AMERICAN UNIVERSITY OF BEIRUT

DETECTING MICRO-PLASTICS (MPS) IN BOTTLED WATER

by
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ABSTRACT
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Title: Detecting Microplastics in Bottled Water

Microplastics are anthropogenic contaminants that have been accumulating in surface water bodies for the past several decades. Little is known on the presence of microplastics in drinking water in the Middle East region in general and Lebanon in specific. In this study, microplastics occurrence in plastic bottled water was assessed. 17 samples were prepared by filtering water through Nucleopore track-etched Polycarbonate filters (Whatman, 0.2 µm pore size and a diameter of 47 mm) using a vacuum filtration setup inside a biosafety cabinet. 12 of those samples were made up of two, two-liter water bottles and 5 of those samples were blank samples that were prepared by filtering one liter of UltraPure water. Filter samples were analyzed using scanning electron microscopy (SEM). 30% of each filter sample area was analyzed to determine the number of micro-particles/L. The plastic bottles contained $23,702 \pm 3793$ micro-particle/L and the blank contained 8353 ± 633 microparticles/L. The micro-particles were divided into four size groups which are: less than 1 µm, 1-5 µm, 5-10 µm, and 10+ µm. Among all the categories, the 1-5 µm showed the highest number of particles accounting for $14,454 \pm 2703$ particle/L. A sample of the micro-particles in each sample were analyzed and using the atomic and weight percentages of each particle, a microplastic (MP) percentage estimate was calculated. Sample analysis reveals MPs in plastic bottles amount to $10,134 \pm 2,184$ MP/L and $3,656 \pm 768$ MPs/L in blank samples. Therefore, around 50% of the total particles detected are plastics. the amount of MPs/L was 86.6 times greater than the study done by (Schymanski, Goldbeck, Humpf, & Fürst, 2017) that reported 118 ± 88 MPs/L. Furthermore, when compared to the study done by (Oßmann, et al., 2018) the number of MPs/L is 3.9 times greater than the single use PET bottles which was $2,649 \pm 2857$ MPs/L and 2.1 times greater than the new reusable PET bottles which was $4,889 \pm 5432$ MPs/L and 1.2 times greater than the old reusable PET bottles which was $8,339 \pm 7043$ MPs/L. This discrepancy in the studies might be due to either different brands used in the studies and/or the difference in methodology. The experiments show that MPs are present in every water bottle sample as well as the blank samples. The abundance of the plastics that are less than 5 µm poses a concern since they can be absorbed by the human body and eventually accumulate in it. Future work includes using µ-Raman spectroscopy or µ-FTIR to identify the type of plastic present in bottled water.

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CHAPTER 1

INTRODUCTION

Microplastics (MPs) are plastics that have a diameter of less than 5 mm and are divided in to two types: primary MPs and the secondary MPs. Primary MPs result from the spread of plastic dust resulting from plastic production. While the secondary MPs are large plastic pieces that degrade into small pieces due to the UV rays from the sunlight or by being ingested by animals (Susanna Cannas, 2017) (Nirban Laskar, 2019). Ever since the 1950s the world entered an era of accelerated spread of industrialization. This resulted in increasing the demand of plastics due to their excellent mechanical properties, low cost, light weight, stability, durability and chemical inertness. The annual plastic production started with around 1.5 million tons and it kept on increasing until it reached around 350 million tons in 2017 (Chaczko, Rodriguez, Kale, & Araujo, 2018) (Noura Raddadi, 2019).

Nowadays plastics are found in most of the products around us such as clothes, cosmetics and healthcare products, transportation, communication, and food packaging (Huppertsberg & Knepper, 2018). Unfortunately, most of the plastics produced end up in water bodies due to the lack of appropriate reuse and recycle schemes in most countries (Huppertsberg & Knepper, 2018). In addition to that, plastic waste disposed in landfills are exposed to photooxidation which causes the production of small fragments that are less than 5 mm in diameter that will eventually leak and concentrate in the water bodies (Noura Raddadi, 2019).

As a result to the accelerated rise of plastic production ,careless littering, leaks from the waste management facilities, and their non-biodegradability that prevents them from decomposing in nature have resulted in the accumulation ofmore than 5 trillion plastic

pieces that weigh more than 250 thousand tons floating in seas around the globe. This accumulation of MPs has caused it to get integrated in the food chain where they have been found in several fish and bird species, sea turtles, marine mammals, salt, and beverages and some of those things are consumed by humans (Banaee, Siyavash Soltanian, & Behzad Nematdoost Haghi, 2019). This means that MPs have been circulating in the environment ever since they have been introduced in the market. Keeping in mind that MPs are neither natural nor biodegradable, their integration in nature will have a disastrous outcome.

Despite increasing research on MPs occurrence, gaps still exist regarding the presence of MPs in drinking water, especially in the Middle East region. The aims of this study are to quantify the number of MPs in representative samples of bottled water sold in the Lebanese market using the scanning electron microscopy (SEM). Then, the type of MPs will be estimated using the SEM combined with energy dispersive X- ray spectroscopy (SEM-EDX). This study aims at producing much needed data pertaining to the size, amount, and type of MPs found in the samples mentioned above.

CHAPTER 2

LITERATURE REVIEW

2.1. Toxicity of MPs on nature

Plastics in the environment degrade by mechanical action, biological activity, and most importantly due to UV exposure. UV radiation is responsible to the fast breakdown of large plastic particles into MPs through photolytic, photo-oxidative, and thermo-oxidative reactions. Furthermore, mechanical action by wave forces and collision of the plastics cause additional fragmentation and breakdown. These two processes are augmented by biological activity where some fungi and microbes produce certain enzymes that can degrade and breakdown plastics (Joana C. Prata, 2020). The longer the plastic stays in the environment, the smaller it gets. This reduction in size increases the bioavailability of plastics in aquatic organisms especially for filter feeder animals (Banaee, Siyavash Soltanian, & Behzad Nematdoost Haghi, 2019).

MPs have been found on the shore, sea surface, municipal and industrial effluents, polar ice, and the seabed from the coast to the open ocean (Banaee, Siyavash Soltanian, & Behzad Nematdoost Haghi, 2019). A study conducted by Li Et al showed that the adsorption of polystyrene (PS) on large suspended sediments in water bodies results in the formation of heteroaggregates which can quickly drag the PS particles down the water column. In addition to that, the smaller the PS particle the faster it would adsorb on the suspended sediments and the faster it would settle at the bottom.

The presence of MPs can can result in a high environmental impact due to their persistency, ubiquity, and their ability to concentrate hydrophobic chemicals from seawater and transferring them to organisms by ingestion (Suman, D, & S.R., 2018). MP debris imitates a vector of chemical contaminants in the marine environment. The

contaminated MPs get carried long distances and can even cross between waterbodies and nearby seas (Suman, D, & S.R., 2018). Plasticizers such as Mono-(2- ethylhexyl) phthalate (MEHP), and di-(2-ethylhexyl) phthalate (DEHP) easily leach out of the plastic and cause an environmental pollution. MEHP and DEHP are active metabolites and they promote the production of reactive oxygen species, modify detoxification enzymes, and act as endocrine disruptors. The plasticizer bisphenol A (BPA) has estrogenic properties acting as an endocrine disrupting chemical but also has the ability to cause oxidative stress and mitochondrial dysfunction; therefore, causing a change in gene expression in the animals that get exposed to it (Banaee, Siyavash Soltanian, & Behzad Nematdoost Haghi, 2019). Chemicals that have estrogenic properties can also cause a dysfunction in reproduction which would decrease the population of the animals that consume it.

