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Mansurat Golden Abdulmalik Ali

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PRESENCE AND CHARACTERIZATION OF MICROPLASTICS IN DRINKING (TAP/BOTTLED) WATER  
AND SOFT DRINKS

by

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Bachelor of Science, Kogi State University, 2010

A Thesis

Submitted to the Graduate Faculty

Of the

University of North Dakota

In partial fulfillment of the requirements

For the degree of

Master of Science

Department of Earth System Science and Policy

Grand Forks, North Dakota

December

2019

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This thesis, submitted by Mansurat Golden Abdulmalik Ali in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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This thesis is being submitted by the appointed advisory committee as having met all of the requirements of the School of Graduate Studies at the University of North Dakota and is hereby approved.

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Water and Soft Drinks

Department Earth System Science and Policy  
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University of North Dakota

Grand Forks, ND

December, 2109

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Mansurat Golden Abdulmalik Al

To my children: Muhsin Sultan and Rahma (Abdulmalik)

(No limit to hard work driven by passion)

## ABSTRACT

This study investigates the presence of microplastics in human consumable products using 33 samples; Fifteen tap water samples, nine bottled water and nine soft drink samples, collected and purchased in Grand Forks, ND.

Tap water analysis confirm all samples contain microplastics, majority of which were fibres and fragments with 48.6% and 41.8% abundance. The average concentration was 182 mpp/L (range 66 mpp/L – 472 mpp/L) with 2.5  $\mu\text{m}$  – 3 mm size range. Bottled water and soft drinks were also contaminated with microplastics with an average of 101 mpp/L (range 49 mpp/L – 166 mpp/L) and particle size ranging from 5  $\mu\text{m}$  – 1.4 mm. Fragments were most abundant with 51.7 % followed by fibres with 38.1 %. Soft drink samples were all contaminated with microplastics averaging 159 mpp/L (range 77 mpp/L – 256 mpp/L) with particles size > 3  $\mu\text{m}$  – 1.2 mm. Morphologic analysis was done for particles > 100  $\mu\text{m}$  thus, fragments were most abundant with 58.7 % followed by fibres with 32.2 %.

Data suggests contamination was at least coming from surface run-off, waste water effluents and packaging or bottling itself. Analysis showed the prevalence of smaller particles less than 100  $\mu\text{m}$  containing 84%, 92 % and 71 % of total microplastics analyzed in tap, bottled water and soft drinks, respectively. Our results give a substantial need for a well targeted

research to better understand microplastic uptake, fate and health effects under relevant exposure scenarios.

## CHAPTER 1

### INTRODUCTION

#### 1.1 Manufacturing of plastics and Applications

Plastics are defined as any synthetic or semi-synthetic polymer with thermoplastic or thermoset properties, which may be synthesized from hydrocarbon or biomass raw materials (UNEP, 2009). In other words, plastics are made of synthetic polymers of high molecular mass, which are usually produced through the polymerization of monomers derived from oil gas, or coal (Ivleva et al., 2017). They come in thousands of varieties with different base chemistries, derivatives, and additives that are formulated to cover a wide range of functional and aesthetic properties. They are the most common materials for end-use parts and products, from consumer products to medical devices.

To simplify the process of finding the material best suited for a given part or product, two main categories of plastics need to be considered: 'Thermoplastics and Thermosetting plastics. 'Thermoplastics' have a distinct feature by having numerous melt and solidification cycles with less degradation. They take the form of sheets or pellets that are heated into desired shapes during manufacturing processes which makes recycling or melting and reusing thermoplastics feasible. Typical thermoplastics include Polyethylene terephthalate (PET), Low Density Polyethylene (LDPE), Polyvinylchloride (PVC), High Density Polyethylene (HDPE), Polypropylene (PP) and Polystyrene (PS).



'Thermoset' undergo a chemical reaction once softened by heat and treated and form a high molecular weight 3D matrix structure that cannot be soften again by heat (UNEP, 2009).

Thermoset decompose when heated rather than melting and will not reform upon cooling therefore it is impossible to recycle thermosets to return into their base ingredients. The common materials of thermoset plastics include epoxy, polyester, cyanate ester, silicone, polyurethane and vulcanized rubber.

