of biological functions to mortality. During the experiment carried out by [26] ,the following affects were observed: oxidative damage, tissue damage, DNA damage, intestine damage, behavioural change, slow down of swimming, growth reduction, dysbiosis, breeding impairment, disruption of digestion, inflammation, alter gene expression, neurotoxicity, organ damage or death. [26]

Figure 7 debris found in the stomach of a fish in Portugal [29]

Since fish are not always the perfect indicators of general microplastic pollution in the marine environment, due to the mentioned selective feeding habits, other lower trophic level bioindicators were assessed whether they can provide reflective information of the pollution of their surrounding environment. Marine sponges are filtering large amounts of water, while they are capturing the surrounding microplastics, as during the analysis, significant amount of microplastic were found in all species **(Figure 8)**, making sponges be the perfect bio-indicator of increasing levels of pollution in the marine environment. [28]

Figure 8 Microplastic found in marine sponge [28]

Humans subject to the inhalation of micro and nano plastic particles from the air, to ingestion from dust, as well as from beverages and food stored in plastic packaging or container. [31] As this study is assessing the pollution of the marine environment, it's important to understand how humanity can be affected by the described environmental conditions. Seafood is an important element of the human diet. In the long term, the previously listed events can cause serious drops in commercial fish stock which will out rule a really important source of vital origin protein for humans. In short term this can cause serious toxicological effect in the human health such as : Translocation to distant tissues, disruption of immunity, metabolism alteration, oxidative stress, cytotoxicity, neurotoxicity, carcinogenicity, reproductive toxicity. [26]

Determination of microplastics

As I previously mentioned, the lack of international standard methods for the sampling and analysis of microplastics in the environment means that comparisons across studies are not as easy in certain cases. Although the increased risk of microplastics have caught the attention of policy makers, effective regulations or standards are still under development due to lack of representative physical evidence, methodologies of sampling, sample preparation or sample analysis. Currently what might be the most important initial step before deployment of any legal act is being completed by the International Organization for Standardization. The ISO 24187 – ‘’Principles for the analysis of microplastics present in the environment’’ is under development possibly to be published by the end of 2023, which will be a game changer in the research field of microplastics.

The first microplastics studies ever conducted are related to the marine environment. Small plastic particles were accidentally discovered while sampling by plankton net. There are several designs of plankton nets, but the most commonly used is so-called Manta net. **(Figure 9)**, The net is attached to a typical 60x18 cm metal frame which is supported by air-filled balloons ensuring continuous floating on the surface of the water, and a mechanical flow meter is fitted to measure the volume of water filtered. The Manta net is usually towed, typically either behind the boat, which is not the most ideal condition due to the generated waves, as this can interfere with the usual distribution of the floating particles, or either along the side of the boat which will ensure noiseless sampling. The pore size of the net used in many publications was 300 µm. [32] [33] [34] However in certain others, we could find different details regarding the size of the metal frame or the pore size of the net, reflecting the incomparability of certain studies. [35] [36]

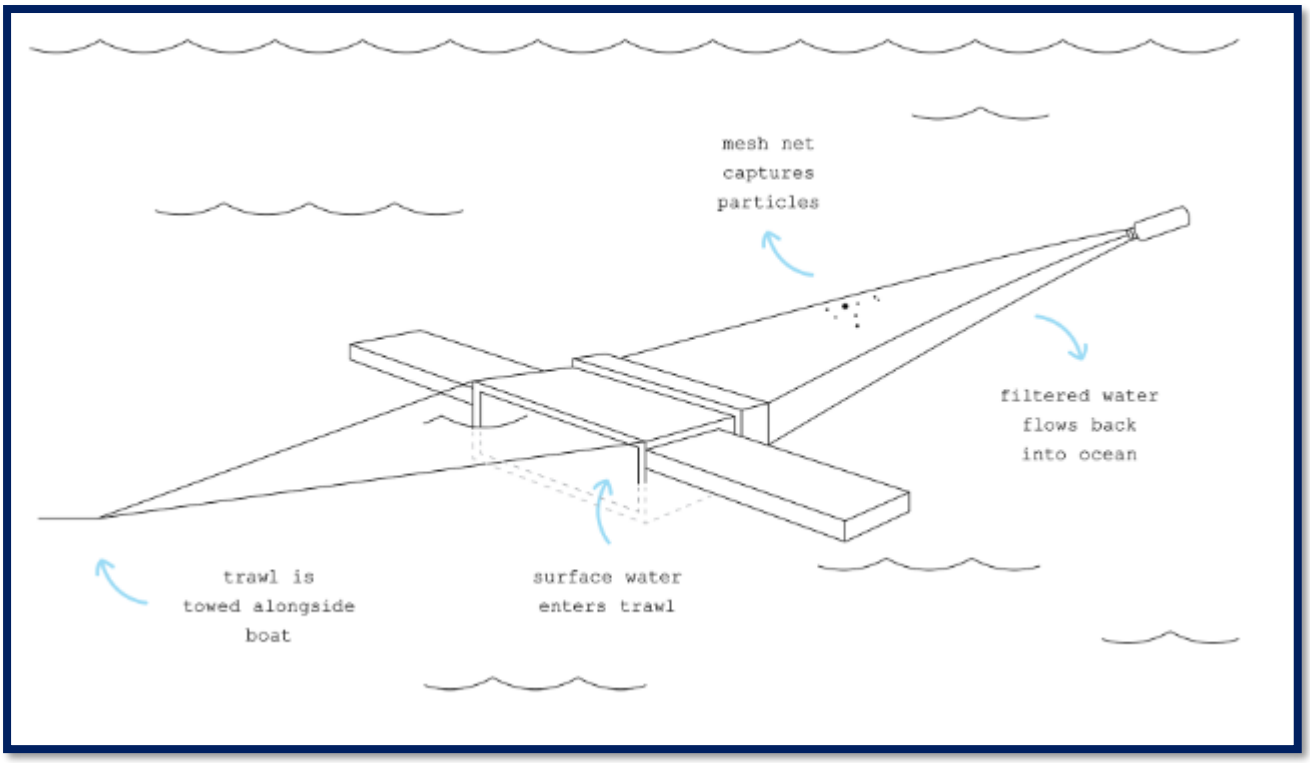


Figure 9 Illustration of Manta net sampling mechanism [37]

The real difficulty in the determination of microplastics in the environment lies in distinguishing microplastics from the complex mixture of natural organic and inorganic particles in any given environmental matrix. These can be, for example, inorganic particles like sand and silt, but also organic particles originating from biofilms, plant, and animal debris [38]. As a first step, the inorganic matters are separated by using density differences. Generally, microplastics have a density close to that of water (0.83–1.1 g/cm^3), whereas most inorganic constituents have higher densities [38], so with controlled conditions, the separation could be done easily by respective density differences. On the other hand, organic matter has a similar density to microplastics and cannot be separated based on density differences. Thus, a matrix rich in organic matter needs to be treated via chemical digestion protocols, such as oxidative, acidic, alkaline, as well enzymatic digestions [39]. When choosing a chemical used to decompose/oxidate organic matter, one has to consider whether it interacts with the polymers to be tested. For example, the use of some strong oxidizing agents could lead to loss of particles that make up polyester and polycarbonate and other polymers.

The most common analytical technique for this purpose is the Fourier Transform Infrared (FTIR) spectroscopy, but in some cases Raman spectroscopy is also used. Both methods are based on the principle of vibrational spectroscopy, thus by the unique properties of a given molecule the test sample can be identified on the basis of a modified signal [40]. In the case of Infra-red spectroscopy, the wavelength of the infrared light used for the study is most typically analytical infrared (4000-650cm^{-1}), but in some cases near infrared (near infrared - NIR, 10000-4000 cm^{-1}) also uses light in this wavelength range to identify polymers [41]. The FTIR microscope examined, on the basis of images taken in the visible light range, is characteristic of microplastics morphology, each particle is designated as a measurement point. After the measurement points are recorded using the device, the measurement results are correlated with a database so to determine how many matches were shown between the selected points with some type of plastic. [40] The presented method for the concentration of microplastics in each case given in particle number referred to the sample volume. This technology's most serious fault is the limit of detection in our case, the instrument cannot recognise fragments size below 10 µm.

Methodology

The project has started with the identification of potential hot zones of the Maltese islands which are being influenced by anthropogenic interactions, typically by certain type of effluent discharge into the sea, combined with a reference site, which can allow for comparison between the influenced sites and relatively un-impacted areas in of the nearshore area of Malta.

The assessed areas were the followings:

- Għar Lapsi (discharge point of RO plant)
- Ħofra ż-Żgħira (Delimara power station discharge point)
- Xgħajra Barkat (Wastewater treatment plant discharge point)
- Selmun (Reference site)

Three parties (Oceanography Malta Research Group, Zibel, and Eurofins Analytical Services Hungary Ltd.) collaborated on the project, with each party participating at various stages of the process. By making a change to the established sampling procedures, our group sought to describe the microplastic contamination of the Maltese nearshore waters from a novel perspective. We aimed to shed additional light on the dynamics of microplastic accumulation within the water column. In order to compare surface microplastic abundances and composition with mid-water ones in the same region, we also collected surface microplastic samples at the same sites in parallel. To demonstrate representativity, data was gathered over a period of two summers, and confirm the campaign's first year's outcomes, we conducted sampling in both previous two year's area in collaboration with our partners, Zibel. After the samples were collected, these were sent to Eurofins Analytical Services Hungary Ltd.'s laboratory, where the support staff completed the whole analytical testing. Following this, I have evaluated the obtained analytical results, according to the data collected during the sampling campaign.

Dates of the sampling campaigns:

- 24-26th August 2022
- 2nd June 2023

Sampling methodology for surface water

To collect the desired microplastic samples, we have used the most common sampling apparatus, which is suitable to capture microplastics from surface water, known as Manta-Net **(Figure 10)**. The limitation of the sample analysis is mainly constituted by the Manta-net mesh size, as under that pore size the results are considered to be unreliable. Our Manta-net mesh size is 50µm which is considered to result in an efficient filtration, as most of the research carried out in the past used a manta-net having 300 µm mesh size. The same apparatus is equipped with a calibrated flow meter, which will give us information about the amount of water filtered. Apart from the Manta-net, we have utilized the research boat of Zibel NGO, which is suitable to connect the Manta-net to the towing access of the boat, also equipped with sensors (GPS coordinates recorder, speed and time recorder) which will play an important role in the evaluation of the results. The sampling methodology contains strictly plastic-free materials to avoid any internal interference involved in the sampling.

Figure 10 Releasing Manta-net into the sea at the nearshore area of Għar Lapsi

Prior to sampling, the Manta-net was cleaned out from any interfering materials. First, the towing facility of the boat was set up, prior to the assembly of the Manta-net. The net was attached to a metal frame, which has been floated by two secure air-filled balloons.At the end of the collector net a removable "Cod-end" was attached, which is responsible for the collections of the particles which have a larger size than the pore-size of the Manta-net. The sampling apparatus was attached to the towing access on the side to the boat to avoid any disbalancing by the created waves. Prior the release of the Manta-net on the starting point of the chosen track. We recorded the initial reading of the flow meter, set timer to monitor the towing-time, recorded the coordinates, and set the speed of the boat with respect of the ideal towing speed of the Manta-net (1-3.5kts). At the end of the track, we carefully removed the net through the metal frame from the water **(Figure 11)**, paying attention to keep all the samples within the net. We stopped the timer, recorded the reading of the flowmeter, and recorded the end coordinates of the route. We carefully washed into the cod end all the stuck particles from the wall of the net. Afterwards, we carefully removed the Cod-end from the Manta-net and, through the glass funnel, we transferred all the sample collected during the collection; later we washed 3 times the residue particles from the bottom. We labelled the bottles according to the initial letter of the location and the replicate number. During the first sampling campaign (2022), we took 2 replicates in opposite direction to each other, in order to sample larger amounts of matrix from the same area; we cleaned the net between two tracks, as we have experienced high level of organic interference ;however, during the 2nd phase (2023), we were able to perform the sampling along one track only for the same period of time, so the results are covering larger surface. This slight difference between the two sampling events is not causing any discrepancy during the evaluation as during the first phase, the samples were merged and analysed together from the same area.

Figure 11 Removing Manta-net from the sea after sampling

Sampling methodology for sub-surface water

As I have mentioned before, the innovative perspective of our research was to clarify if there could be any possible accumulation of microplastics in the sub-surface areas of the water column, since the majority of the existing studies are focusing on the pollution at the surface or studying the sediment. Firstly, the setup of the Manta-Net was modified prior the sampling. The buoys from the side have been removed, and the trawling metal ropes were connected to the top connection point of the metal frame. At the bottom connection points, two 4kg weight has been attached to keep the balance of the frame straight during the trawling as per **Figure 12**.

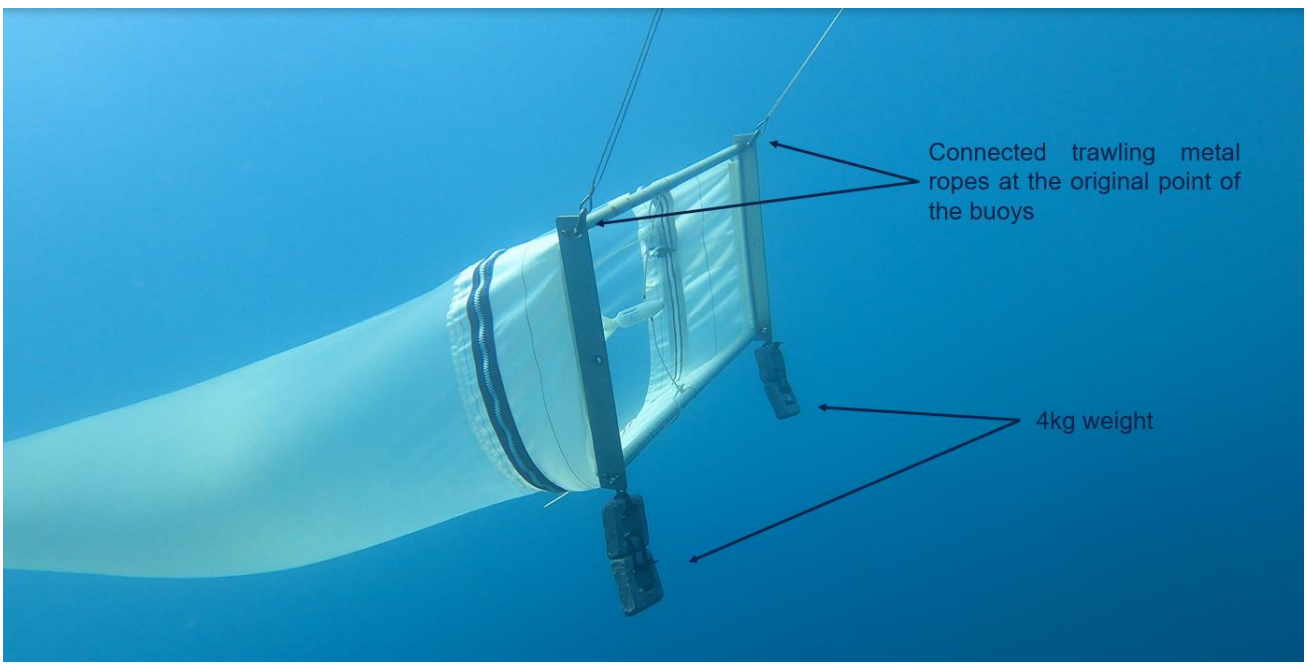


Figure 12 Underwater setup of the manta-net ensuring the balance

To the back of the boat, two large buoys has been connected by two 3m trawling ropes to ensure the same distance from the boat. At the top, connection points to the Manta-net through two 12m sailing ropes have been made to the safety buoys as per **Figure 13**.