MPs and nanoplastics (NPs) can interfere with the surface charge of the water and thus affecting the organisms that live there. A study done by (Juan Saavedra, 2019) on two types of polystyrene (PS) that have a diameter of 200 nm which are positively charged (zeta potential +50 mV) Amidine PS and negatively charged (zeta potential -50 mV) carboxyl PS. This study showed that both types were ingested by zooplanktons and accumulated in their guts and both were toxic. However, the positively charged Amidine PS were much more toxic to the zooplanktons than the negatively charged carboxyl PS. Hence, the MPs and NPs have the potential to affect the zooplanktons that live on the surface which are also food to a lot of fish.

(Marc Long, 2013) conducted experiments to see the effects of PS MPs on phytoplankton. The study used 2 μm PS particles on dinoflagellate, heterocapsa triquetra and a diatom Chaetoceros neogracile. The algal growth, fluorescence, and the

MP distribution were determined using cytometry. They concluded that PS MPs did not influence algal growth and fluorescence.

(E. Bergamia, 2017) did a study on the effect of surface charged PS NPs on the following planktonic species: Dunaliella tertiolecta and Artemia franciscana. Negatively charged MPs aggregates did not affect the growth of micro-algae up to 50 µg/ml and brine shrimps 10 µg/ml. The negative charge was detected on micro-algae and in brine shrimps. The positively charged PS MPs the EC_{50} value at 12.97 µg/ml and mortality in brine shrimps on the fourteenth day LC_{50} was 0.83 µg/ml. In addition to that, the positively charged PS MPs were able to trigger the apoptotic pathway by cathepsin L like protease in brine shrimps.

(Prabhu Kolandhasamy, 2018) studied the adherence of MPs using mussels. The study showed that several organs of the mussel had MPs and the intestines contained the highest amount. The authors stated that adherence is the novel way for MP uptake after ingestion.

(Chiara Gambardella, 2017) studied the effects of PS MPs on A. amphitrite nauplii and A. franciscana InstarI larvae. The authors studied the MP build up, mortality, swimming speed, and enzyme activity. The results showed that the PS MPs only affected the swimming speed and the enzyme activity and the MPs were found in the guts of both crustaceans.

(C. Alomar, 2017) examined mullus suruletus for MP ingestion. MPs were found in the liver of the fish. There were no verifications that MPs cause oxidative stresse in the fish but there was an increase in glutathione stransferase.

(Carlo Giacomo Avio, 2015) found that both virgin and contaminated MPs cause cellullar responces in mussels such as immune responces, peroxisomal proliferation,

neutrotoxic effects, and a change in the gene expression profile which was exhibited through a new DNA microarray platform.

In conclusion, plasticizers such as MEHP and DEHP easily leach out of the plastic and cause environmental pollution. In addition to that, they are active metabolites and they promote the production of reactive oxygen species, they modify detoxification enzymes, and they act as endocrine desruptors. The plasticizer BPA has estrogenic properties, acts as an endocrine desruptor, and can cause oxidative stress and mitochonrial dysfunction. Chemical that have estrogenic properties can also cause a dysfunction in reporoduction in the animal species that consume and which would lead in a decrease in its population.

2.2. Toxicity of MPs on humans

MPs can be transferred to humans by several paths such as: seafood consumption, MP contaminated sea-salt, and MP contaminated beverages. The smaller the MP the easier it would penetrate and translocate inside the human organs (Dinuka Kankanige, 2020). MPs that have a diameter less than 130 µm can translocate inside human tissues, trigger a localized immune response, and release constituent monomers, toxic chemicals added during plastic production, and pollutants adsorbed from the environment such as heavy metals and persistent organic pollutants (Kieran D. Cox, 2019). PE, PET, and PS have cytotoxic effects at the cellular level in the human gastrointestinal tract. MPs can translocate to the lymphatic and circulatory systems and eventually aggregate in secondary organs such as liver, spleen, and kidneys (Dinuka Kankanige, 2020). PET in the size range of 0.5-20 µm have been found in the cytoplasm of the histiocytes in a joint capsule and PET in the size range of 20-100 µm have been found in the tissue of a joint capsule (Stephanie L. Wright, 2017).

A study done by (Kieran D. Cox, 2019) used all the studies done in literature regarding the presence of MPs to try and approximate the amount of MPs consumed by humans and the following results were obtained: Male children consume 41,106±7,124 MPs/year and inhale 40,225±4,730 MPs/year, male adults consume 51,814±8,171 MPs/year and inhale 61,928±68865 MPs/year, female children consume 38,722±6,977 MPs/year and inhale 35,338±39296 MPs/year, and female adults consume 46,013±7,755 MPs/year and inhale 48,270±53676 MPs/year. On the other hand, the study done by (Jangsun Hwang, 2019) assessed the toxicity of polypropylene (PP) in human cells. First a hemolysis test showed that PP may not induce hemolysis in the body. Then a cytokine profiling and macrophage polarizing test showed that PP particles larger than 25 µm weren't identified as pathogens and did not trigger cell mediated immune response while PP particles less than 25 µm might be considered as pathogens by the human immune system. The histamine profiling test showed that the cells that were introduced with PP particles (500 µg/ml) showed an increase levels of histamine compared with the control and this indicates that PP particles trigger acute inflammation and possibly induce tissue damage with the help of histamines. Finally, the ROS profiling test demonstrated that PP particles with a size of 25 µm and a concentration of 1000 µg/ml increased ROS levels by approximately 30% while larger PP particles did not induce any change.

The study done by (Dennis Brennecke, 2016) showed that in a 14-day experiment copper and zinc leached from an antifouling paint to virgin PS beads and PVC fragments in sea water. Another study done by (Hisashi Hirai, 2011) gathered plastic pieces from open ocean and beaches from central pacific gyre, Pacific Ocean, and Marbella beach. After those pieces were studied it turned out that they have polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, dichloro-

diphenyl-trichloroethane and its metabolites, polybrominated diphenyl ethers, alkylphenols and bisphenol A were detected in the fragments at concentrations from 1 to 10,000 ng/g.

In a study that was conducted by (Etsuko Nakashima, 2012) plastic particles were gathered from Ookushi beach in Japan. The tests revealed that those plastic particles have chromium, cadmium, tin, antimony, and lead. A similar study was done by (Turner, 2016) in SW England showed that the plastic particles collected encompassed a concentration of 17,500 µg/(g of plastics) of lead and a concentration that exceeded 1000 µg/(g of plastics) of cadmium.

All those materials found on the plastics in the above-mentioned studies are all toxic not just to humans but to any organism that would consume them.

Plastics are unnatural chemicals that have been integrated in the food chain. MPs less than 130 µm can trigger a localized immune response in the human body. In addition to that, smaller particles have the ability to penetrate the human tissues and accumulate inside secondary organs such as liver, spleen, and kidneys. The smaller the plastic piece is, the more danger it imposes such as raising the histamine levels and release constituent monomers, toxic chemicals added during plastic production, and pollutants adsorbed from the environment such as heavy metals and persistent organic pollutants.

## 2.3. The quality and quantity of MPs in water

After exploring the effects of MPs on humans and on nature, it is important to see the quantity and quality of MPs that humans consume on daily basis due to their presence in drinking water.