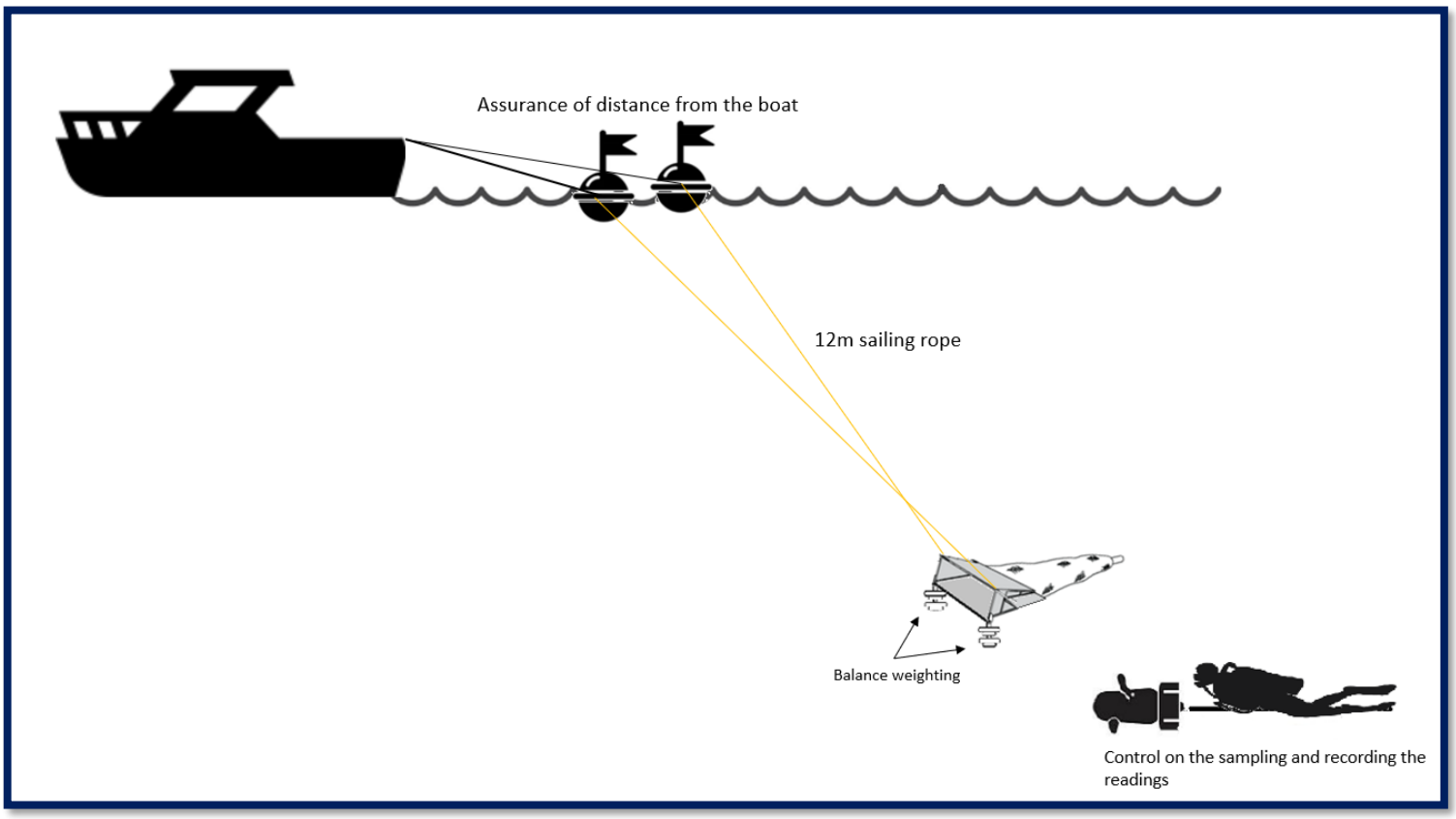


Figure 13 sub-surface sampling setup schematic illustration

Before and after the sampling time, the mouth of the manta net has closed **(Figure 14),** to make sure that the net is not capturing any samples during the time while it is being positioned into the sampling track. To control the balance of the Manta-net, the sampling mouth was opened and closed so as to check the stability, the position of the trawling ropes was checked and the flowmeter reading and coordinates were taken, one diver assisted the process, following the net with a sea-scooter. During our first trial, the boat speed was set to 2kts; however, with the generated sea current the diver couldn`t follow the net, but from a distance he was assuring that the sampling was spotless. So, the second time we set the boat for 1.2 kts, which was considered as not fast enough to keep the net straight. We identified that the ideal speed is 1.5-1.6kts so as to keep the net horizontal and so as to give enough room to the diver to have full control over the trawling. **(Figure 15)** After the indicated time has passed, the diver took the reading of the flowmeter connected to the net and recorded the end coordinates. As well as closed the Cod-end to preserve the collected samples and assured that the metal frame is being transferred to the surface in the right position to not lose any stuck particles from the walls.

Figure 14 Closed manta-net prior the commencement of the sampling.

After the net was transferred to the boat **(Figure 16)**, we carefully washed into the cod end all the stuck particles from the wall of the net. Afterwards, we carefully removed the Cod-end from the Manta-net and through the glass funnel we transferred all the sample collected during the collection, later we washed in 3 times the residue particles from the bottom. We labelled the bottles according to the initial letter of the location and the replicate number, indicating that the sample were taken from the sub-surface.

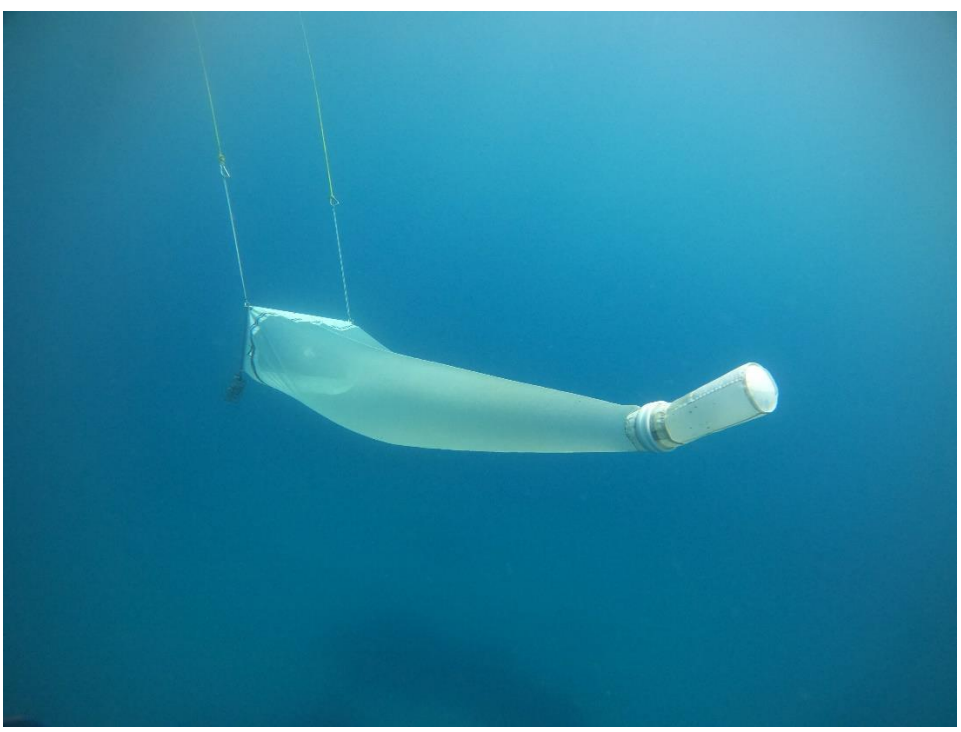


Figure 15 Manta-net towed in 10m depth

Figure 16 (left) preparation for sampling (right) towing of manta-net during sub-surface sampling.

Sample preparation and analytical characterisation

The samples collected during both surveys carried out in 2022 and in 2023 had been sent to our project partner Eurofins Analytical Services Hungary Ltd by currier services to ensure a safe delivery. The samples did not require any specific preservation, as the microplastic particles are not being affected in a closed container. The original sample volumes were calculated according to the recorded data during the sampling, as this is an essential part of the final report received from the laboratory.

Larger particles (1000‑5000 µm) were filtered out of the samples using a stack of sieves. Particles in the fraction between 1000-5000 µm were picked manually, captured by a digital camera first, then their chemical composition was analysed with FTIR-ATR. Resulting spectra were compared to a reference polymer spectra database and particles showing correlation >70% were considered as microplastics. The remaining sample in the fraction between 1000-50 µm went through a Fenton

oxidation process using 30% hydrogen-peroxide and a subsequent density separation with 1.6 g/cm^3 zinc-chloride solution. The resulting sample was filtered on 25 mm Anodisc filters (0.2 µm pore size) **(Figure 17)**. The filters were then analysed by a Thermo Scientific Nicolet iN10 MX FTIR imaging microscope with 25 µm pixel size in transmission mode, 4 scan numbers and 8 cm^{-1} spectral resolution was applied. The spectral data was evaluated with the "siMPle" software, designed for microplastic analysis. Data were compared with a reference polymer spectra library and particles with >70% correlation was considered as microplastics. Estimated mass concentration of different polymers in the samples was also reported. It is calculated by the "siMPle" software based on the estimated volume of the particle (assuming an ellipsoid shape) and the density of its material.

To prevent sample contamination, general laboratory precautions were taken. Cotton lab coats were used and samples were prepared under a laminar flow hood to minimise airborne contamination. For washing steps, deionised water was used that was previously filtered on 0.7 µm pore size glass filters. Laboratory blanks were measured to determine background contamination. Average values were 1.3 polyethylene, 1 polypropylene and 0.3 polystyrene particles per sample.

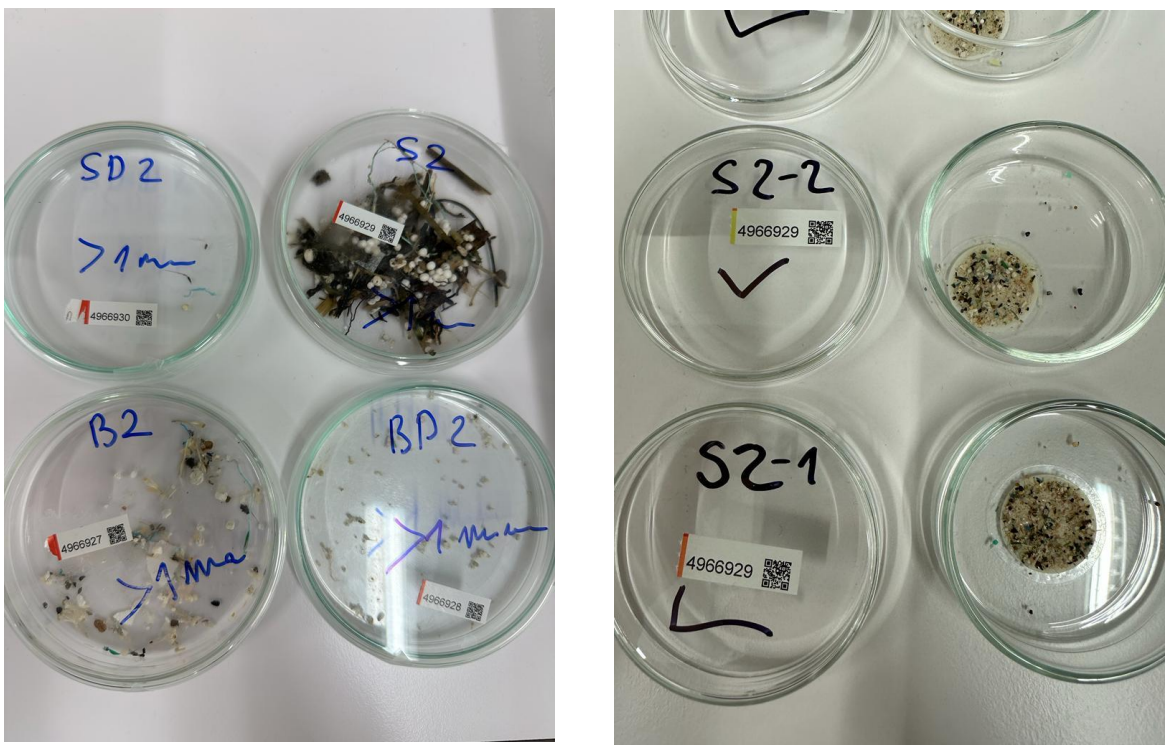


Figure 17 (left) separated samples between 1000-5000 µm (right) Samples after preparation on filter between 1000-50 µm

Data and findings

Sampling

As mentioned above, the samplings were carried out over two consecutive years. During 2022, we have sampled all 4 indicated sites to gain information about their microplastic contamination on the surface, and we tested our pilot sampling methodology on 2 sites, namely Xghajra and Selmun. As the results of the fist sampling campaign showed really interesting trends in comparison of the microplastic pollution levels of the surface with the sub-surface levels of the sea, we have repeated the sampling in 2023 inat Xghajra and Selmun, for both surface and sub-surface level, so as to confirm the first results and compare the pollution over two different year. The samples were coded as per the table below:

Table 1 Sample identification

Sampling area	24-26th August 2022		2nd June 2023	
	Surface sampling	Sub-surface sampling	Surface sampling	Sub-surface sampling
Għar Lapsi	L1	-	-	-
Ħofra ż-Żgħira	H1	-	-	-
Xghajra, Barkat	B1	BD1	B2	BD2
Selmun	S1	SD1	S2	SD2

As previously mentioned, during the first survey, we encountered challenges with longer sampling distances due to the collecting of large organic particles, which complicated the safe transfer of the samples. As a result, we chose to divide the planned sampling tracks into two, but this did not compromise inter-annual comparability since the final evaluation of the results was primarily expressed as to a common unit, allowing us to make a general comparison between sites. Additionally, we observed non-representative readings in the data from the flow meter, which were primarily caused by wave action. As a result, when we arrived at the calculation of the total quantity of filtered samples to obtain the closest estimated value, I took into account the distance between the starting and finishing coordinates as well as the precise parameters of the support metal frame (0.045

m^2) in order to calculate the amount of water within the given column. I applied the same methods for all the samples to demonstrate continuity. The sampling timeframe in each case of the surface sampling was 20 min, except in the case of Ħofra ż-Żgħira (8 min), where the site-specific characteristis were not permitting to perform such long distances. In the case of the sub-surface samples in 2022, the sampling time was 10 min, while in 2023 we have carried out sampling for 20 min to cover larger column. The whole set of data acquired throughout the sample campaigns is shown in the tables below.

Table 2 Total amount of filtered seawater in m^3

Sampling area	24-26th August 2022		2nd June 2023	
	Surface sampling (m^3)	Sub-surface sampling (m^3)	Surface sampling (m^3)	Sub-surface sampling (m^3)
Għar Lapsi	83.51055	-	-	-
Ħofra ż-Żgħira	16.95465	-	-	-
Xghajra, Barkat	76.455	21.20805	104.4	69.75
Selmun	**83.99565**	**21.22245**	**106.200**	**66.150**

Table 3 Total distance in meters

Sampling area	24-26th August 2022		2nd June 2023	
	Surface sampling (m)	Sub-surface sampling (m)	Surface sampling (m)	Sub-surface sampling (m)
Għar Lapsi	1855.79	-	-	-
Ħofra ż-Żgħira	376.77	-	-	-
Xghajra, Barkat	1699	471.29	2320	1550
Selmun	**1866.57**	**471.61**	**2360**	**1470**

Table 4 Register of starting and finishing coordinates of the sampling routes

Sampling area	Track	24-26th August 2022		2nd June 2023	
		Surface sampling	Sub-surface sampling	Surface sampling	Sub-surface sampling
Għar Lapsi	Start	35°49.602'N. 14°24.768'E	-	-	-
	Finish	35°49.471'N. 14°25.522E 35°49.545N. 14°25.351'E 35°49.491'N. 14°24.871E	-	-	-
Ħofra ż-Żgħira	**Start**	35°50.134N. 14°33.649E	-	-	-
	Finish	35°50.131N. 14°33.823E 35°50.229N. 14°33.792E 35°50.137N. 14°33.761E	-	-	-
Xghajra, Barkat	Start	35°53.225N. 14°33.105E	35°53.168'N. 14°33.383E	35°53.391'N. 14°32.983E	35°53.324'N. 14°33.217E
	Finish	35°53.028`N. 14°33.647E 35°52.938N. 14°33.623E 35°53.268N. 14°33.215E	35°52.984'N. 14°33.598E	35°52.641'N. 14°34.225E	35°52.788'N. 14°34.018E
Selmun	**Start**	35°58.240'N. 14°22.972'E 35°58.201'N. 14°23.564E	35°58.364'N. 14°22.888E	35°58.382'N. 14°22.309'E	35°58.119'N. 14°23.816E
	Finish	35°58.179N. 14°23.583'E 35°58.276'N. 14°22.940E	35°58.405'N. 14°23.197E	35°58.217N. 14°23.866'E	35°58.380'N. 14°22.891E

Quantitative and qualitative characterization - Phase 1 (2022)

As described previously, the analytical characterisation was conducted along two size ranges, as the analytical methodology has limitations; so to avoid generating non-reliable data the samples were analysed after separation for particles between 5000-1000 µm, and separately for 1000-50 µm. **(Figure 18)** As the larger fragments (5000-1000 µm) were often not collected in the sub-surface water column samples, but have rather almost solely in the surface samples, this comparison was out of the scope of our research and we instead concentrated on the analysed microplastics lower size range (1000-50 µm) during the evaluation and during the 2nd phase of sample collection. The examination of each particle would take months, since it involves extensive manual interactions for particles between 5000 and 1000 µm. We had obtained some broad information about this size range during the initial survey, even if it occasionally did not provide a complete picture because some samples included hundreds of particles while others only a few. In these cases, the representative sub-samples were only analysed to have comparable results with the other samples. In **Table 5**, I provide a summary of the findings from the qualitative characterization of the collected particles, indicating the kind of polymer and demonstrating that the match with the reference samples for each material type was discovered.

Table 5 Summary of the identified microplastics with FTR-ATR. (5000-1000 µm) 2022

Sample code											
B1 (10% of total)		BD1		H1		L1		S1		SD1	
Match (%)	Polymer type	Match (%)	Polymer type	Match (%)	Polymer type	Match (%)	Polymer type	Match (%)	Polymer type	Match (%)	Polymer type
85.16	Stearate	25.05	PE	24.3	PROTEIN	93.27	PE	84.1	PE	89.34	STYRENE
56.2	Stearate	47.94	CELLULOSE	33.61	CELLULOSE	28.51	CELLULOSE	93.23	PE	50.48	CELLULOSE
91.71	PE					84.13	PE	94.17	PP	72.61	PE
82.15	Stearate					96.51	PE	93.94	PP		
60.99	Zein							92.94	PE		
67.14	Proteins							82.01	PE		
49.55	Glycerol triolate							93.42	PP		
64.92	Stearate							91.06	PE		
61.82	Proteins							90.15	PP		
69.45	Stearate							9.68	CASO4		
92.39	Styrene							78.17	PE		
								93.52	PP		
								89.42	PE		

Figure 18 Selmun surface sample (S1) visual example of found Microplastic samples (5000-1000 µm) 2022

Our primary attention was on the microplastic particle size range of 1000–50 µm, since the initial survey results revealed some very intriguing trends. **Figure 19** shows the distribution of the types of polymers that were discovered in the samples and which ones were the most prevalent.

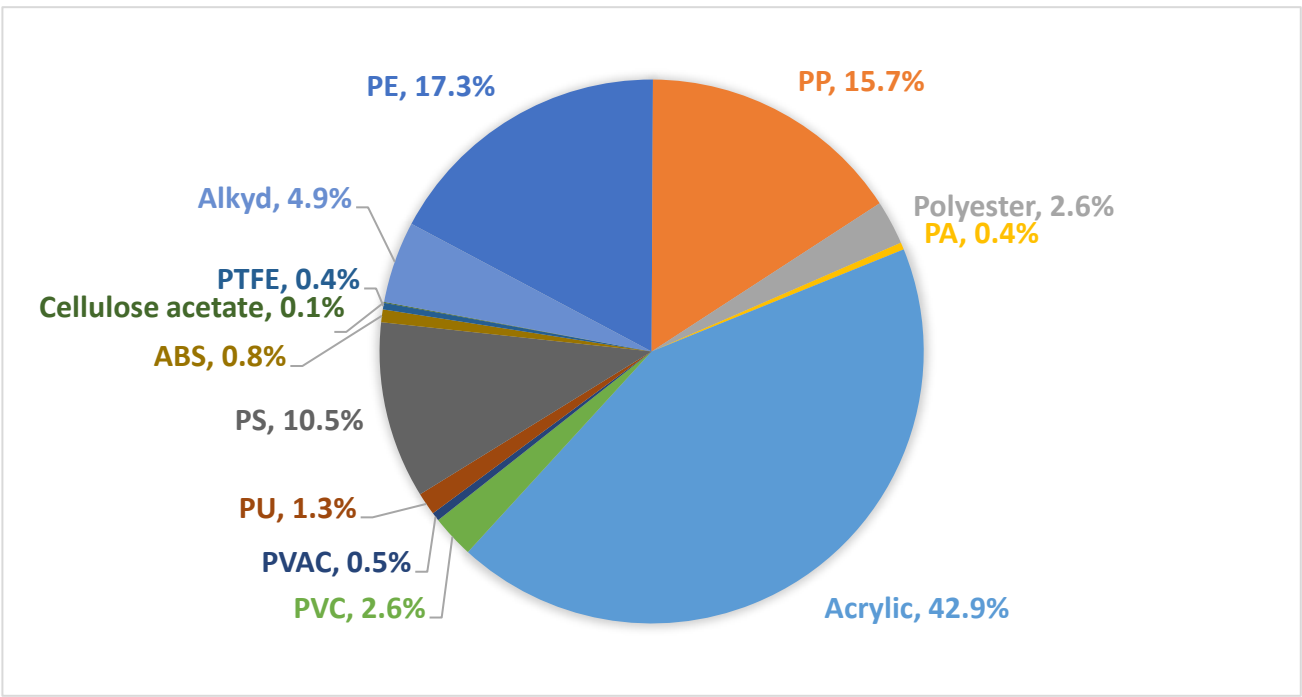
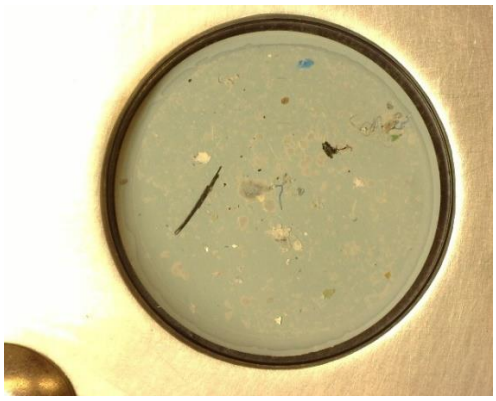


Figure 19 Polymer type distribution (%, all sample average from phase 1- 2022 samples)

Before analysis, the filtered samples were photographed **(Table 6)** under a microscope, so to provide a visual identification of the collected microplastics because some of them were invisible to the naked eye.

Table 6 Photo documentation of samples 1000- 50 µm of Phase 1 - 2022

B1	BD1
	
H1	**L1**
	
S1	**SD1**
	

While analysing the sample, the program used to identify the microplastics generates two different spectral maps, one of which serves as a showcase with a false-colour image to represent the sample's total absorbance and the other which represents the microplastics that were found in the sample. In **Figure 20** and **Figure 21,** I will illustrate thus by using one of our sample from Phase 1.

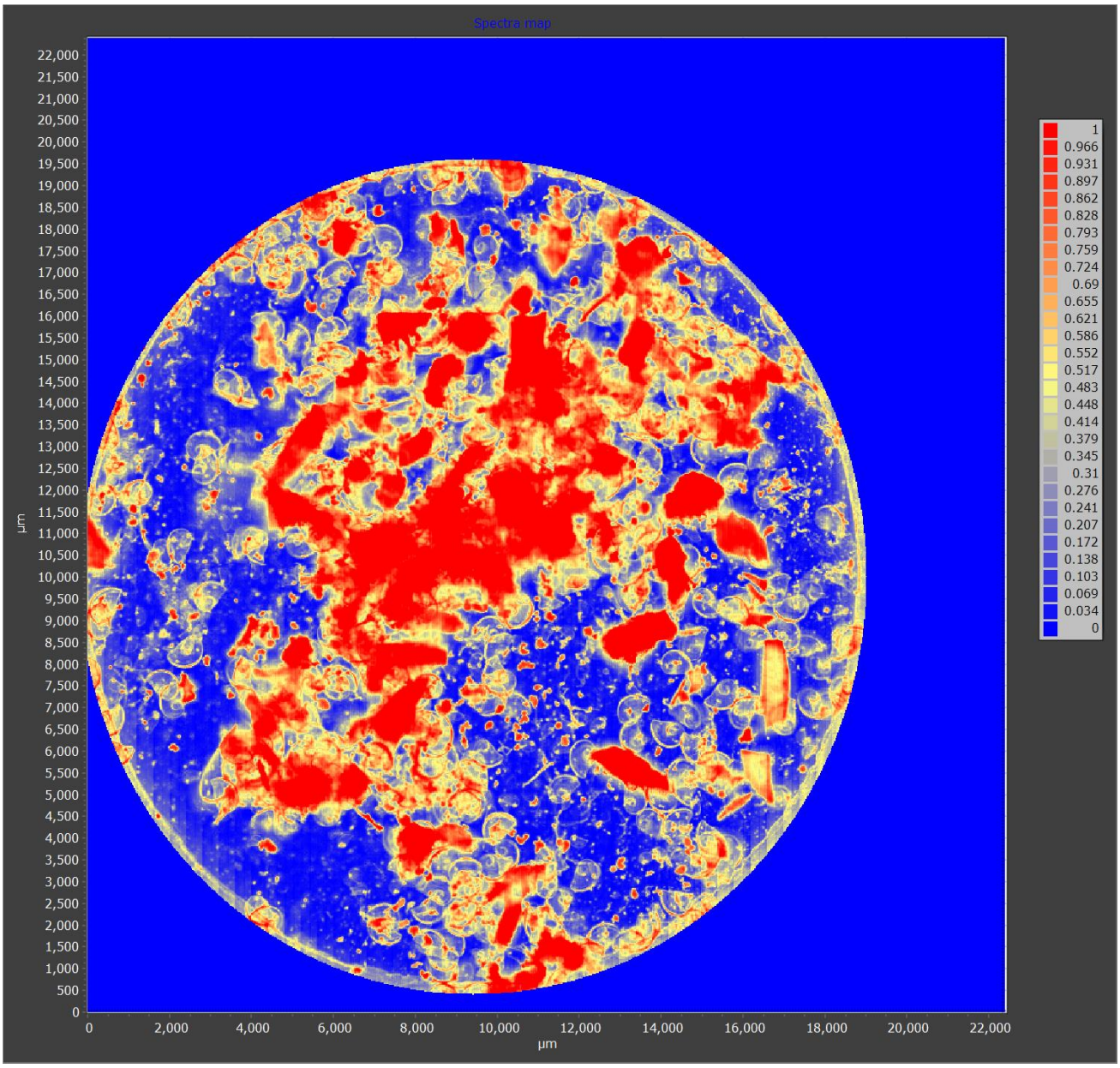


Figure 20 false-colour image showing the total absorbance of the sample L1

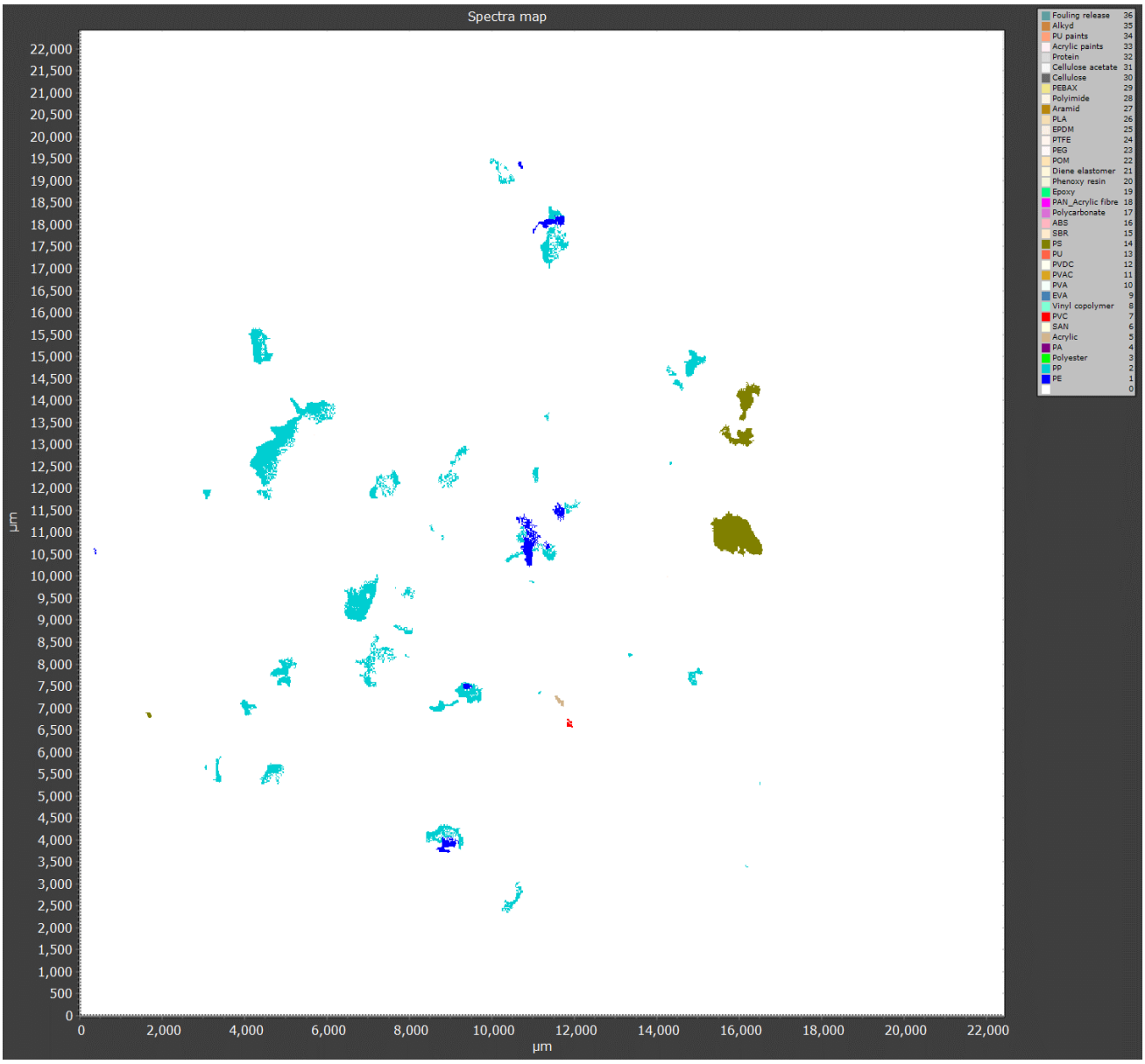


Figure 21 false-colour image represents the microplastics that were found in the sample L1

Following the spectral analysis, the program gathers all the data about the detected matches to the standard (polymer type, size, weight etc) for each microplastic found, which we further analysed and gathered in **Table 7** and **Figure 22-23.**

Table 7 Qualitative and quantitative characterization of Microplastics in each sample (1000-50 μm) 2022

	Sample code	B1	BD1	H1	L1	S1	SD1
Identified MPs (particles/sample)	PE	74	9	8	8	6	6
	PP	45	14	1	7	11	5
	Polyester	34	6	0	0	0	0
	PA	2	1	1	0	0	0
	Acrylic	58	83	65	2	10	38
	PVC	8	6	0	0	2	2
	PVAC	0	1	0	0	1	0
	PU	3	4	2	0	0	1
	PS	1	14	5	4	12	0
	ABS	1	1	1	0	1	0
	PTFE	0	0	2	0	0	0
	Cellulose acetate	1	0	0	0	0	0
	Alkyd	70	1	1	0	0	2
	Sum	**297**	**140**	**86**	**21**	**43**	**54**

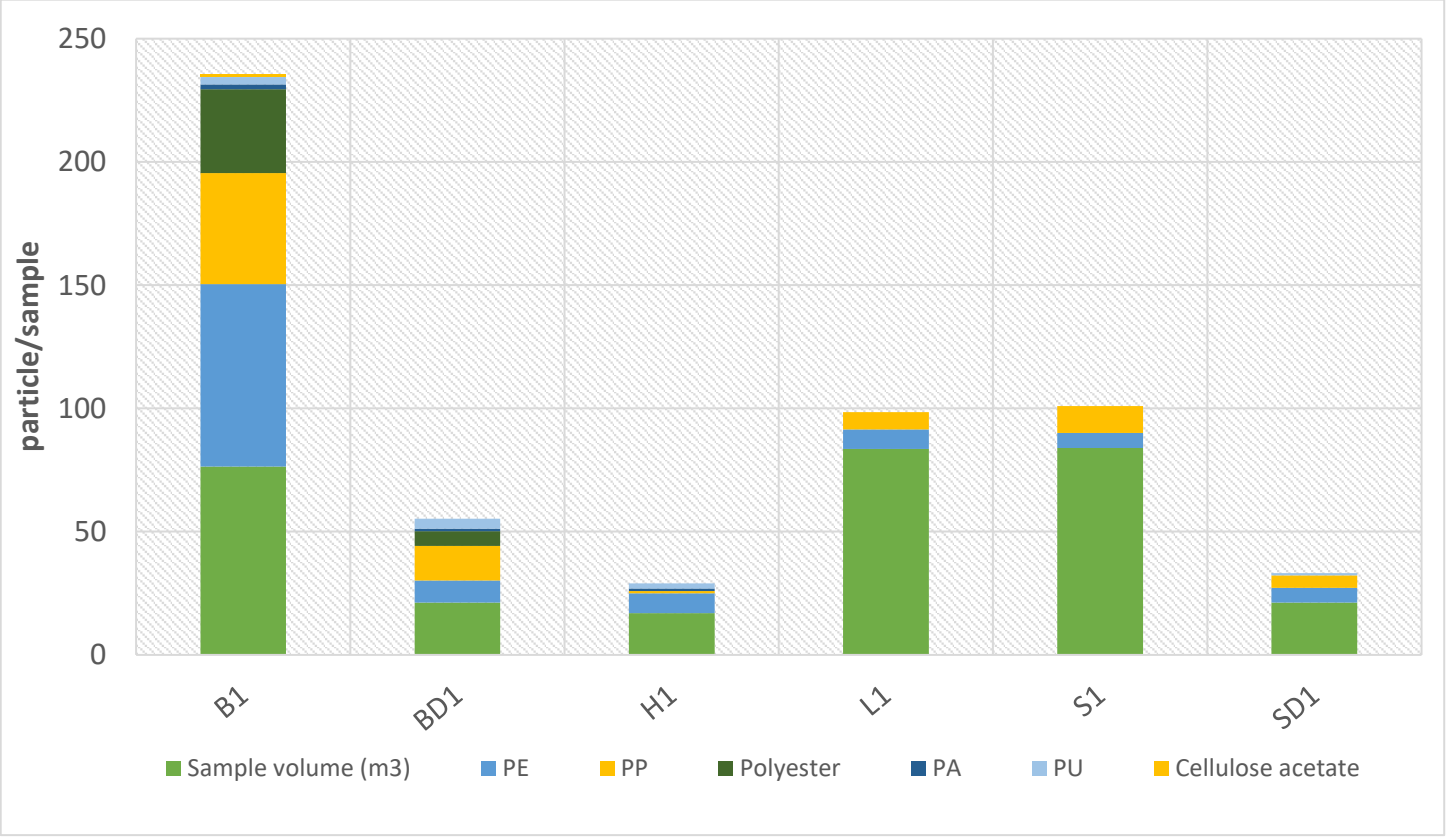


Figure 22 Microplastics content in the tested samples between 1000-50 μm 2022

As part of the characterisation, the program captures the size of the microplastics measured taking into consideration their longest dimension, as per **Figure 23**.