A study conducted on single use and reusable PET bottles and glass bottles in Germany using a µ-Raman spectroscope (Oßmann, et al., 2018) shows that for single

use PET all of the MPs were less than 10 µm and for reusable PET only 1.7% of MPs were larger than 10 µm and for glass only 6.9% of the MPs were larger than 10 µm. In a study done on raw and treated water in the Czech Republic using a µ-Raman spectroscope by (Pivokonsky, et al., 2018) shows that MPs exceeding the 10 µm in size were slightly less than 10% whereas, 40-60% of the MPs had a size between 1-5 µm. Another study also done in Germany using a µ-Raman spectroscope by (Schymanski, Goldbeck, Humpf, & Fürst, 2017) on bottled water shows that 45% of the MPs fell in a size range of 5-10 µm and 32% had a size between 10-20 µm. A study done by (S.M., M.G.J., S., & G., 2018) analyze drinking water coming from ground water sources using FTIR and in this study no MPs were detected in the ground water but it is unclear if there are no MPs or if the MPs present are less than 10-20 µm. Another study which was done by (Sherri A. Mason V. G., 2018) on bottled water using NR-FTIR, focused on the type and morphology of the MPs, however; this technique was only able to detect MPs of 100 µm and larger. On one hand, this paper showed that the MPs detected were 54% polypropylene, 16% Nylon, 11% polystyrene, 10% polyethylene, 6% polyester, and 3% others. On the other hand, the morphologies of the MPs were 65% fragments, 14% film, 13% fiber, 5% foams, and 3% pellets. A study done by (Maria Kazour, 2019) in the Mediterranean Sea along the Lebanese coastal waters shows that the MPs found consist of 77.5% fragments, 15.25% fibers, and 7.25% microbeads. Where in Tripoli 88% of the MPs are less than 1000 µm and in Beirut 69.3% of MPs are less than 1000 µm and in Sidon 87% of MPs are less than 1000 µm. As for MPs concentration, in the water samples 6.7 MPs/m^3 and in sediments 4.68 MPs/g. A study done by (Hal, Ariel, & Angel, 2016) in Israel on the coastal water using visual identification showed that there were 2.21-20.87 ±32.56 MPs/m^3. **Error! Reference source not found.** s ummarizes the mentioned researches.

On the other hand, the study done by (Zhong-MinWang, 2017) was done on ocean trawl and fish guts. The MPs in this study were detected using SEM/EDS. Fish samples were collected and gutted and their guts were washed and filtered. Then using the SEM/EDS the MPs were observed and then their elements were analyzed. As can be seen by **Error! Reference source not found.**a and c the high carbon peak obtained f rom the EDS indicates the presence of PP and PE. Whereas the high calcium peak in **Error! Reference source not found.** b indicates that the particle scanned is fish bone s ince bones are very high in calcium. Therefore, in this study they determined MPs by their bright appearance under the SEM and they were differentiated using the EDS.

a)
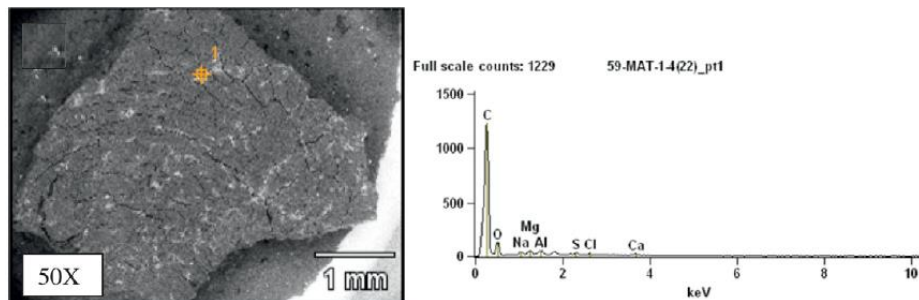
b)
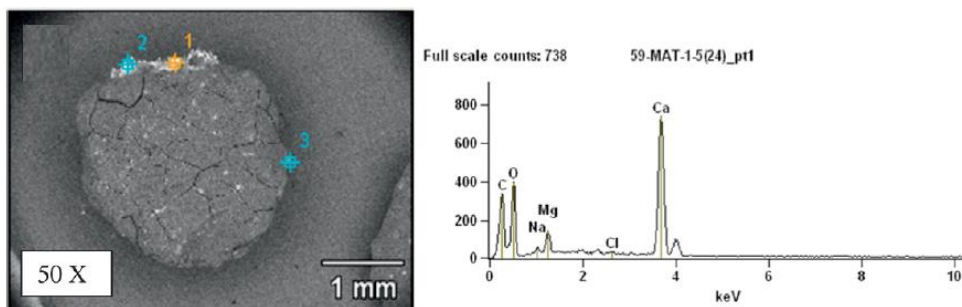
c)
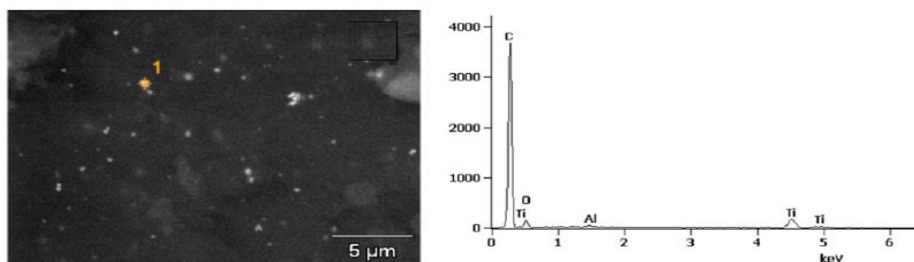
Figure 1: a) plastic fragment in a fish gut, b) fish bone in a fish gut, c) plastic particles in fish gut (Zhong-MinWang, 2017)

Table 1: Summary of Previous Studies

	Country	Type of Water Tested	Method Used	Quantity of MPs (MPs/L)	Size of MPs (µm)	Type of MPs
(Pivokonsky, et al., 2018)	Czech Republic	Raw and treated drinking water coming from 3 rivers	FTIR (for MPs larger than 10 µm) and µ-Raman (for MPs less than 10 µm)	River1: 1473+-34 River2: 1812+-35 River3: 3605+-497 WTP1: 443+-10 WTP2: 338+-76 WTP3: 628+-28	40-60% between 1-5 µm 30-40% between 5-10 µm 10% larger than 10 µm	PET: 60, 68, and 27% from WWTP 1, 2, and 3 respectively PP: 12-26% PE: 24% only in WWTP 3
(Schymanski, Goldbeck, Humpf, & Fürst, 2017)	Germany	Several types of bottled water: Single-use, reusable, glass, and beverage cartons	µ-raman spectroscopy	Single use bottles: 14+-14 Reusable bottles: 118+-88 Glass bottles: 50+-52 Beverage cartons: 11+-8	Single use bottles: 41% between 5-10 µm 30% between 10-20 µm 22% between 20-50 µm 7% larger than 50 µm Reusable bottles: 56% between 5-10 µm	Single use bottles: 59% PEST 9% PE 1% PP 1% PA Reusable bottles: 84% PEST 7% PP 5% PE 2% PA Glass bottles: 41% PEST 35% PE 8% PP 12% PA Beverage cartons:

					29% between 10-20 µm	38% PE
						32% PEST
						26% PP
					12% between 20-50 µm	
					3% larger than 50 µm	
					Glass bottles:	
					45% between 5-10 µm	
					32% between 10-20 µm	
					14% between 20-50 µm	
					10% larger than 50 µm	
					Beverage cartons:	
					39% between 5-10 µm	
					28% between 10-20 µm	
					16% between 20-50 µm	
					17% larger than 50 µm	
(Oßmann, et al., 2018)	Germany	Several types of bottled water:	µ-raman spectroscopy	Single use bottles: 2649 +- 2857	Single use bottles:	Single use bottles:

		Single-use PET, reusable PET, and glass		Reusable bottles: 4889+-5432 Glass bottles: 3074+-2531	53.6% less than 1.5 µm 44.7% between 1.5-5 µm 1.7% between 5-10 µm Reusable bottles: 47% less than 1.5 µm 48.4% between 1.5-5 µm 2.9% between 5-10 µm 1.7% larger than 10 µm Glass bottles: 16.4% less than 1.5 µm 61.4% between 1.5-5 µm 15.4% between 5-10 µm 6.9% larger than 10 µm	78% PET 11% PET+Olefin 0.7% PE 10% PP 1% others Reusable bottles: 74% PET 7.7% PET+Olefin 5.4% PE 10% PP 2.6% others Glass bottles: 3.6% PET 46% PE 23% PP 14% Styrene-Butadiene-Copolymer 13% others
(S.M., M.G.J., S., & G., 2018)	Germany	Ground water and drinking water coming	FTIR	Ground water: 0.7 MPs/m³ Drinking water: 4.3 MPs/L	The sizes ranged between 50 and 150 µm	The plastics detected are PEST, PVC,

		from ground water				PE, PA, and epoxy resins
(Hal, Ariel, & Angel, 2016)	Israel	Coastal Water	Visual Identification	2.21-20.87 +-32.56 MPs/m^3	0.3 mm to 2.5 cm	Not specified
(Serranti, Palmieri, Bonifazi, & Cózar, 2018)	Italy	Ocean	Hyperspectral Imaging	Not specified	2.3% between 0.5-1 mm 18.87% between 1-2 mm 23.77% between 2-3 mm 17.29% between 3-4 mm 12.1% between 4-5 mm 9.36% between 5-6 mm 4.76% between 6-7 mm 4.18% between 7-8 mm 2.59% between 8-9 mm 1.3% between 9-10 mm	PP: 23.6% PE: 70.6% PS: 5.8%

					1.44% between 10-11mm	
					1.3% between 11-12 mm	
					0.58% between 12-13mm	
					0% between 13-14 mm	
					0.14% between 14-15mm	
(Maria Kazour, 2019)	Lebanon	Eastern Mediterranean	stereomicroscope Micro-Raman	6.7 MPs/m³ in water samples 4.68 MPs/g in sediments	Tripoli: 48.78% less than 200 µm 88% less than 1000 µm Beirut: 69.3% less than 1000 µm 31.7% greater than 1000 µm Sidon: 51.8% less than 200 µm 87% less than 1000 µm	PE PP PS PA PET Polyurethane (PUR) acrylonitrile butadiene styrene (ABS) PVC

2.4. Techniques to measure MPs

2.4.1. Optical techniques

Optical techniques were used initially due to their simple operation. Optical identification enables the user to see the plastics with the aid of a visual microscope (Silva, et al., 2018). Characteristics such as color, shape, and surface texture may contribute in distinguishing MPs from other particles in the sample (Silva, et al., 2018). The selection criteria of optical identification are the following: the suspected particles or fibers should have no visible organic or cellular structures, the fibers should have a consistent color and thickness along its length, the particles are clear and uniformly colored, and transparent and white particles should be further confirmed under a high magnification microscope. However, this technique always has a potential for a bias when identifying MPs. The quality of identification results depends on many factors such as the subjectivity of the examiner, the sample matrix, the particle shape and size, and the microscope used for inspection. In addition to that, as the particle size decreases the possibility of misidentification increases (Wang & Wang, 2018).

2.4.2. Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) can provide high resolution images of a sample by firing a high intensity electron beam at the sample surface. SEM can produce extremely clear and high magnification images of plastic particles and thus allowing the discrimination between MPs and other particles (Silva, et al., 2018). This method can show the surface details of the sample at high magnification which can reach around 0.5 nm. SEM can also analyze the weathering of MPs by examining the featured surface textures. SEM can be combined with energy-dispersive X-ray spectroscopy (SEM-EDS) to provide detailed information

regarding the elemental composition of MPs and the inorganic additives that they contain. However, this technique requires extensive sample pre-preparation which makes it inapplicable for handling large number of samples (Wang & Wang, 2018).

2.4.3. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared (FTIR) can provide a unique infrared spectrum for a specific chemical bond. This technique allows the identification of an unknown substance by comparing its spectrum with spectra of known materials. FTIR is mainly used in two ways which are scanning all suspected particles or analyzing a set of subsamples to validate the visual identification results. In addition to accurately identifying the polymer types of MPs, FTIR can provide information about physiochemical weathering of MPs by analyzing their oxidation intensity. On the downside, FTIR can only identify particles that are larger than 10-20 µm and may lose applicability in cases where the target particles are smaller than its aperture size. In addition to that, FTIR requires a significant time and experienced operators for analysis (Wang & Wang, 2018).

2.4.4. µ-Raman spcetroscopy

µ-Raman identifies MPs by irradiating monochromatic laser beam onto a sample which results in a different frequency of the backscattered light due to absorption, scatter, or reflection by the specific molecular structure and atomic composition of the sample. µ-Raman can produce a unique spectrum for each polymer in a non-destructive way which would be helpful in cases where the sample will be needed for later analyses. This technique can identify particles down to 1 µm in size which is extremely challenging for other techniques to achieve. However, this method produces low signal to noise ratio which makes the spectrum analysis more difficult.

2.4.5. Pyrolysis-gas chromatography-mass (Pyr-GC-MS) spectrometry

Pyrolysis-gas chromatography-mass (Pyr-GC-MS) spectrometry is a destructive technique that can identify MPs by analyzing their thermal degradation products. The polymer types of MPs can be identified by comparing their pyrograms by reference pyrograms generated by known pure polymers. This technique requires minimum sample pre-treatment. Pyr-GC-MS can simultaneously provide detailed information regarding the chemical composition and the additives contained by the polymer. In addition to that, Pyr-GC-MS is not sensitive to the shape, size, and the organic and inorganic contaminants of the analyzed sample. Only a small amount of 100-500 µg from the sample is needed for one measurement. However, this technique requires only one particle to be analyzed per cycle which requires a duration of 30 to 100 minutes and this makes it very time consuming for the analysis of large samples (Wang & Wang, 2018).

2.5. Comparison between identification techniques

A comparison between some of the common methods mentioned above would allow the selection of the most efficient one.

2.5.1. FTIR vs Raman spectroscope

FTIR and µ-Raman spectroscopy are the two most commonly used techniques for the characterization of MPs (Silva, et al., Microplastics in the environment: Challenges in analytical chemistry - A review , 2018). They enable non-destructive, possibility for high throughput screening, high reliability, low sample amount requirement, and environmental friendliness. Nevertheless, µ-Raman is

advantageous over FTIR since it has a higher spatial resolution, wider spectral range, narrower spectral bonds, and lower sensitivity to water interference (Wang & Wang, Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis, 2018). One of the most important things that favors µ-Raman over FTIR is the limit of detection where µ-Raman can detect MPs down to the 1 µm size whereas FTIR can only detect MPs larger than 10-20 µm. According to a study done on single use and reusable PET bottles and glass bottles by (Oßmann, et al., 2018) it was found that for single use PET all of the MPs were less than 10 µm and for reusable PET only 1.7% of MPs were larger than 10 µm and for glass only 6.9% of the MPs were larger than 10 µm, and another study done by (Pivokonsky, et al., Occurrence of microplastics in raw and treated drinking water, 2018) shows that MPs exceeding the 10 µm in size were slightly less than 10% whereas, 40-60% of the MPs had a size between 1-5 µm. Another study by (Schymanski, Goldbeck, Humpf, & Fürst, 2017) on bottled water shows that 45% of the MPs fell in a size range of 5-10 µm and 32% had a size between 10-20 µm. A study done by (S.M., M.G.J., S., & G., 2018) analyze drinking water coming from ground water sources using FTIR and in this study no MPs were detected in the ground water but it is unclear if there are no MPs or if the MPs present are less than 10-20 µm. Therefore; those studies show that it is very important to use a method that allows the identification of MPs that have a size less than 10 µm and or the problem of MPs in drinking water will be underestimated thus µ-Raman is favored over FTIR.