Identifier	Coord. [pixels]	Coord. [μm]	Max score	Group	No. of pixels [-]	Area on map [μm²]	Major dim [μm]	Minor dim [μm]	Feret min [μm]	Volume [μm³]	Mass [ng]
MP_1	[1.434]	[25.10850]	0.635	PP	4	2500	75	42.4	50	42441.32	40.31925

Figure 23 Identification of microplastics with "siMPle" program

Figure 24 gives us an indication what is the main size range found in our samples, where it is showing that 70% of the found particles are between 50-350 µm.

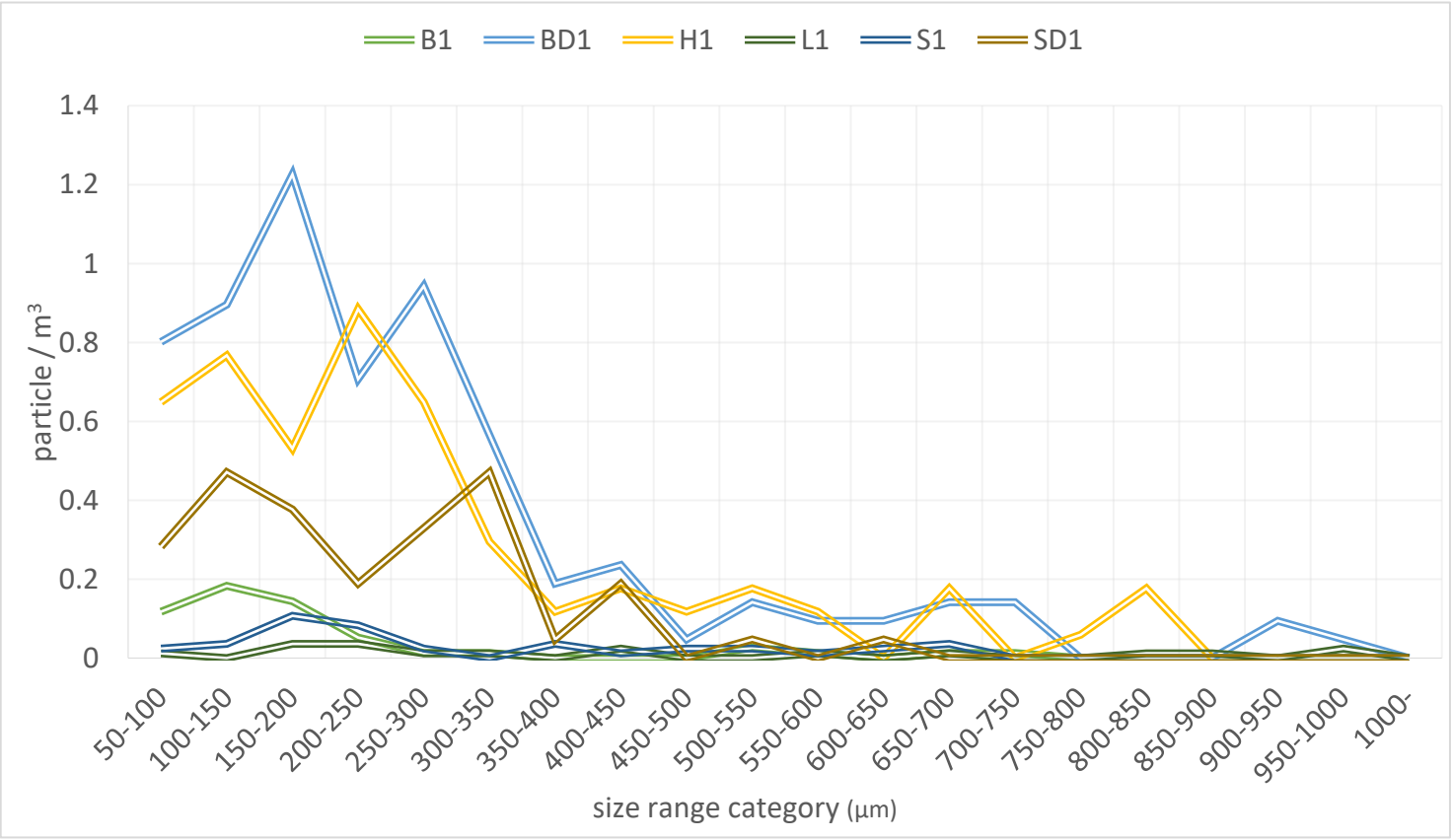


Figure 24 Relative particles size distribution in the samples 2022

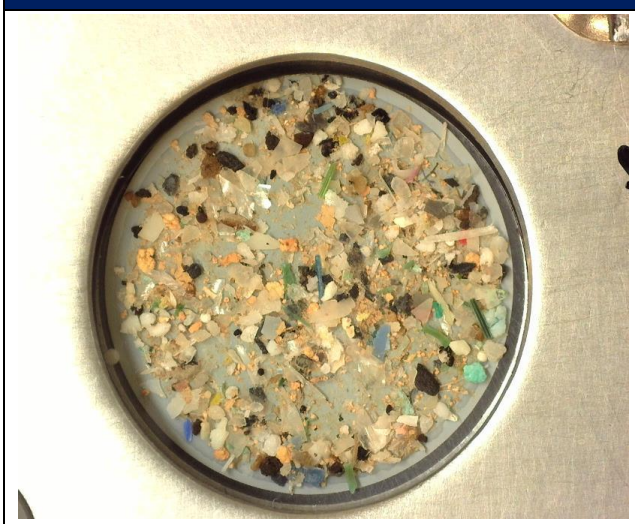
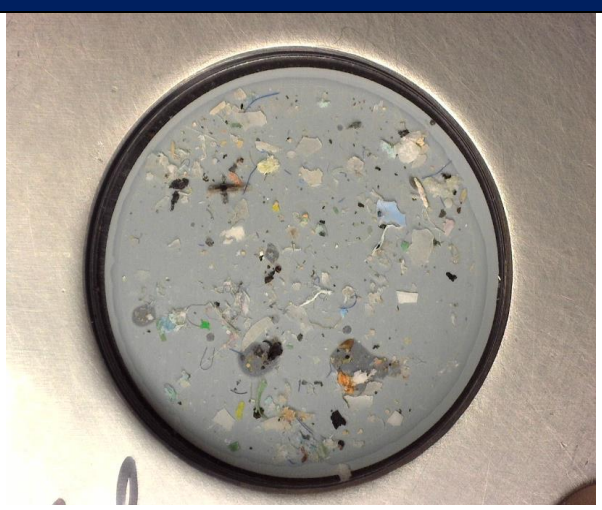
Quantitative and qualitative characterization - Phase 2 (2023)

During the 2nd phase of the sampling campaign, we focused on covering larger surfaces sampling and volumes to be collected, in order to have a better picture and be able to understand the results of the 1st phase. We optimized the towing speed according to our experience from the sampling in 2022 and increased the sampling time on the same set-up, so we obtained significantly higher sampled volumes. These modifications presented difficulties during the sample analysis. Since the sampled amount of water was much larger than in 2022, the captured amount of microplastics and the organic interference were also higher. For this reason, the laboratory had to take extra measures

during the sample preparation phase in order to attain comparable results to those of the 1st phase. The sample preparation includes several separations based on oxidation (removal of organic matter) and density (removal of non-plastic particles of inorganic matter). One of the difficulties of sample preparation is that strong oxidizing agents cannot be used in order so as not to damage the plastics, so, if the samples contain a high amount of organic matter, the method does not work properly and the program may report incorrect values, which is why we did not take into consideration the reported weights of the microplastic samples when evaluating the results, in order to allow us to obtain comparable results. The IR frequently does not pass through the polymers if there is too much plastic in the sample and we are unable to distinguish plastic from plastic, or if they are too large, in which case the information is lost or the individual parts are treated as one by the software, partly due to overlap. For that reason, since we have captured significantly higher amounts of microplastics in certain cases, or the organic interference was larger in the laboratory, they have created sub-samples from the original samples and analysed them separately, which will be showed in separated filters in **Table 8.** The pictures perfectly illustrate the yellowish (sand-like) digested organic interference, which caused the difficulties during the analytical process.

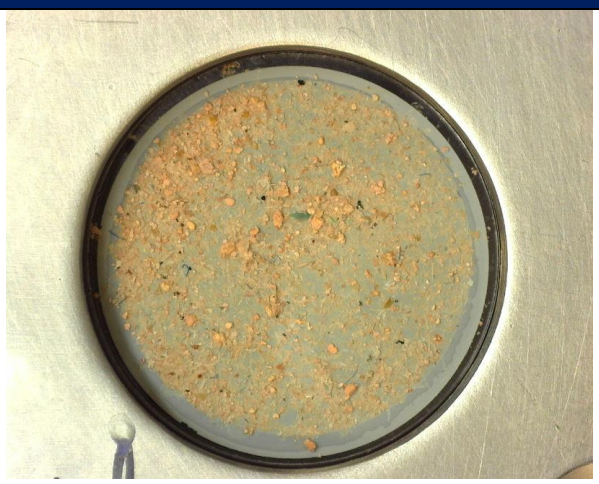
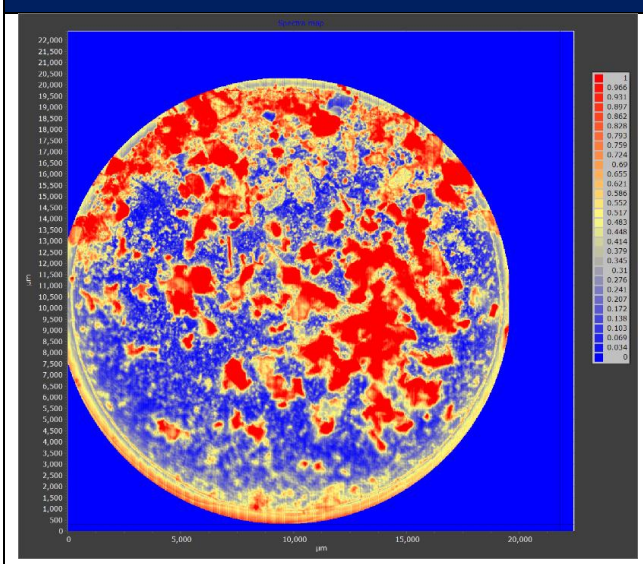
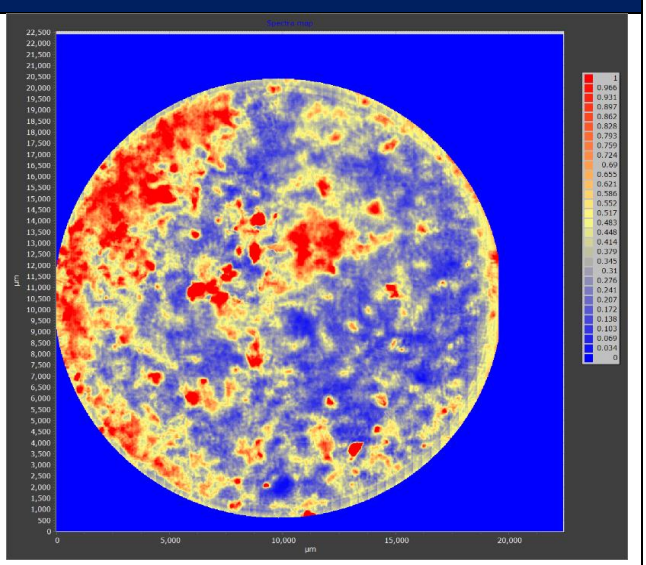
Table 8 Photo documentation of samples 1000- 50 µm of Phase 2 - 2023

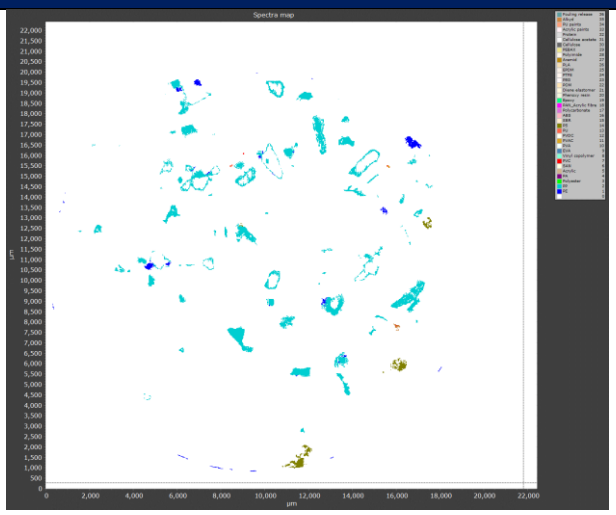
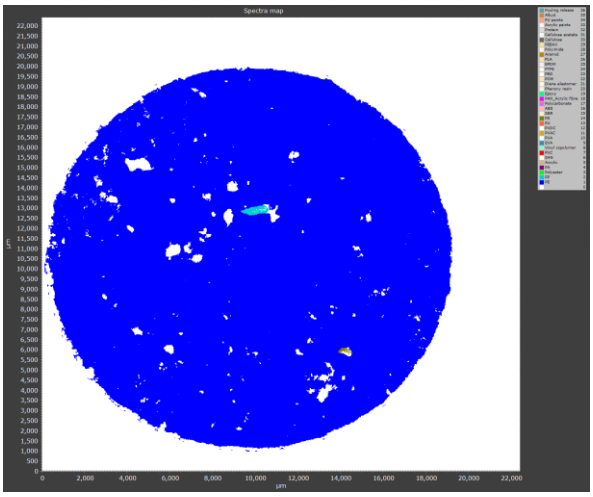
B2_1	B2_2
	

BD_1	BD_2
	
S2_1	S2_2
	
S2_3	SD2
	

While analysing the sample, the program used to identify the microplastics, generates two different spectral maps, one of which serves as a showcase with a false-colour image to represent the sample's total absorbance and the other which represents the microplastics that were found in the sample compared to the data lab of the program, which is going to illustrate the reason why we cannot consider the mass of the samples as a comparison base to the previous year, **Table 9** illustrates a clean sample (left) and a highly contaminated sample (right) with digested organic materials both on the absorbance map and the identification.

Table 9 Illustration of digested organic interference showing on the Spectra Maps

B2_1	BD_1
	
B2_1 total Absorbance Spectra Map	BD_1 total Absorbance Spectra Map
	

B2_1 total Microplastic Spectra Map	BD_1 total Microplastic Spectra Map
	

Following the spectral analysis, the program gathered all the data about the detected matches to the standard (polymer type, size, weight etc) for each microplastic found, which we further analysed and gathered in **Table 10** and **Figure 25**.

Table 10 Qualitative and quantitative characterization of Microplastics in each sample (1000-50 µm) 2023

	Sample code	B2	BD2	S2	SD2
Identified MPs (particles/sample)	PE	87	41	309	75
	PP	92	23	224	93
	Polyester	0	1	0	5
	PA	0	0	0	1
	Acrylic	10	0	71	75
	PVC	6	0	14	2
	PVAC	3	0	7	0
	PU	1	0	1	5
	PS	17	6	145	16
	ABS	1	2	0	1
	Alkyd	1	3	11	3
	Sum	**218**	**76**	**782**	**276**

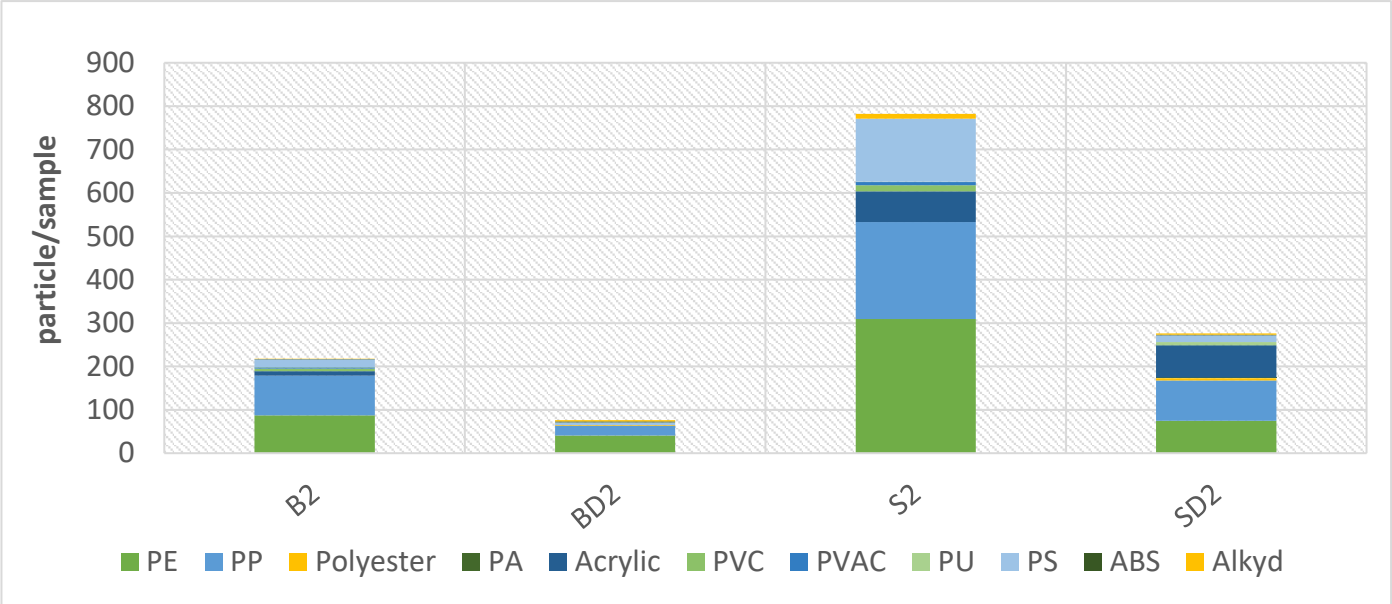


Figure 25 Microplastics content in the tested samples between 1000-50 µm 2023

During the 2nd phase sampling, we found significantly higher numbers of plastic particles in certain cases. Whereas only 45% of the total number of particles were found to between 50-350 µm as illustrated in **Figure 26**. **Figure 27** demonstrates the prevalence of the different polymer types that were found in the samples and their distribution.

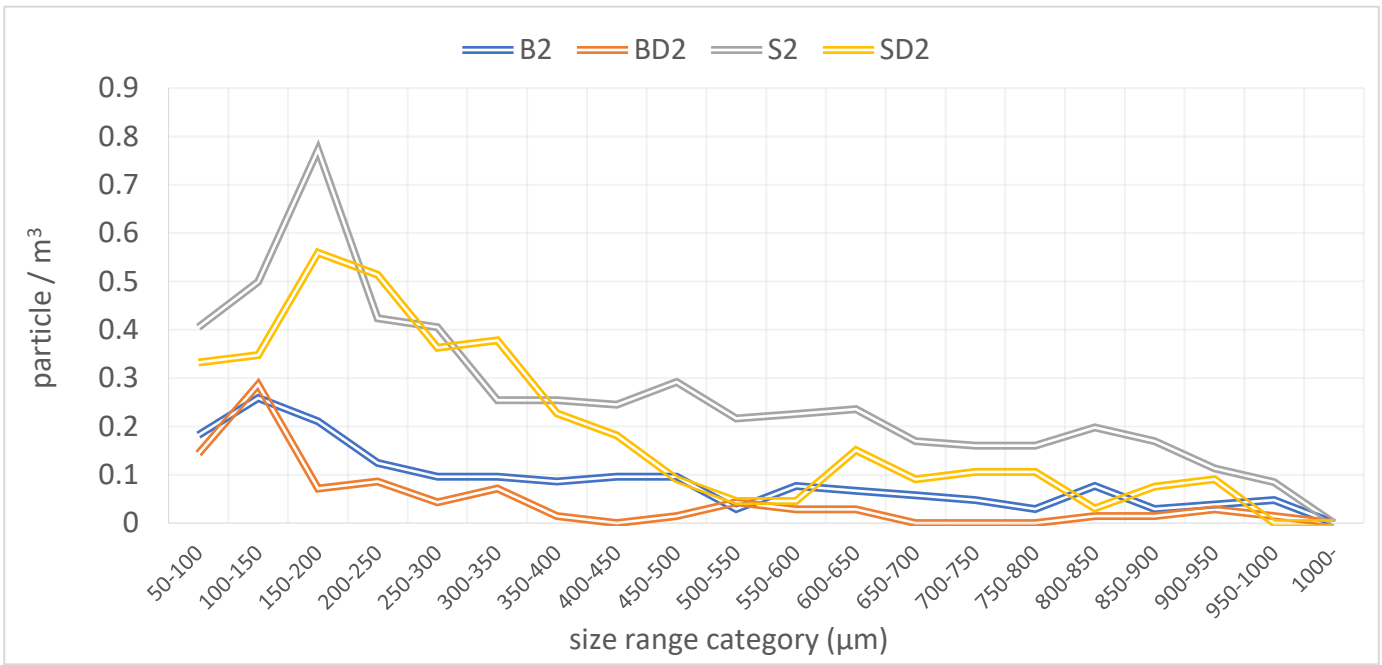


Figure 26 Relative particles size distribution in the samples 2023

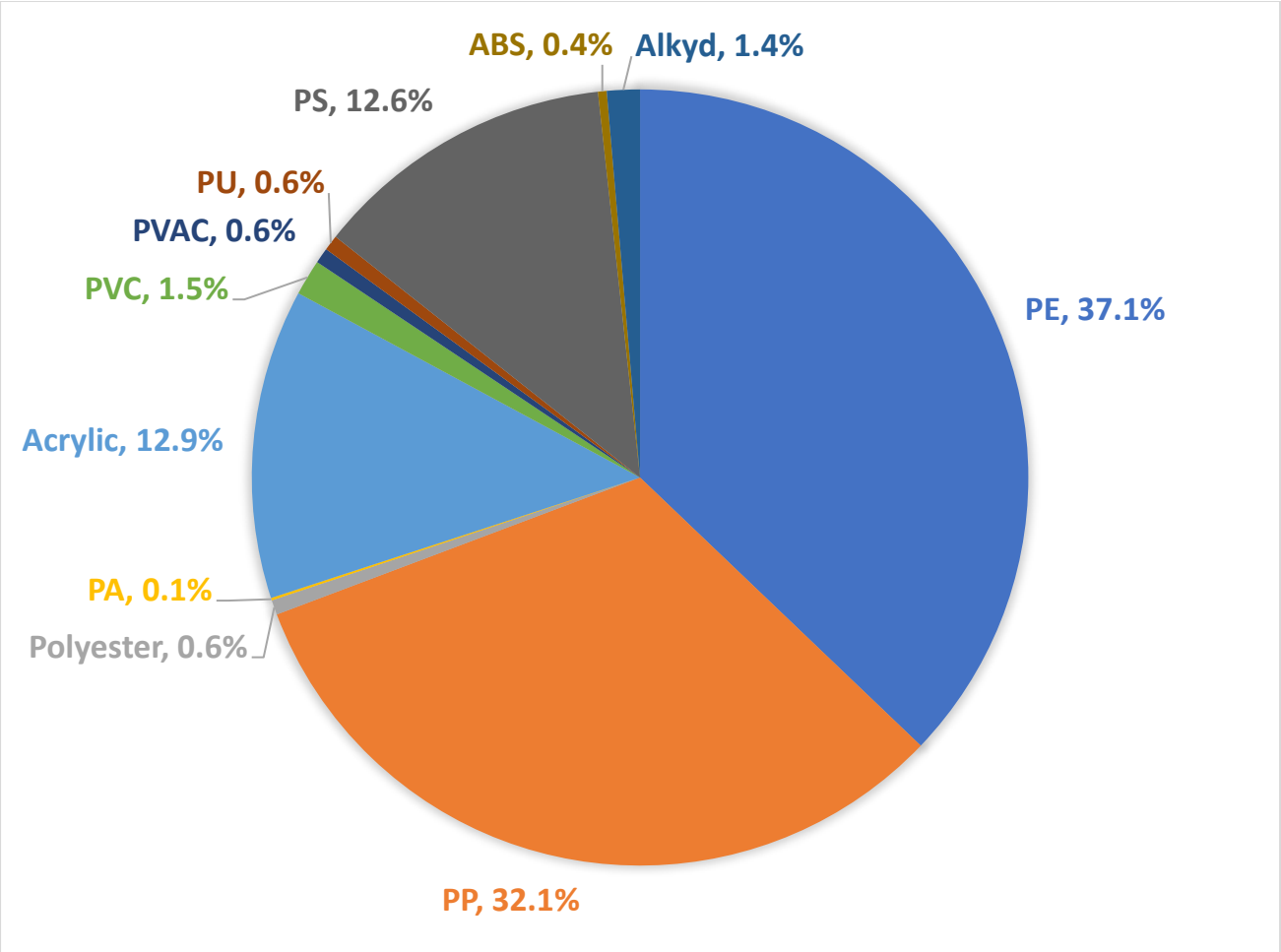


Figure 27 Polymer type distribution (%, all sample average from 2023 samples)

Evaluation of the obtained results

The results of the first sampling are showing fascinating trends with regards to our research goal of developing a methodology which is able to sample the microplastic contamination 10 meters below the surface level, and to compare it to the contamination of the surface. Our hypothesis was that the lower sea levels might be more contaminated in the 1000-50 µm size range.

Quantitative analysis

As already indicated, the primary problem with all studies that have been done on microplastic contamination in the marine environment is that the results are sometimes incomparable because of variations in methodology and reporting. To make it more evident how the pollution level has evolved over time and what the differences between sites and depths are, in addition to the general data acquired in the previous chapter, I have compiled the major results' highlights as generic relative units. In order to make a meaningful comparison and to obtain a general concentration of microplastics in these studies, the sampling conditions played an important role. To standardize our concentrations, I report data measure in **particles/filtered meter cube** (particles/m^3) of sea water. As **Table 11** and **Table 12** is showing, in 2022 the most polluted according to our findings with regards to the contamination of surface water was Ħofra ż-Żgħira, with 5.07 particles/m^3; this result can origin at from the fact that the area is a closed bay, which is less affected by the external conditions of the sea, however also the volume of the sample collected is very low in comparison with the other sites, where the conditions were more convenient. Microplastic concentration in each area 2022

Table 11 Microplastic concentration in each area 2022

	Sample code	B1	BD1	H1	L1	S1	SD1
	Sample volume (m^3)	76.455	21.20805	16.95465	83.51055	83.99565	21.22245
Identified MPs (particles/m^3)	PE	0.97	0.42	0.47	0.10	0.07	0.28
	PP	0.59	0.66	0.06	0.08	0.13	0.24
	Polyester	0.44	0.28	0.00	0.00	0.00	0.00
	PA	0.03	0.05	0.06	0.00	0.00	0.00
	Acrylic	0.76	3.91	3.83	0.02	0.12	1.79
	PVC	0.10	0.28	0.00	0.00	0.02	0.09
	PVAC	0.00	0.05	0.00	0.00	0.01	0.00
	PU	0.04	0.19	0.12	0.00	0.00	0.05
	PS	0.01	0.66	0.29	0.05	0.14	0.00
	ABS	0.01	0.05	0.06	0.00	0.01	0.00
	PTFE	0.00	0.00	0.12	0.00	0.00	0.00
	Cellulose acetate	0.01	0.00	0.00	0.00	0.00	0.00
	Alkyd	0.92	0.05	0.06	0.00	0.00	0.09
	Sum	**3.88**	**6.60**	**5.07**	**0.25**	**0.51**	**2.54**

However, it is important to highlight that, according to the survey, in both cases where we applied our experimental methodology to collect samples at 10-meter depths from the surface significantly higher contamination levels compared to the same area`s surface water were recorded. In the case of Xgħajra, the overall pollution level in the sub-surface sample (6.60 particles/m^3) was **70% higher** compared to the surface (3.88 particles/ m^3), while in the case of Selmun, which was originally indicated as a reference site, the pollution of the sub-surface water column (2.54 particles/$m^{3)}$ was **498% higher** than that of the surface water (0.51 particles/m^3). It is evident that the pollution level of Xgħajra is higher in every sense than that at our reference site Selmun.

Table 12 Microplastic concentration in each area 2023

	Sample code	B2	BD2	S2	SD2
	volume (m3)	104.400	69.750	106.200	66.150
Identified MPs (particles/m^3)	PE	0.833	0.588	2.910	1.134
	PP	0.881	0.330	2.109	1.406
	Polyester	0.000	0.014	0.000	0.076
	PA	0.000	0.000	0.000	0.015
	Acrylic	0.096	0.000	0.669	1.134
	PVC	0.057	0.000	0.132	0.030
	PVAC	0.029	0.000	0.066	0.000
	PU	0.010	0.000	0.009	0.076
	PS	0.163	0.086	1.365	0.242
	ABS	0.010	0.029	0.000	0.015
	Alkyd	0.010	0.043	0.104	0.045
	Sum	**2.088**	**1.090**	**7.363**	**4.172**

In 2023, we focused on the repetition of the sampling at those sites where we have performed the experimental sampling methodology. The analytical results showed an opposite outcome compared to those of 2022. The pollution level in both cases was lower at the sub-surface level than at the surface. In the case of Selmun, the microplastic concentration at the surface (7.363 particles/m^3) was **76% higher** than at the sub-surface level (4.172 particles/m^3), while, at Xgħajra, it was **92 % higher** at the surface (2.088 particles/m^3), than at 10m from the surface (1.090 particles/m^3). One possible explanation for the difference between the two years is that there may have been variations in the amount of matrix that was analysed, or changes in environmental conditions such as fluctuations in currents, waves, and wind patterns. However, it is important to conduct further evaluation, such as emissions modelling, to gain a better understanding of the statistical behaviour of microplastics.

Qualitative analysis

During this research, we collected in total 10 samples from 4 different sites, and we have identified a total of 1,993 Microplastics. Within this amount of plastic particles, the four most abundant types of plastic were **27.9%** Acrylic , **27.2%** PE (Polyethylene) , **23.9%** PP (polypropylene) and **11.6%** PS (polystyrene) as **Figure 28** illustrates. Between these four main contributors, there was a change in the distribution over the two sampling years; however, during both years, these four were found to be the most prevalent.

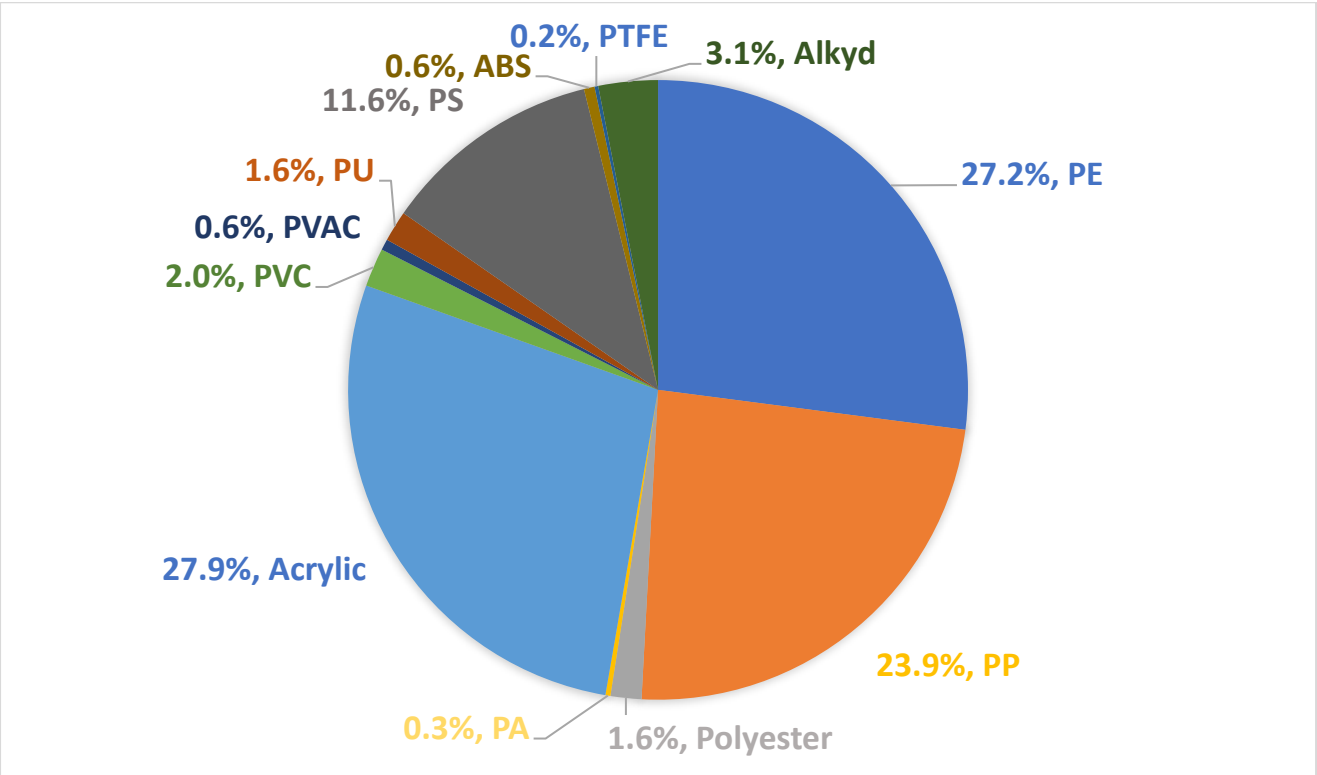


Figure 28 polymer type distribution in all samples collected during 2022 and 2023

The abundance of these main polymers found are however interesting, such as Acrylic polymers were mainly found at the sub-surface level more than at the surface, while in the case of the PS and PE, it was significantly higher at the surface. (**Figure 29**)

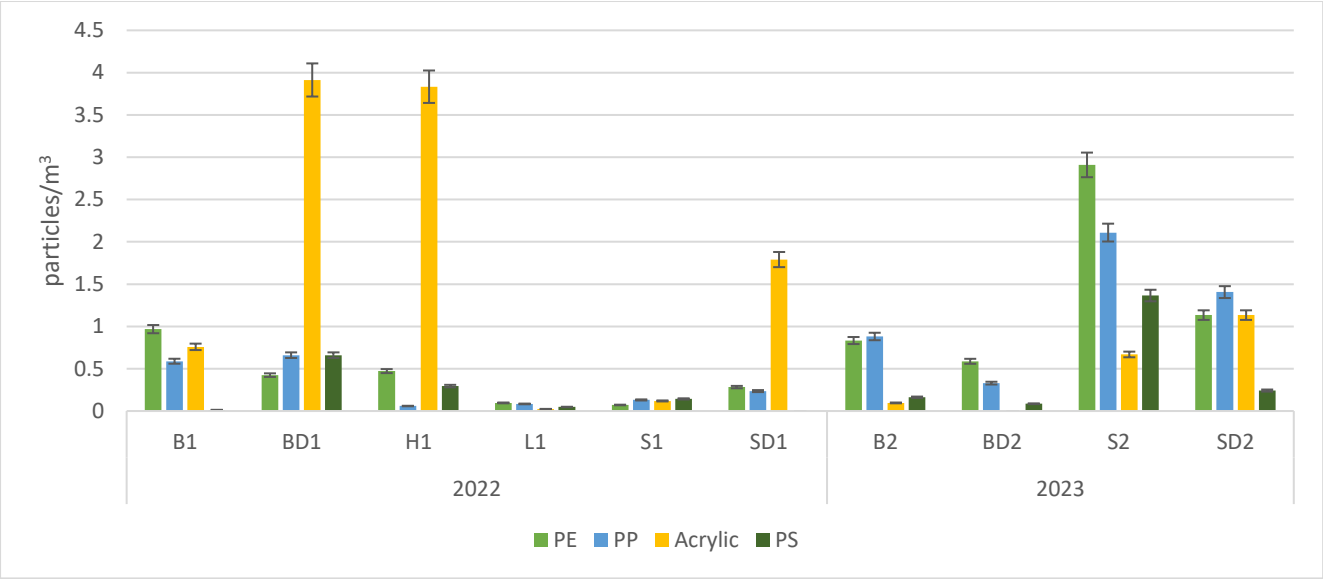


Figure 29 Most abundant polymer distribution in all samples

Conclusion

According to the surveys conducted between 2022 August and 2023 June, we have observed that the Microplastic pollution level of the surface waters was **0.25-7.363 particles/m^3**, while in the sub-surface water column at 10 meters from the surface we found **1.090-6.60 particles/m^3**.(**Table 13**) During the past two years, we have seen significant inter-annual deviations in the microplastic pollution levels in the case of Xghajra and Selmun which was our main scope to evaluate.