2.5.2. μ-Raman vs visual identification

Visual identification is suitable to identify MPs down to 500 µm (Renner, Schmidt, & Schram, 2017) which is much higher than that of µ-Raman which is 1 µm. A study that was done by (Lenz, Enders, Stedmon, Mackenzie, & Nielsen, 2015) compares visual identification with µ-Raman. In this study a total of 452 fibers and 827 particles where visually identified as plastic of which 75% and 64% were confirmed by the Raman spectra respectively. 63% of particles that had a size less than 50 µm were confirmed as plastic whereas in the group of 50-100 µm 67% of the particles were confirmed as plastics. For particles larger than 100 µm 83% were confirmed. Thus, as the particle size increased the successful visual identification increased with it. Therefore, as can be seen by this study using visual identification methods would overestimate the problem.

2.5.3. μ-Raman vs thermal decomposition

A study done by (Dümichen, et al., 2017) required 1500 L of river water to be filtered in order to get a sufficient weight to use in the thermal decomposition method. This wouldn't be infeasible for bottled water since it will require 3000 bottles of volume 0.5 L and 1500 bottles of volume 1 L. However, for µ-Raman spectroscopy a single bottle of water would be sufficient to yield results.

2.5.4. The result of the comparison

The comparison between the analytical methods shows that the µ-Raman is the most suitable to obtain the best results in the easiest way possible.

CHAPTER 3

RESEARCH QUESTION

The objective of this project is to determine the occurrence, type, and size of MPs in bottled water in Lebanon.

Various samples were collected from various plastic. The samples were filtered through 0.2 µm pore sized filters using a vacuum filtration setup. The filters were placed in glass Petri dishes to be transferred for analysis.

Samples were analyzed using Scanning Electron Microscopy (SEM) for quantitative analysis to determine the number and size of the plastics.

Energy-dispersive X-ray spectroscopy (EDX) was used with the SEM to approximate the percentage of plastic of the total MPs observed.

CHAPTER 4

MATERIALS AND METHODS

4.1. Materials

The morphology of the plastics was studied using a Scanning Electron Microscope (SEM) (Oxford instruments, XMax), model number? EDX details?

Filtration will be done using a 47 mm setup that has the following characteristics: a 250 ml graduated glass funnel, an aluminum clamp, a 47 mm fritted glass support base, and a silicone stopper. Each setup was connected to a pump that will create a vacuum in the fritted glass support base.

Nuclepore track-etched Polycarbonate filters (Whatman, 0.2 µm pore size and a 47 mm) will be used.

4.2. Types of water

Different brands of mineral plastic bottled water were purchased from supermarkets. 12 samples that consisted of two two-liter water bottles and 5 blank samples that consisted of 1 liter of UltraPure water were prepared. The bottled water samples were compared with samples that have been prepared by filtering UltraPure water.

4.3. Airborne and waterborne contamination

A complex cleaning process was used to assure that the filtration setups and the glass vessels didn't contribute to particle input. Even the smallest irregularities in the glass vessels could have been a source of particle accumulation. Therefore, all vessels were checked for any cracks or scratches.

In the first step, glass funnels and vessels were intensively cleaned with UltraPure water and detergent. Then they were rinsed five times with UltraPure water. The vessels were left to air dry inside the laminar flow hood to prevent airborne contamination. This guaranteed that the glassware didn't contribute to any particle input.

Before filtration, the outside of the water bottles was washed with UltraPure water and detergent to reduce the risk of contamination from the outside. Then the bottle was placed inside the biosafety cabinet and allowed to air dry before filtration.

4.4. Filtration

The entire water volume was filtered by turning the bottle upside down and pouring through the vacuum filtration setup. After filtration is complete, the filters were carefully removed to avoid any scratches or damage to it and then they were stored in a glass petri dish which was dried in a vacuum furnace at a temperature of 60 degrees Celsius for 5-10 minutes to prevent any noise or disturbance from water in the analyses. Then the petri dish was closed and stored until the filter was analyzed.

4.5. Counting and identification of MPs with SEM

4.5.1. Quantitative analyses

30% of each filter was used by sticking it on four aluminum slabs using carbon double tape. Since plastics are non-conductive, the filters were placed in a sputtering machine that applied 15 nm of platinum on the sample surface to enable the SEM to visualize the morphology of the particles.

4.5.2. Qualitative analyses

The SEM-EDX shows the weight% and atomic% of the elements in each particle scanned. Similar to the work done by (Zhong-MinWang, 2017) those percentages were

compared to plastics such as polyethylene terephthalate (PET), polyether sulfone (PES), and Polyamides (PA). However, polypropylene (PE) and polypropylene (PP) can't be distinguishing using the SEM-EDX since they both give 100% carbon for the weight% and atomic%.

The weight% and atomic% of each particle scanned were compared to the plastics mentioned above and if they were similar, then the particle would be considered as a plastic. If not, then they would be considered as non-plastic. Then after scanning several particles, the percentage of plastics was calculated and generalized over the entire sample.

CHAPTER 5

EXPERIMENTAL WORK

5.1. Raman spectroscopy qualitative analyses

The limit of detection of the Raman machine was tested. 6 different samples of decreasing PE concentrations were prepared: 19.4 mg/L, 11.3 mg/L, 7.4 mg/L, 4 mg/L, 2.6 mg/L, and a 6L of plastic bottled water using 4 1.5 L bottles. The PE powder which was on the filter were easily observed with the naked eye; however, the 6L filter had no visible particles.

Each sample was placed under the Raman microscope and observed under x10 magnification. Then after some PE particles are observed, the microscope will be switched to x40 magnification to do proper focusing on the chosen area. After that the Raman laser was fired and the spectra was observed on the computer screen.
The Raman was able to detect the PE from the samples with 19.4 to 4 mg/L. However, when the 2.6 mg/L sample was tested only noise was obtained since the particles weren't close enough to be detected.

when the 6-liter sample was placed under the Raman microscope, no particles were observed neither under the x10 nor x40 magnification. Regardless how much the filter was moved, when the laser was fired only random noise were obtained. Therefore, the Raman limit of detection is much higher than the concentration and size of the actual water sample.

5.2. ATR spectroscopy qualitative analyses

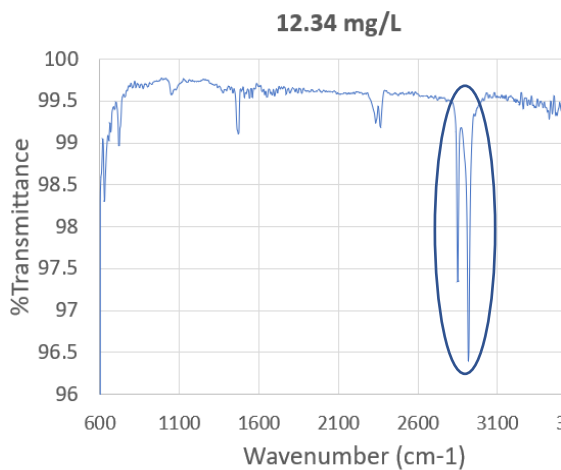*5.2.1. First test for the limit of detection*

The limit of detection of the ATR machine was tested. 7 different samples of decreasing PE concentrations were prepared: 12.34 mg/L, 8.5 mg/L, 2.6 mg/L, 1.3

mg/L, 0.6 mg/L, 0.3 mg/L, and 0.13 mg/L. The PE powder was visible in all filter samples.

Each sample was flipped over and placed above the ATR laser source. Then the laser was fired and the spectra was observed on the computer screen.