Table 13 Concentration on Microplastic pollution in all samples 2022 and 2023

Sampling area	24-26th August 2022		2nd June 2023	
	Surface sampling (particles/m^3)	Sub-surface sampling (particles/m^3)	Surface sampling (particles/m^3)	Sub-surface sampling (particles/m^3)
Għar Lapsi	0.25	Not collected	Not collected	Not collected
Ħofra ż-Żgħira	5.07	Not collected	Not collected	Not collected
Xghajra, Barkat	3.88	6.60	2.088	1.090
Selmun	**0.51**	**2.54**	**7.363**	**4.172**

This difference can originate from many factors, considering that as microplastics are entering the marine environment through many different ways (effluent discharge, atmospheric deposition, surface run-off etc), these affects are strongly dependant on environmental conditions, also, since microplastics are solid contaminants, their presence or structure in the marine matrix can be altered by external factors, such as waves, wind, currents, sedimentation, degradation etc. Since in 2023 we optimized our sampling methodology, this allowed us to collect larger volume of water so as to acquire a broader picture of the environment at both locations, which could statistically also change the results compared to the samples taken in 2022. In the case of Selmun's surface waters, the average microplastic pollution level was **3.9365 particles/m^3** and in the case of Xghajra this was **2.984 particles/m^3**. In the case of Selmun's sub-surface water's (10m from the surface) the average pollution level was **3.356 particles/m^3** and in the case of Xghajra this was **3.845 particles/m^3**. (**Figure 30**)

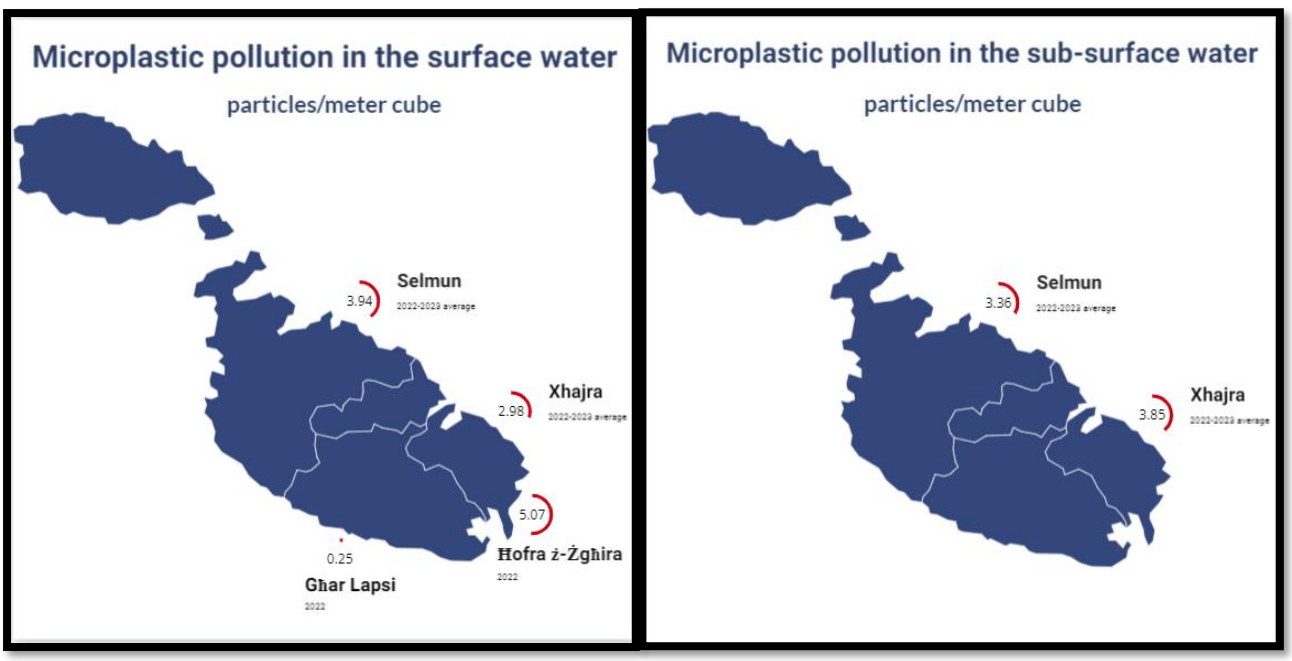


Figure 30 Comparison between the pollution level of the nearshore Maltese surface water and the water column 10 meters from the surface

The phenomenon that the microplastic pollution level in certain cases was observed to be higher in the water column 10 meters from the surface than on the surface can originate from various factors. It could be based on an equilibrium state between the size and the density of certain polymers with the linked environmental conditions, such as salinity and buoyancy. The most significant difference

between the microplastic pollution levels at the surface waters and those recorded in sub-surface samples was detected in terms of acrylic pollution, which happens to be the densest polymer among the four most comonly dectected ones. As most of the polymers has lower density than sea water, this equilibrium between the sedimentation process and the buoyancy can be influenced by other external factors such as the occurance of microorganisms (or other contaminants), which form biofilms on the surface of the particles, which can cause change in the relative density of the polymers. That might might mean that the equilibrium state might cause the accumulattion of microplastics in the lower sea levels. Acrylic polymers, in fact, have the highest density (1.15-1.19 g/cm^3), while PE (0.86-1.00 g/cm^3), PS (0.96-1.05 g/cm^3), and PP (0.90-0.92 g/cm^3), have considerably lower densities, which might explain some of the results obtained in this study. The recorded polymers include : Acrylic ones are frequently used for lighting, electronics screen, automotive components, clothing industry and outdoor glazing in architecture and construction, PE (Polyethylene) are the most commonly-used plastic all over the world mainly as packaging material, PP (Polypropylene) is used in a wide variety of applications such as packaging, automotive parts, fibers, and textiles, and PS (Polystyrene) mainly used as protective packaging (foam) or as plastic containers, bottles, and lids. The apperance in Maltese nearshore areas of these polymers can be due to transboundary origins, however, Malta's antropogenic context (strong wind exposing a landfill areas, heavy rains causing surface run-offs, non-efficient wastewater treatment) can also contibute to the change in the pollution level of the sea.

During the analytical characterization, an interesting fragment was observed, which can be possibly originating from a cloth tagging pin. The shape and state shows it was hardly exposed to external conditions, suggesting a a local origin. (**Figure 31**)

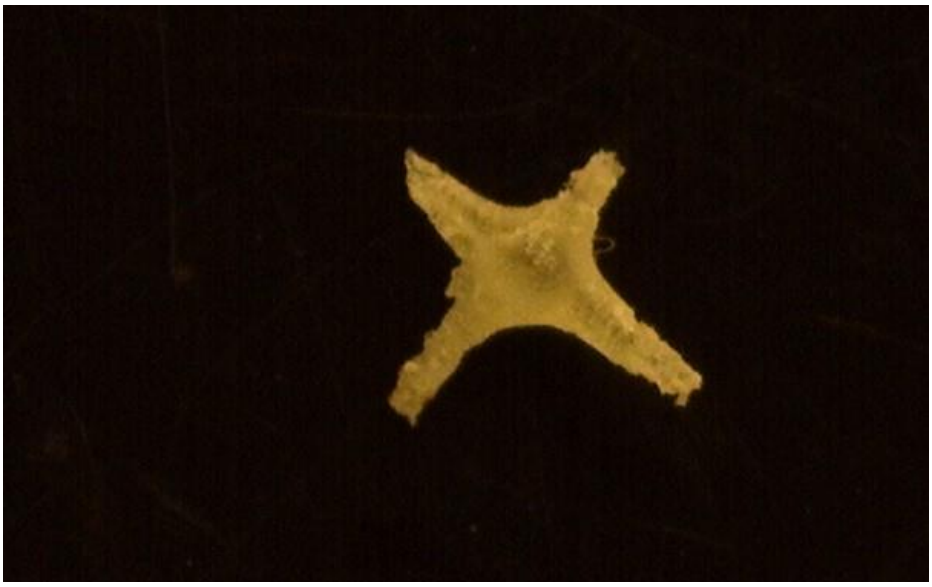


Figure 31 Microplastic particle originating suspectedly form a cloth tagging pin.

(Image: Eurofins Analytical Services Hungary Kft.)

The major conclusion of our research was that the monitoring of the baseline microplastic pollution should not only focus on the level of microplastics floating in the sea, or sedimented at the bottom, as we have clearly proved that in the subsurface water column, significantly under the surface, one can still find accumulation of microplastics as well. This can effect negatively the living marine environment (mainly omnivore fish species) , which will in the long-term effect humanity, either through a decline of commercial fish species stock, or due to the consequences of microplastics entering into the food chain. The results we have obtained, have a significantly higher analytical value than those recorded in other studies carried out in other Mediterranean Sea areas, as **Table 14**:

Table 14 Comparison of Surface water pollution in the Mediterranean Area

Study Area	Mean Density
Sardinian Sea [42]	0.16 (±0.31) particles/m^3
Ligurian Sea [42]	0.49 (±1.66) particles/m^3
Calabrian costs [43]	0.52 (±0.778) particles/m^3
Malta [20]	0.58 (±0.72) particles/m^3
Malta- Ħofra ż-Żgħira (This research)	**5.07 particles/m^3**

Malta- Xhajra, Barkat (This research)	**2.984 particles/m^3**
Malta- Selmun (This research)	**3.9365 particles/m^3**
Malta- Għar Lapsi (This research)	**0.25 particles/m^3**

These results should be duly considered for integration in the national environmental policies as well as within a revised Water Framework Directive and Marine Strategy Framework Directive monitoring strategy, which are standing for the approach for managing the marine environment and aims to protect the marine environment across Europe while encouraging the continuance of ocean sustainable uses.

Reference list

[1] https://nso.gov.mt/themes_infographics/plastic-pollution/

[2] Gesamp (2015): Sources, fate and effects of microplastics in the marine environment: a global assessment

[3] [6] THOMPSON, R. C., OLSEN, Y., MITCHELL, R. P., DAVIS, A., ROWLAND, S. J., JOHN, A. W.,MCGONIGLE, D. RUSSELL,A. E. (2004): Lost at sea: where is all the plastic?

[4] World Health Organization (2019), Microplastics in drinking-water

[5] Environmental and health risks of microplastic pollution (European Commission, 2021)

[6] Biotechnology Advances, (GESAMP, 2016; HORTON ET AL., 2017, GIGAULT ET AL., 2018; FRIAS & NASH, 2019; HILDEBRANDT ET AL., 2020)

[7] SHAH, A. A., HASAN, F., HAMEED, A., AHMED, S. (2008): Biological degradation of plastics: A comprehensive review.

[8] Gábor Bordós (2021) MIKROMŰANYAGOK KÖRNYEZETI ELŐFORDULÁSÁNAK VIZSGÁLATA

[9] Hind El Hadri, (2020) Nanoplastic from mechanically degraded primary and secondary microplastics for environmental assessments

[10] Bela Pukanszky, Janos Moczo (2011) Plastics

[11] Olagoke O., Kolapo A (2016) Handbook of Thermoplastics

[12] Hanna Dodiuk (2022) Handbook o Thermoset Plastics

[13] Geyer et al. (2017), published in the journal Science Advances.

[14] Tania Martellini,Cristiana Guerranti,Costanza Scopetani,Alberto Ugolini,David Chelazzi,Alessandra Cincinelli (2018) A snapshot of microplastics in the coastal areas of theMediterranean Sea

[15] Froukje A.E.Lotsa Paul Behrensab Martina G.Vijverb Alice A.HortonbcThijs Boskerab (2017) A large-scale investigation of microplastic contamination: Abundance and characteristics of microplastics in European beach sediment

[16] H. Kaberi, C. Tsangaris, C. Zeri, G. Mousdisd, A. Papadopoulos and N. Streftaris (2013) Microplastics along the shoreline of a Greek island (Kea isl., Aegean Sea): types and densities in relation to beach orientation, characteristics and proximity to sources

[17] Lourens J. J. Meijer, Tim van Emmerik, Ruud van der Ent, Christian Schmidt, Laurent Lebreton (2021) More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean

[18] Miquel Canals, William P. de Haan, and Anna Sànchez-Vidal(2019) Polyethylene, polypropylene and polystyrene are the most abundant microplastics in the Mediterranean coastal waters, according to a new study
[19] Deidun, Alan,Axiak, Victor, Bezzina, Michelle, Lomax, Corinne, Edwards, Kristina(2017)

First full investigation of microplastics on Maltese sandy beaches - MEDCOAST 2017

[20] Project Impact (2021), IDENTIFYING MICROPLASTIC HOTSPOTS IN THE MALTESE WATERS

[21] Boucher, J., & Friot, D. (2017). Primary Microplastics in the Oceans: Global Evaluation of Sources. InternatIonal UnIon for ConservatIon of Nature and Natural Resources. Gland, Switzerland: International Union for Conservation of Nature and Natural Resources

[22] Laurent C. M. Lebreton, et al., "Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic," Scientific Reports 8, no. 4666 (March 2018),

[23] https://theoceancleanup.com/great-pacific-garbage-patch/

[24] Shivika Sharma, Vikas Sharma, Subhankar Chatterjee (2021) Microplastics in the Mediterranean Sea: Sources, Pollution Intensity, Sea Health, and Regulatory Policies

[25] SAPEA, Science Advice for Policy by European Academies. (2019). A Scientific Perspective on Microplastics in Nature and Society. Berlin: SAPEA. https://doi.org/10.26356/microplastics

[26] Md. Simul Bhuyan (2022) Effects of Microplastics on Fish and in Human Health https://doi.org/10.3389/fenvs.2022.827289

[27] Teresa Romeo, Battaglia Pietro, Cristina Pedà , Pierpaolo Consoli , Franco Andaloro , Maria Cristina Fossi (2015) First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea

[28] Andrea Corti, Giulia Pagano, Angelina Lo Giudice,Maria Papale , Carmen Rizzo , Maurizio Azzaro, Virginia Vinciguerra, Valter Castelvetro, Stefania Giannarelli (2023) Marine sponges as bioindicators of pollution by synthetic microfibers in Antarctica

[29] https://www.theguardian.com/environment/2017/aug/16/fish-confusing-plastic-debris-in-ocean-for-food-study-finds

[30] Ana I. Catarino, Valeria Macchia, William G. Sanderson, Richard C. Thompson, Theodore B. Henry School of Energy, Geoscience, Infrastructure and SocietyInstitute for Life and Earth Sciences (2018) Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal

[31] Kurunthachalam Kannan, Krishnamoorthi Vimalkumar (2021) A Review of Human Exposure to Microplastics and Insights Into Microplastics as Obesogens

[32] FAURE, F., DEMARS, C., WIESER, O., KUNZ, M., FELIPPE DE ALENCASTRO, L. (2015): Plastic pollution in Swiss surface waters: nature and concentrations, interaction with pollutants. (Environmental Chemistry)

[33] MANI, T., HAUK, A., WALTER, U., BURKHARDT-HOLM, P. (2015): Microplastics profile along the Rhine River.Scientific Reports 5.