As can be seen by Figure 2a when the sample was at a high concentration, the ATR gave a proper and clear spectrum which is identical as that of PE which is signified by a strong peek at around 3000 wavenumber cm^{-1} which has been circled in each spectra. However, as the concentration kept on decreasing, more noise started to appear and the peek started to get distorted. When the concentration went from 0.6 mg/L to 0.3 mg/L, the PE signal became very weak and the finger print region was lost, and the spectrum obtained was that of an empty filter as can be seen by Figure 2d and the zoomed spectra in Figure 3. Therefore, the experiments showed that the limit of detection of the ATR is between 0.6 mg/L of PE or less which is higher than the concentrations of MPs and have a size in the μm scale and isn't visible by the naked eye.

a)   b)
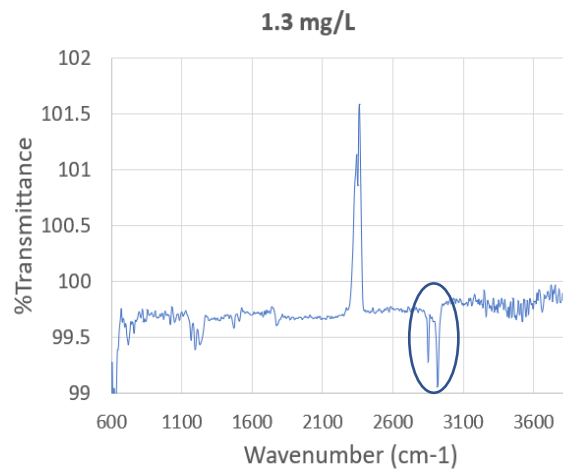
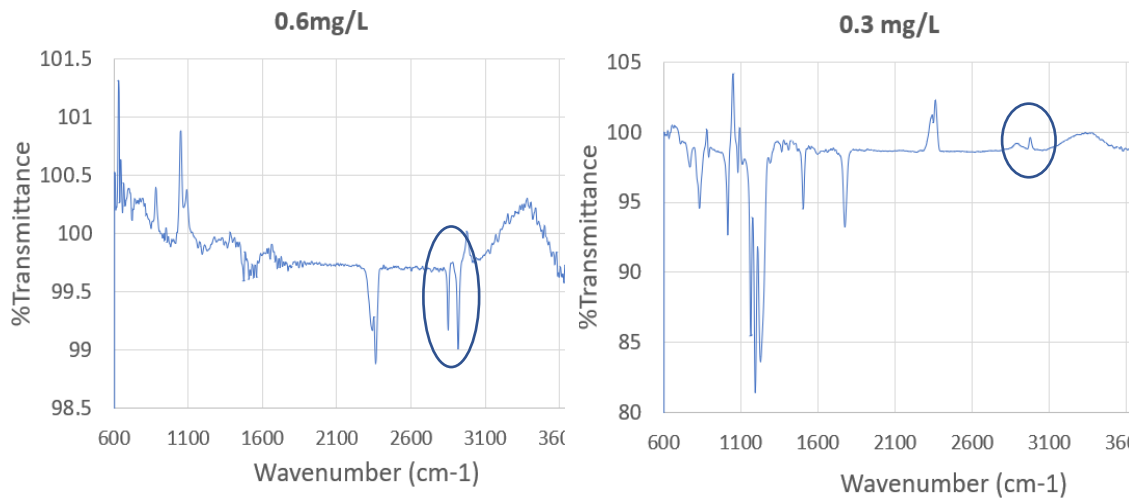c)   d)

Figure 2: IR spectra for a) 12.34 mg/L, b) 1.3 mg/L), c) 0.6 mg/L, d) 0.3 mg/L
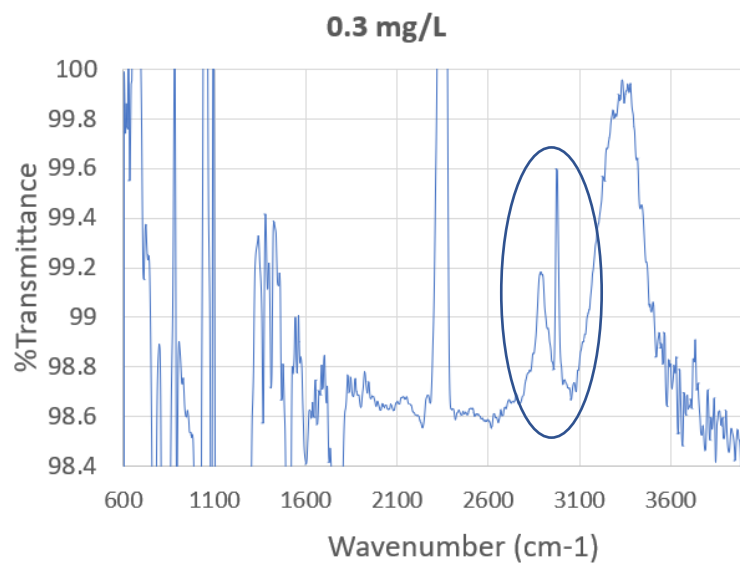
Figure 3:0.3mg/L zoomed at around 3000 cm-1

5.2.2. Second test for the limit of detection

To attempt to verify the limit of detection, a second test was done. In this test 5 different samples were prepared: 1.54 mg/L, 0.64 mg/L, 0.5 mg/L, 0.3 mg/L, and a 6 liter of plastic bottled water using 4 1.5-liter bottles. The PE powder which was on the filter were easily observed with the naked eye; however, the 6L filter had no visible particles.

As can be seen by Figure 4 as the concentration decreased the PE spectrum got weaker and the filter spectrum was becoming more dominant. In all four spectra in Figure 4 the PE fingerprint region can't be observed and only the filter finger print region was

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observed. However, in the 6L water sample only the filter spectrum was obtained, and no other peeks can be observed.

When the particles can be observed by the naked eye, then the filter can be placed in a way that puts the visible particles directly above the laser source and this guarantees at least a minimal signal. On the other hand, when the particles can't be observed like the 6-liter sample, then the particles will be too small to detect and it would be impossible to tell whether there are particles above the laser source or no. Therefore, the ATR limit of detection is much higher than the concentration and size of the actual water sample.

a)

b)

c)

d)

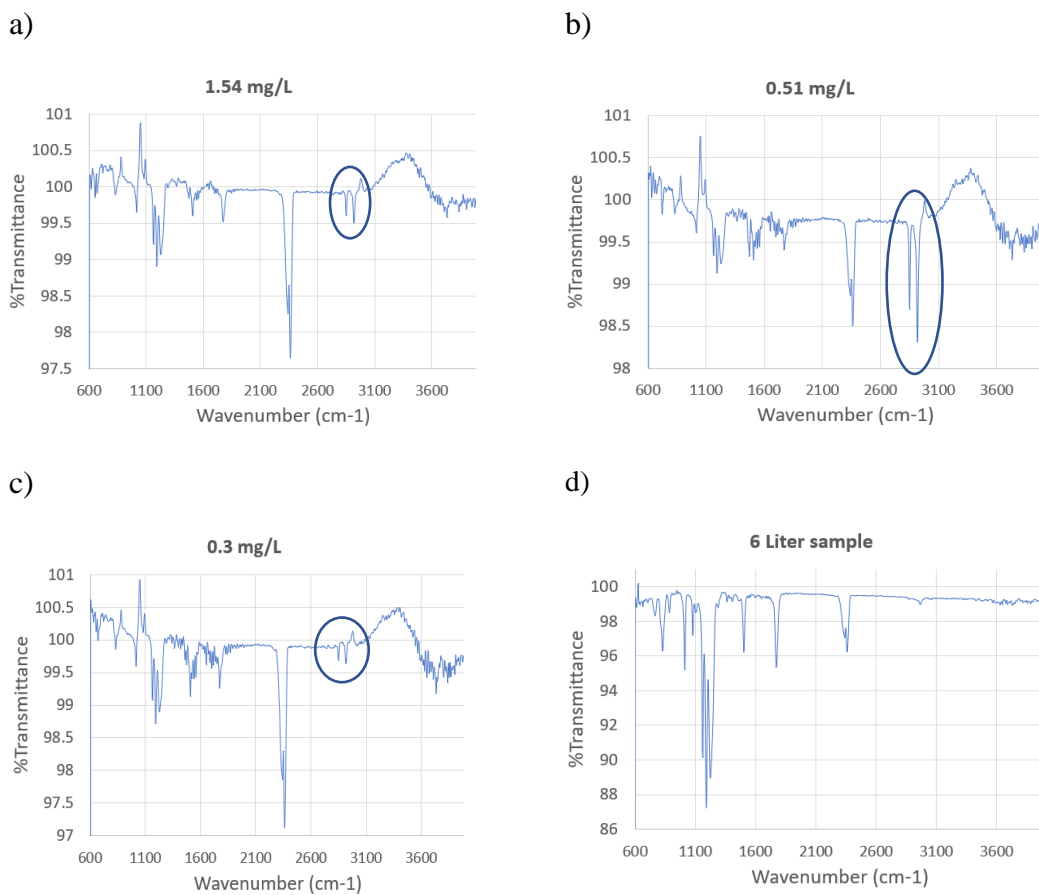Figure 4: IR spectra for a) 1.54 mg/L, b) 0.51 mg/L, c) 0.3 mg/L, d) 6 Liter sample