[34] FISCHER, E. K., PAGLIALONGA, L., CZECH, E., TAMMINGA, M. (2016): Microplastic pollution in lakes and lake shoreline sediments: A case study on Lake Bolsena and Lake Chiusi (central Italy). Environmental Pollution,

[35] STOCK, F., KOCHLEUS, C., BÄNSCH-BALTRUSCHAT, B., BRENNHOLT, N., REIFFERSCHEID, G. (2019): Sampling techniques and preparation methods for microplastic analyses in the aquatic environment ‐

[36] SCHERER, C., WEBER, A., STOCK, F., VURUSIC, S., EGERCI, H., KOCHLEUS, C., ARENDT, N., FOELDI, C., DIERKES, G., WAGNER, M., BRENNHOLT, N., REIFFERSCHEID, G. (2020): Comparative assessment of microplastics in water and sediment of a large European river.

[37] https://parley.tv/journal/2020/4/6/tavake-pakomio-science-at-sea

[38] Mohammed S. M. Al-Azzawi 1, Simone Kefer 2 , Jana Weißer 3 , Julia Reichel 1 , Christoph Schwaller 1 , Karl Glas 3 , Oliver Knoop 1,* and Jörg E. Drewes 1 (2020) Validation of Sample Preparation Methods for Microplastic Analysis in Wastewater Matrices− Reproducibility and Standardization

[39] Tagg, A.S.; Sapp, M.; Harrison, J.P.; Ojeda, J.J. Identification and quantification of microplastics in wastewater using focal plane array-based reflectance Micro-FT-IR imaging. Anal. Chem. 2015,

[40] PRIMPKE, S., WIRTH, M., LORENZ, C., GERDTS, G. (2018): Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy.

[41] PAUL, A., WANDER, L., BECKER, R., GOEDECKE, C., BRAUN, U. (2019): High-throughput NIR spectroscopic (NIRS) detection of microplastics in soil.

[42] Maria Cristina Fossi a, Letizia Marsili a, Matteo Baini a, Matteo Giannetti a b, Daniele Coppola a, Cristiana Guerranti a, Ilaria Caliani a, Roberta Minutoli c, Giancarlo Lauriano d, Maria Grazia Finoia d, Fabrizio Rubegni e, Simone Panigada f, Martine Bérubé g, Jorge Urbán Ramírez h, Cristina Panti (2016) Fin whales and microplastics: The Mediterranean Sea and the Sea of Cortez scenarios

[43] Marcus Eriksen , Laurent C M Lebreton , Henry S Carson , Martin Thiel , Charles J Moore5, Jose C Borerro , Francois Galgani , Peter G Ryan , Julia Reisser (2014) Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea

[44] https://www.statista.com/statistics/1073286/global-share-plastic-consumption-globally-by-class/

[45] Meijer et al. (2021). More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Science Advances.

Table of figures

Table content

Appendix A (Report of first analysis)

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INVESTIGATION REPORT

Customer: University Of Malta
2080 Msida, University of Malta, Maths and Physics Building, Room 211A

Project: Analysis of microplastics (2022/K/10242)

Report no.: 767450/1

Beginning of analysis: 13. 10. 2022.
End of analysis: 09. 12. 2022.

1. Introduction

In cooperation with the University of Malta, WESSLING Hungary Ltd. conducted analysis of microplastics in concentrated seawater samples that have been delivered to the laboratory in glass jars by the customer (**Figure 1**). Sample volumes were recorded by Malta University as shown in **Table 1**.

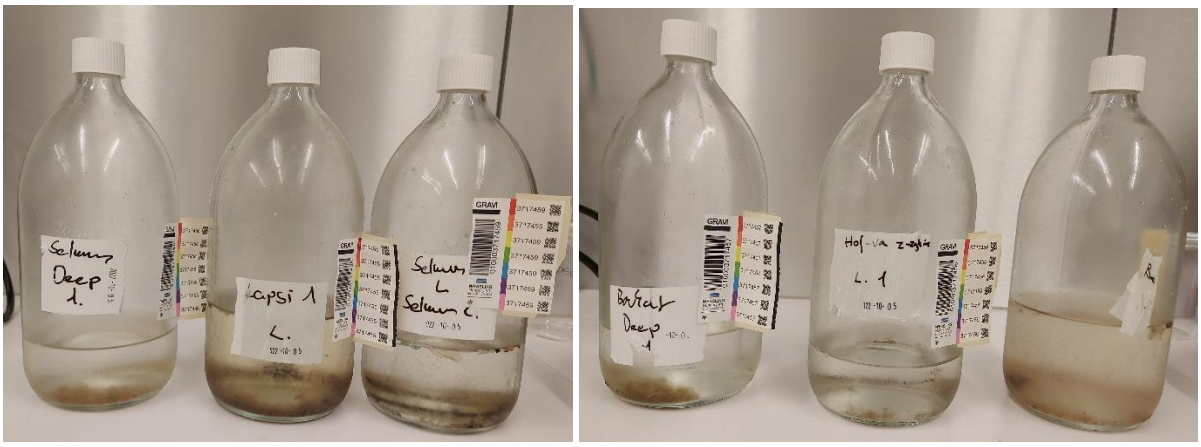

Figure 1: Samples received in the lab.

2. Sample preparation and analysis

The samples were filtered on a stack of sieves to remove larger pieces of particles. Particles in the fraction between 1000-5000 µm were picked manually, captured by a digital camera first, then the chemical composition was analysed with FTIR-ATR. Resulting spectra were compared to a reference polymer spectra database and particles showing correlation >70% were considered as microplastics.

The sample in the fraction between 1000-50 µm went through a Fenton oxidation process using 30% hydrogenperoxide and a subsequent density separation with 1.6 g/cm^3 zinc-chloride solution. The resulting sample was filtered on 25 mm Anodisc filters (0.2 µm pore size).

The filters were then analysed by a Thermo Scientific Nicolet iN10 MX FTIR imaging microscope with 25 µm pixel size in transmission mode, 4 scan numbers and 8 cm^{-1} spectral resolution was applied. The spectral data was evaluated with the “siMPle” software, designed for microplastic analysis. Data were compared with reference polymer spectra library and particles with >70% correlation was considered as microplastics.

To prevent sample contamination general laboratory precautions were taken. Cotton lab coats were used and samples were prepared under a laminar flow hood to minimise airborn contamination. For washing steps deionised water was used that was previously filtered on 0.7 µm pore size glass filters. Laboratory blanks are measured to determine background contamination. Average values are 1.3 polyethylene, 1 polypropylene and 0.3 polystyrene particles per sample.

3. Results

3.1. FTIR imaging (1000-50 µm particles)

The analysis results are shown in **Table 1** and **Figure 2**. Polymer type distribution is presented on **Figure 2** and microplastic particle size distribution on **Figure 4**.

Detailed results of the particle analysis of the sample are attached in **Annex 1** (including visual image, heat map after FTIR analysis, microplastic map, details of the identified particles).

Table 1: Identified microplastics in the samples (particle/m^3).

Sample code	B1	BD1	H1	L1	S1	SD1
Sample volume (m^3)	76,455	21,20805	16,95465	83,51055	83,99565	21,22245
PE	0,97	0,42	0,47	0,10	0,07	0,28
PP	0,59	0,66	0,06	0,08	0,13	0,24
Polyester	0,44	0,28	0,00	0,00	0,00	0,00
PA	0,03	0,05	0,06	0,00	0,00	0,00
Acrylic	0,76	3,91	3,83	0,02	0,12	1,79
PVC	0,10	0,28	0,00	0,00	0,02	0,09
PVAC	0,00	0,05	0,00	0,00	0,01	0,00
PU	0,04	0,19	0,12	0,00	0,00	0,05
PS	0,01	0,66	0,29	0,05	0,14	0,00
ABS	0,01	0,05	0,06	0,00	0,01	0,00
Sample code	B1	BD1	H1	L1	S1	SD1
PTFE	0,00	0,00	0,12	0,00	0,00	0,00
Cellulose acetate	0,01	0,00	0,00	0,00	0,00	0,00
Alkyd	0,92	0,05	0,06	0,00	0,00	0,09
Sum	**3,88**	**6,60**	**5,07**	**0,25**	**0,51**	**2,54**

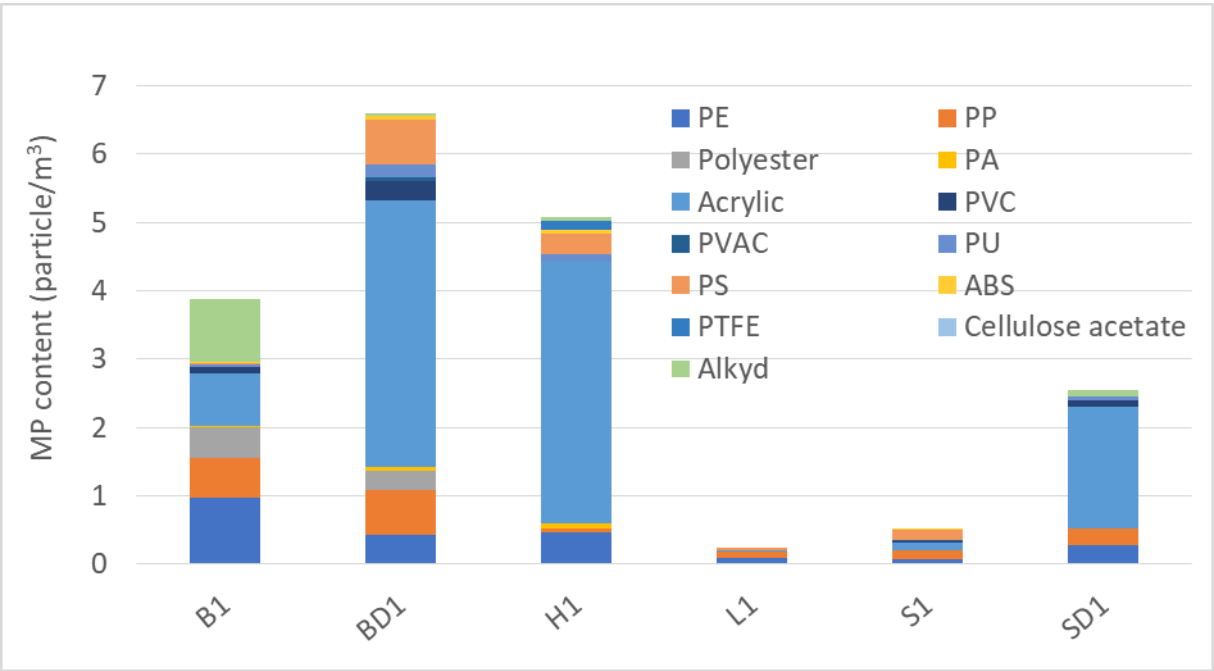

Figure 2: Microplastic content in the tested samples (particle/m^3).

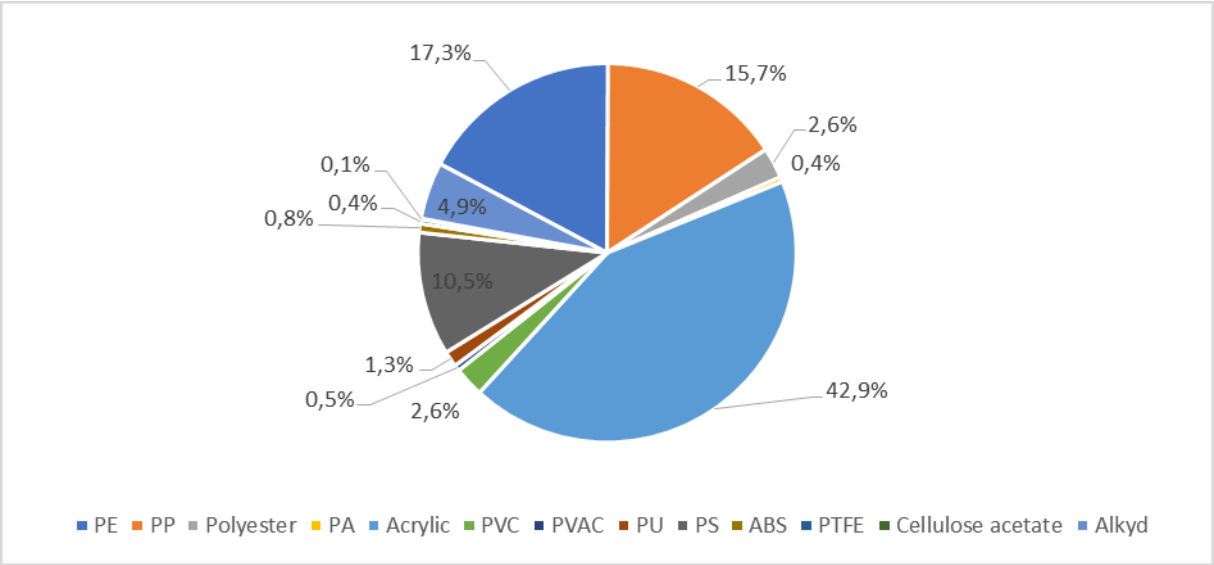

Figure 3: Polymer type distribution (%, all sample average).

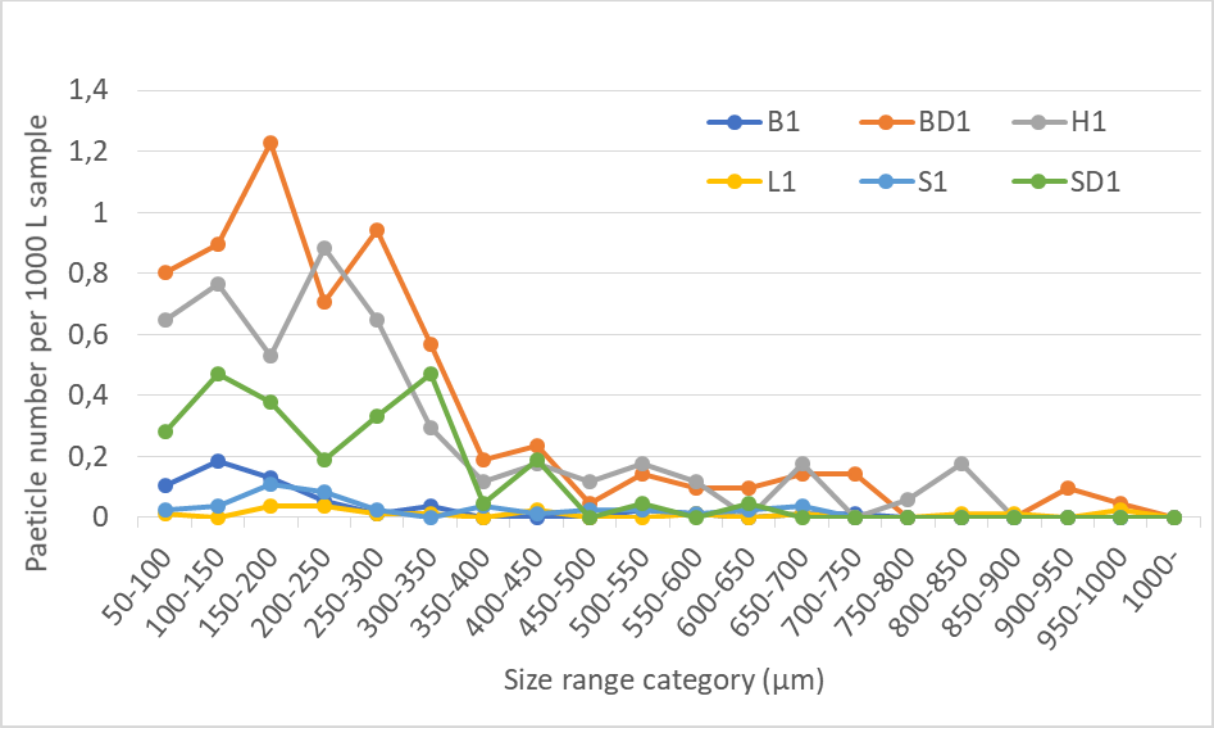

Figure 4: MP particles size distribution in the samples.

3.2. FTIR-ATR (1000-5000 µm particles)

Particles were picked manually from the samples in a range of 2-13 particle/sample and all particles were analysed by FTIR-ATR. In sample “B1” 108 particles were picked and only a subsample of 11 randomly chosen particles were analysed (10% of the whole sample). Identified MPs are summarised in **Table 2**, detailed particle analysis results and visual images of the particles are shown in **Annex 2**.

Table 2: Summary of the identified microplastics with FTR-ATR.

Identified polymer type	**Sample code**					
	B1 (10% subsample)	**BD1**	**H1**	**L1**	**S1**	**SD1**
PE	1	0	0	3	7	1
PP	0	0	0	0	5	0
PS	1	0	0	0	0	1
Sum	**2**	**0**	**0**	**3**	**12**	**2**

This report was compiled by Gábor Bordós, Phd.

09. 12. 2022.

Gábor Volk Deputy Head of Laboratory

This test report was generated from a validated system and is valid without a signature.

Appendix B (Report of second analysis)

INVESTIGATION REPORT

Customer: Ede Kossari Tarnik

SLM 3021 Sliema, 30 triq San Antnin flat 11 Project: Analysis (2023/K/07545)

Report no.: 818732/1

Testing laboratory accredited by NAH under reg. no. NAH-1-1398/2019.