CHAPTER 6

RESULTS AND DISCUSSION

6.1. Microplastic abundance

All plastic water bottles samples contained micro-particles with a quantity that varied from 14,835 particle/L to 26,673 particle/L with an average of 20,348 particles/L. The difference between the number of particles/L from bottle to bottle can be due to several reasons such as: the source of the water, bottling process, quality of the plastic bottle, and the way of storage. On the other hand, the blank sample had 8,353±633 particle/L. The high number of particles found in the blank samples can be due to contamination during sample preparation or during sample analyses or the filter used in the UltraPure water machine was slightly worn out (S.M., M.G.J., S., & G., 2018).

6.2. Size range

6.2.1. Plastic bottles

The micro-particles were divided into four size groups which are: less than 1 µm, 1-5 µm, 5-10 µm, and 10+ µm. As can be seen by **Error! Reference source not found.** among all the categories, the 1-5 µm showed the highest number of particles accounting for 14,454±2,703 particle/L. Directly below it was the less than 1 µm category which has 3,817±1,329. In addition to that, the bulk of micro-particles are 5 µm or less which is similar to the results obtained by (Oßmann, et al., 2018) and (Schymanski, Goldbeck, Humpf, & Fürst, 2017) and this means that most of the particles present in the bottle have the potential to get absorbed and accumulate in the human body.
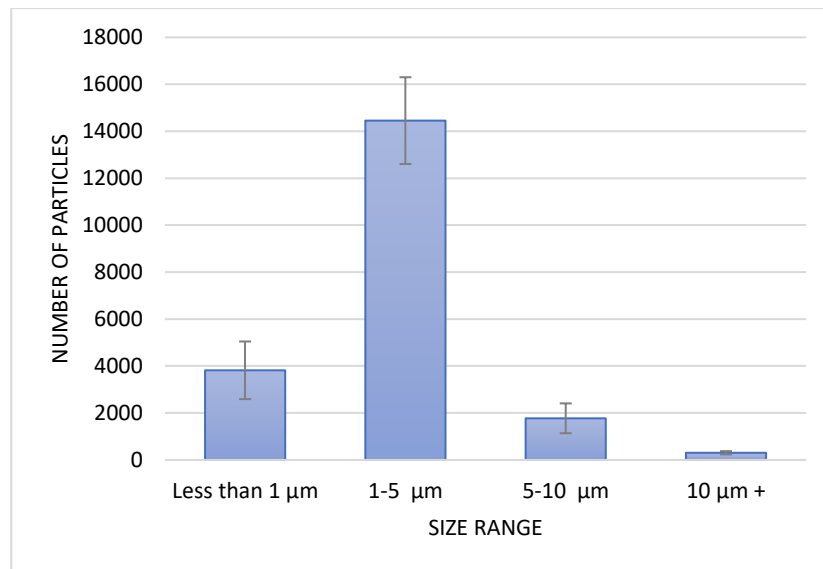
Figure 5:Number of Particles Per Size Range

6.2.2. Blank sample

The blank samples contained 8,353±633 particles/L which is 41% the total number of particles found in the plastic bottles. Figure 6 shows that micro-particles in the blank samples showed a similar distribution as that of the plastic bottles. The majority being in the 1-5 µm with a quantity of 5,504 ±1,316 particle/liter and directly below it is the less than 1 µm which accounts for 2,418 ±957 particle/liter.

Figure 6: Number of Particles Per Size Range in Blank samples

6.3. Qualitative analyses using the SEM-EDX

6.3.1. Plastic identification

Not all the particles detected are plastics. The analysis of the SEM-EDX results will allow a scientific guess on the nature of the particles. As can be seen by Figure 7 the presence of nitrogen might be due to the presence of nitrates in the drinking water and the aluminum is because of the aluminum slab that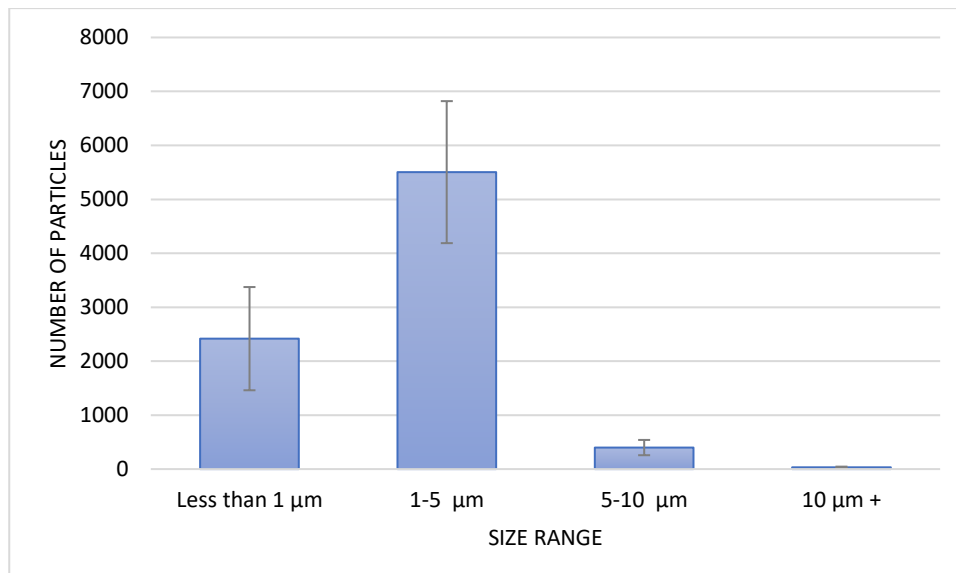 is used as a sample holder. On the other hand, the similar percentages of carbon and oxygen makes it difficult to tell if it is a plastic. This particle can't be nylon because the weight and atomic percentages don't match at all. In a PET the percent weight of carbon should be 1.88 times more than that of oxygen and the atomic percentage of carbon should be 2.5 times more than that of oxygen. This particle might be a PET with altered weight and atomic percentages due to the presence of nitrate which also has oxygen that resulted in an increase in the oxygen level of the particle.