Beginning of analysis: 2023. 07. 14.

End of analysis: 2023. 08. 22.

The laboratory is not responsible for information provided by the customer.

In the case of samples not taken by the laboratory, results refer only to the samples delivered to the laboratory.

Introduction

FTIR imaging analysis of microplastics were conducted in concentrated seawater samples that have been delivered to the laboratory in glass jars by the customer. Original sample volumes were recorded by the customer.

Sample preparation and analysis

The samples were filtered on a stack of sieves to remove larger pieces of particles (1000-5000 µm). The remaining sample in the fraction between 1000-50 µm went through a Fenton oxidation process using 30% hydrogen-peroxide and a subsequent density separation with 1.6 g/cm^3 zinc-chloride solution. The resulting sample was filtered on 25 mm Anodisc filters (0.2 µm pore size).

The filters were then analysed by a Thermo Scientific Nicolet iN10 MX FTIR imaging microscope with 25 µm pixel size in transmission mode, 4 scan numbers and 8 cm^{-1} spectral resolution was applied. The spectral data was evaluated with the “siMPle” software, designed for microplastic analysis. Data were compared with reference polymer spectra library and particles with >70% correlation was considered as microplastics.

Estimated mass concentration of different polymers in the samples are also reported. It is calculated by the “siMPle” software based on the estimated volume of the particle (assuming an ellipsoid shape) and the density of its material.

To prevent sample contamination general laboratory precautions were taken. Cotton lab coats were used and samples were prepared under a laminar flow hood to minimise airborn contamination. For washing steps deionised water was used that was previously filtered on 0.7 µm pore size glass filters. Laboratory blanks are measured to determine background contamination. Average values are 1.3 polyethylene, 1 polypropylene and 0.3 polystyrene particles per sample.

Results

3.1. FTIR imaging (1000-50 µm particles)

The analysis results are shown in **Table 1** and **Figure 1**. Polymer type distribution is presented on **Figure 2** and microplastic particle size distribution on **Figure 3**. Estimated polymer mass is shown in **Table 2** and in **Figure 4**. Detailed results of the particle analysis of the sample are attached in **Annex 1** (including visual image, heat map after FTIR analysis, microplastic map, details of the identified particles).

Table 1: Identified microplastics in the samples (particle/m^3).

Sample code	B2	BD2	S2	SD2
Sample volume (L)	**52200**	**34875**	**35400**	**66150**
PE	1,667	1,176	8,729	1,134
PP	1,762	0,659	6,328	1,406
Polyester	0,000	0,029	0,000	0,076
PA	0,000	0,000	0,000	0,015
Acrylic	0,192	0,000	2,006	1,134
PVC	0,115	0,000	0,395	0,030
PVAC	0,057	0,000	0,198	0,000
PU	0,019	0,000	0,028	0,076
PS	0,326	0,172	4,096	0,242
ABS	0,019	0,057	0,000	0,015
Alkyd	0,019	0,086	0,311	0,045
Sum	**4,176**	**2,179**	**22,090**	**4,172**

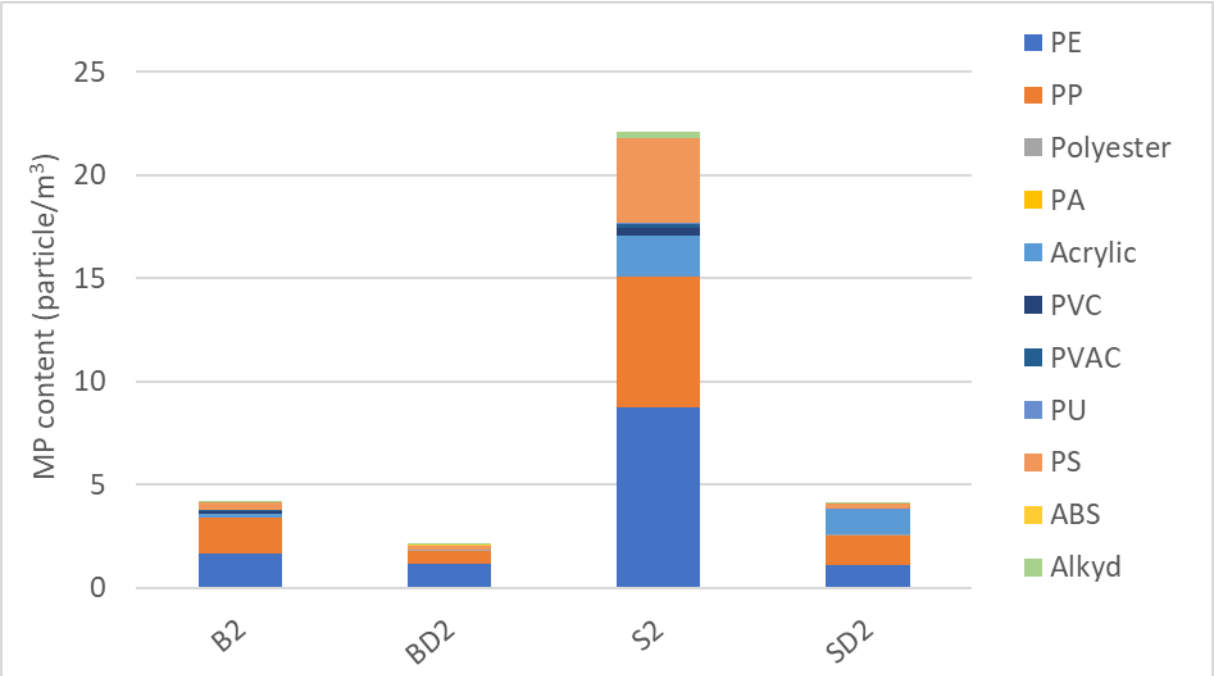

Figure 1: Microplastic content in the tested samples (particle/m^3).

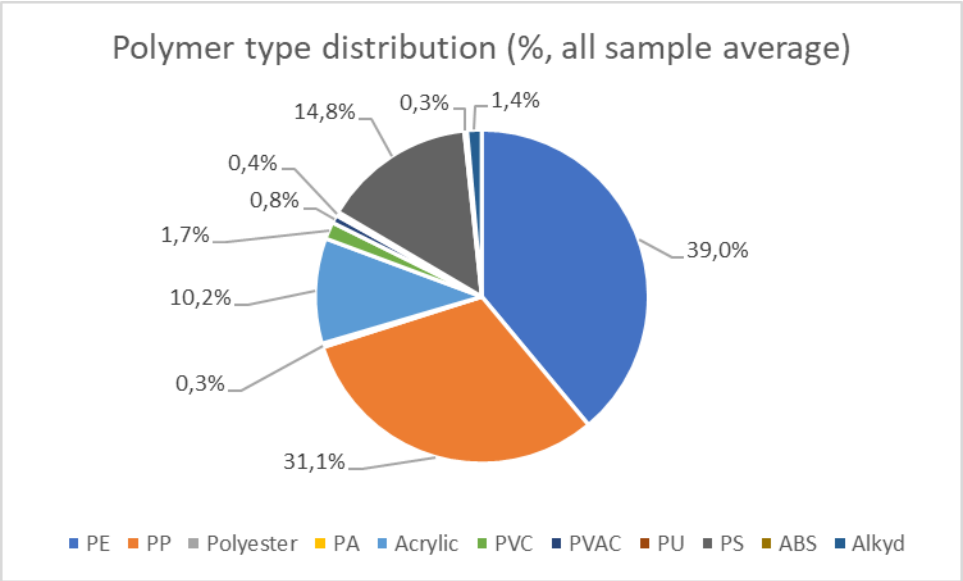

Figure 2: Polymer type distribution (%, all sample average).

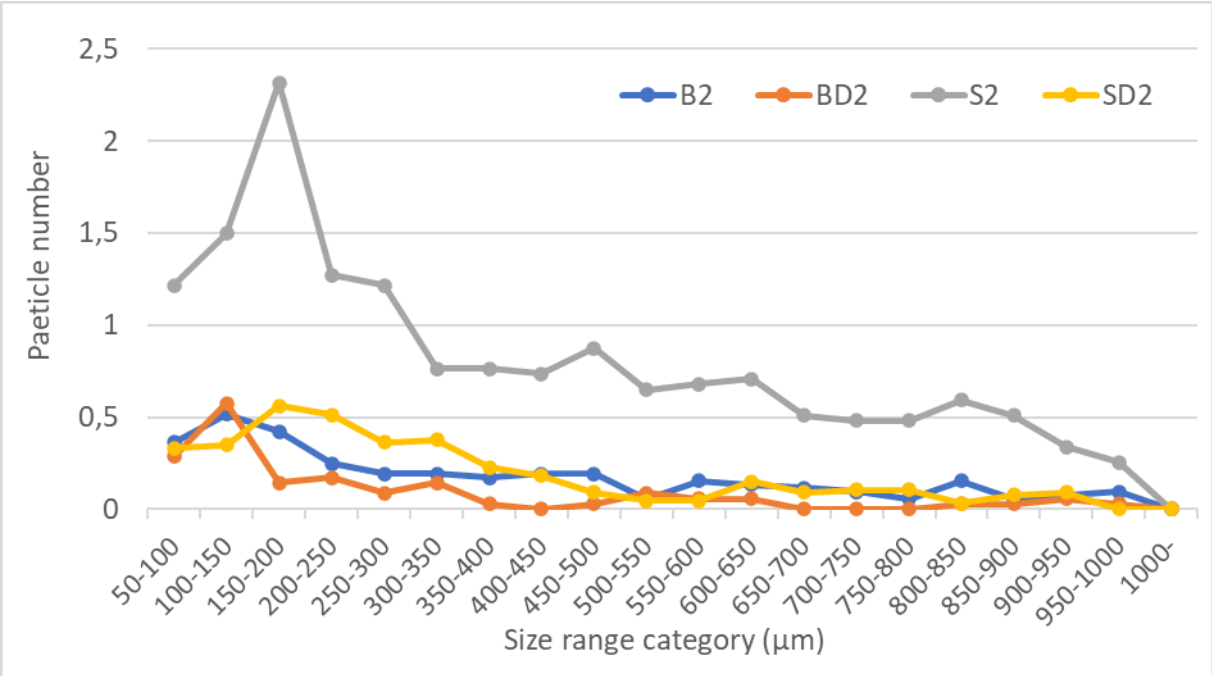

Figure 3: MP particles size distribution in the samples (per m^3).

Table 2: Estimated polymer mass in the tested samples (µg/m^3 sample).

Sample code Sample volume (L)	B2 52200	BD2 34875	S2 35400	SD2 66150
PE	336,0	73264,3	1096,3	15,9
PP	37,2	10,9	676,8	51,7
Polyester	0,0	16515,6	0,0	0,0
PA	0,0	0,0	0,0	0,0
Acrylic	0,2	0,0	20,9	2,2
PVC	0,0	0,0	0,1	0,0
Vinyl copolymer	0,0	0,0	0,0	0,0
PU	0,0	0,0	0,0	0,0
PS	3,8	35062,0	2327,1	4,9
PTFE	0,0	0,0	0,0	0,0
Cellulose acetate	0,0	0,0	0,0	0,0
Alkyd	0,1	3,3	10,4	0,3
Sum	**377,3**	**124856,2**	**4131,6**	**75,1**

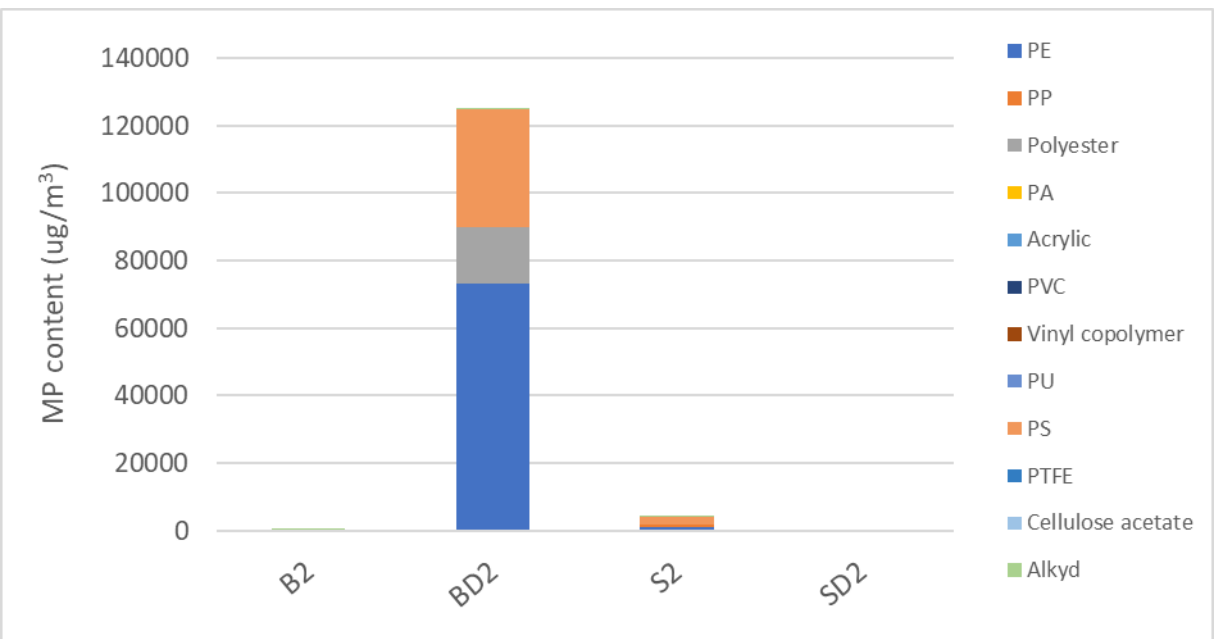

Figure 4: Estimated polymer mass in the tested samples (µg/m^3 sample).

This report was compiled by Gábor Bordós, Phd.

23. 08. 2023.

Zoltán Filep Head of Laboratory

Appendix C (Manta-Net Data Sheet)

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HYDRO-BIOS

Apparatebau GmbH

'MANTA' MICROPLASTIC NET

CATALOGUE NO: 438 217

Edition 08/20

GENERAL DESCRIPTION

Plastic debris in the environment - a problem which is drawing more and more attention. One reason for this is that plastic will never really biologically degrade and disappear from our environment but become so called microplastic. Microplastics are small particles ranging from 1µm to 5mm.

Tons of those particles get into our natural waters and end up in our oceans. Up to now it is not yet fully understood what physical and chemical impacts microplastic is having on any kind of living organism.

The establishment of a reliable, verified and standardized method to quantify the amount of microplastic particles in the environment plays a key role to assess the consequences of plastic debris in aquatic ecosystems.

- Large variety of mesh sizes
- Light weight but robust Easy to handle

SCOPE OF DELIVERY

1. metal frame with mouth opening 30 x 15 cm
2. Nylon webbing with zip fastener
3. Net Bag of synthetic material with zip fastener front opening 30 x 15 cm back opening Ø 11 cm length 200 cm
4. Fixing ring for Soft Net Bucket
5. Soft Net Bucket with side window, covered with sieve gauze
6. two lifting bodies (‘wings’)
7. Allen key 4 mm
8. Spanner 8 mm
9. 12 bolts with shims and nuts
10. one bridle with thimble and two shackles
11. one pin incl. nuts for centred mounting of the optional **Flow Meter** (438 110)
12. Spanner 5,5 mm

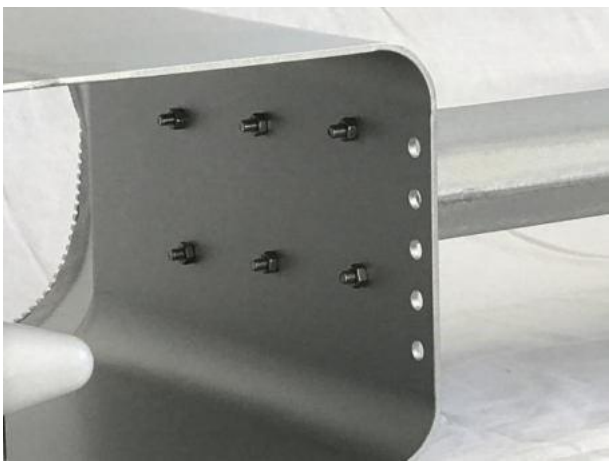
ASSEMBLY INSTRUCTION

The **MICROPLASTIC NET** is delivered pre-assembled. Thus there is no need for extensive preparations to have it ready for operation.

1. Mount the two lifting bodies (‘wings’) onto the net frame by using the provided bolts

   Make sure the rounded edge of the wings’ mounting plate is facing the front opening of the net frame.
2. Zip in the net bag
3. Fit the fixing ring for the soft net bucket to the end of the net bag
4. Mount the soft net bucket to the fixing ring
5. Fix the bridle with the shackles in one of the five holes on each side of the metal frame depending on the angle of the dragline.
6. Optionally mount the pin for the flow meter in the centre whole of the net frame bay using the provided bolt

Now the **‘MANTA’ MICROPLASTIC NET** is ready for deployment.

OPERATION

The **'MANTA' MICROPLASTIC NET** is made for operation at inland waters, coastal areas and the open sea under calm conditions.

The maximum speed is rated to 3 knots.

As it should be done with all offshore equipment the user should **check all screwing and all hose clamps regularly**.