Element	Weight%	Atomic%
C	30.27	36.61
N	19.74	20.47
O	43.32	39.33
Al	6.67	3.59
Totals	100.00	
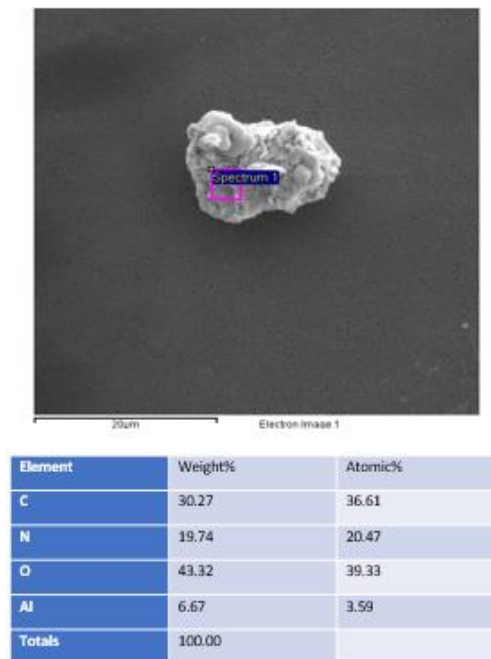
Figure 7:SEM-EDX of a particle

The fiber found in Figure 8**Error! Reference source not found.** is most probably a PET fiber since the carbon and oxygen atomic and weight percentage are very similar to that of a PET.

Element	Weight%	Atomic%
C	63.76	70.10
O	36.24	29.90
Totals	100.00	
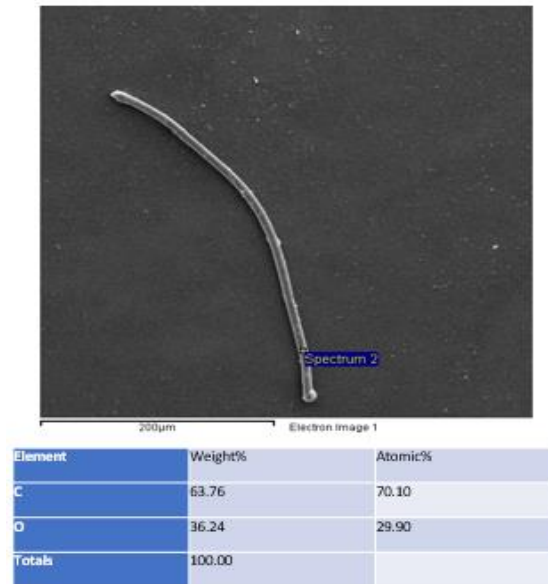
Figure 8: SEM-EDS of a fiber found in drinking water

Not all the particles detected are plastics, for example the particle found inhas a very low carbon weight and atomic percentage and a high oxygen and a significant amount of calcium. In addition to that, the particle is highly crystalline. Therefore, this particle I most probably calcium carbonate.

Element	Weight%	Atomic%
C	17.27	26.99
O	48.61	57.03
Ca	34.12	15.98
Totals	100.00	

Figure 9:Figure 15: SEM-EDS of a crystalline structure found in drinking water

6.3.2. Quantity of MPs/L

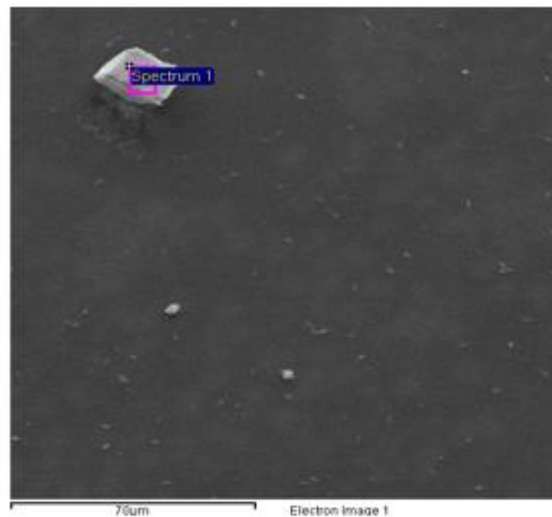The total number of micro-particles detected was 20,348±3,404 micro-particle/L. As can be seen in the section above not all those particles are plastics. Some of them are calcium carbonate, nitrates, sulfates, or other non-plastic particles that are present in drinking water.

Table 2 shows that the percentage of MPs varied between samples where bottle samples 4, 5, and 6 have a higher percentage of MPs than bottle samples 1, 2, and 3 and that can be ether due to the lower area scanned in bottles 1, 2, and 3 or due to the water source and manufacturing process. On the other hand, although the bottle sample 7 has the highest area scanned among all the samples, it has the lowest plastic percentage. This shows that higher areas scanned doesn't necessarily mean that there will be an increase in the percentage of plastics.

After approximating the % of MPs in each sample the number decreased to 10,217±2,348 MPs/L which is around 50.2% of the total number of particles detected. Similarly to the work done by (Oßmann, et al., 2018) and (Schymanski, Goldbeck, Humpf, & Fürst, 2017) MPs were found in every plastic bottle sample analyzed. However, the amount of MPs/L was 86.6 times greater than the study done by (Schymanski, Goldbeck, Humpf, & Fürst, 2017) that reported 118±88 MPs/L. Furthermore, when compared to the study done by (Oßmann, et al., 2018) the number of MPs/L is 3.9 times greater than the single use PET bottles which was 2,649±2,857 MPs/L and 2.1 times greater than the new reusable PET bottles which was 4,889±5,432 MPs/L and 1.2 times greater than the old reusable PET bottles which was 8,339±7,043 MPs/L. This discrepancy in the studies might be due to either different brands used in the studies or the difference in methodology or a bit of the two combined. In addition to that, the number of plastics found in blank samples was 3,656±768 MP/L which is 35.8% of the total MPs found in plastic bottles.

Table 2: MP summary of the six bottled water samples

	% of area Scanned	% of MPs	MPs/L
Bottle Sample 1	0.05	53.66	13,645
Bottle Sample 2	0.02	40	8,262
Bottle Sample 3	0.016	48.3	10,782
Bottle Sample 4	0.11	56.5	11,713
Bottle Sample 5	0.003	62.3	13,174
Bottle Sample 6	0.2	52.5	7,788
Bottle Sample 7	0.6	39	5,776
Bottle Sample 8	0.11	46	9,447

6.3.3. Limitations of the method

The SEM was able to detect the particles that were present on the filter. However, the SEM-EDX doesn't have the ability to properly distinguish the particles observed and to determine the exact number of plastics in the sample. What the SEM-EDX does is give the weight and atomic percentages of the elements that are present on each particle detected. Sometimes there might be interferences on the particle that would cause to a misidentification of plastics. The presence of sulfates and nitrates on a particle would cause an increase in the oxygen weight and atomic percentages that would result in misidentifying it as a non-plastic. On the other hand, sometimes the emission from the SEM-EDX might penetrate the sample and reach the carbon double tape and this would lead to an increase the weight and atomic percentages of carbon and thus misidentify it as a plastic.

In addition to that, mineral drinking water has a lot of non-plastic particles and the SEM-EDX can't scan the entire sample area. Therefore, taking very small areas that include a total of 100-120 particles and scanning them won't give a realistic percentage of MPs in the sample. Consequently, the actual MPs/L would be much lower than the one reported in the study.

The limitation of this technique is the reason why there is such a big discrepancy between this study and the studies mentioned in the previous section. If the entire area of each sample would be fully scanned and properly analyzed, then most probably the results would be closer to the results obtained in literature.

CHAPTER 7

CONCLUSION

With the constant increase in plastic production and the lack of efficient collection schemes and proper waste management facilities, the number of MPs in water bodies is expected to keep on increasing. After the issue of MPs started to gain a global concern, research started to be done on drinking water since it directly affects humans especially because the average person drinks around 1-1.5 L of water a day. The SEM machine which is coupled with an EDX detector was able to observe and detect the particles and fibers which were present in bottled water samples. Several types of plastics were found such as PET, PP, and PE. In addition to that, non-plastic particles were also detected such as calcium carbonate. Finally, the SEM-EDX was used to approximate the percentage of MPs out of all the particles detected to estimate the number of MPs per liter of bottled water were 14323±2527 MPs/L detected. Future work will include glass bottled samples and the analytical technique will expand and use an FTIR spectroscope or a μ-Raman spectroscope.

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