Plastics have become an essential feature of human life; from the bottles we use to the clothing we wear. A world without plastics or synthetic organic polymers seems unimaginable today because they are inexpensive when compared to the alternatives and, they are lightweight, safer and durable which can readily be molded into a variety of products that have a wide range of applications (Mwanza & Mbohwa, 2017). Plastics are used in textile, computers, car parts, refrigerators, etc. because they are sturdy without degradation for hundreds of years. They also play a vital role in hospital and medical fields as plastics are used for disposable syringes, intravenous sets, glucose bottles, disposable plastic aprons, catheters and cannulas (Raman Sharma & Sharma, 2014). They have taken over from paper, glass and cardboard in packaging, usually reducing cost and providing better care of the items.

The use of plastics has increased nearly 20-fold during the last 60 years and production in the 21st century has increased from 200 million tons in 2002 to over 311 million tons in 2014 (Plastics Europe, 2015). Production has increased by approximately 8.7 % annually, evolving into a \$600 billion global industry with China, the European Union and North America being the major contributors (Jambeck et al., 2015). In 2009, around 230 million tons of plastics were

produced and about 25% of these plastics were used in the European Union (Mudgal et al., 2011).

## 1.2 Plastic Waste

The production and consumption of plastics has also resulted to an increase in plastic waste (UNEP, 2009). Plastics make up an estimated 10% of household waste, most of which is disposed in landfill (Barnes, 2005), however, 60 % to 80 % of plastic waste are found on beaches, floating on ocean or sea (Derraik, 2002). It was predicted that a business as usual scenario with continuing increase in plastics consumption would produce around 220 million tons of plastic waste annually in 2025 (Wagner et al., 2014). Currently, approximately 8 million metric tons of plastics enter the oceans annually (Gourmelon, 2015), majorly through run-offs, dumping and fishing.

A combination of increased production, slow degradation process of discarded plastics, and long residence time of materials has caused a tremendous rise of plastic waste in the ocean (Barboza & Gimenez, 2015). It is estimated that at least 5.25 trillion of plastics are currently circulating in ocean surface waters (Eriksen et al., 2014). Plastics in surface water undergoes marine processes such as surface circulation and mixing while large pieces are constantly broken down into smaller pieces rather than completely degrading over time (Moore 2008). As a result, plastics persist in the ocean for decades while releasing toxic chemicals into water bodies.

### 1.3 Microplastics

When plastics of all shapes and sizes enters the waterways, its exposure to the sun, reaction to oxygen, and degradation from physical impacts by waves and sand causes it to break down into tiny pieces. These microscopic pieces of plastics are called 'Microplastics'. The term 'Microplastics' has only surfaced relatively recently and has taken different definitions by different researchers. Gregory, (2003) defined microplastics as plastic particles of size 0.06 - 0.5 mm in diameter with larger particles barely visible called 'mesoplastics' and visible particles referred to as 'macroplastics' (Steven B. 2019). The National Oceanic and Atmospheric Administration (NOAA) defines 'Microplastics' as particles less than 5 mm in size (Andrady, 2011). This definition of microplastics as less than 5 mm in size is used in this study.

Microplastics is evolving and it is expected to be the most numerically abundant items in the ocean (Thompson, 2015). Its concentration will continue to increase as large plastics in the oceans continue to degrade into millions of microplastic pieces (Law & Thompson, 2014). This degradation evolves from environmental exposure to public littering, construction wastes, river run-off and catastrophic events (natural environmental hazards) which, all summed up to direct pathways of plastics in the waterways. Microplastics are generated from variety of sources either primary or secondary sources as shown in Figure 1.

Primary sources are generated through intentional introduction in by-products of items for example, the small plastics called 'microbeads' often applied in cosmetics and personal care products such as, facial scrubs and shower gels acting to enhance or increase the abrasive

effect while improving exfoliation and cleaning properties of the treatment (Juliano & Magrini, 2017). Since microbeads are microscopic when washed down through the sinks, they find their way into water systems and later into natural waterways (Cole et al., 2011).

Secondary sources of microplastic generation are unintentionally introduced which occur on surface water due to harsh solar radiation and exposure to wind and waves causing bulk plastics to break down into smaller particles (weathering and fragmentation of larger plastics) (Andrady, 2011; Song et al., 2017). Synthetic textiles and clothing are also large sources of microplastics generation for instance, the abrasion during laundry through exposure to chemicals and detergents causes the breakdown of synthetic fibres into smaller microfibrils (Browne et al., 2015). The microscopic size of the fibres allows them to find their way into the air, rivers, lakes and larger water bodies.

The presence of plastics in the marine environment present a number of challenges that obstruct economic development such as aesthetic issue resulting from plastic littering the shorelines creating negative impacts for tourism (Jang et al., 2014). Economic losses are associated with reduced tourism revenues, recreational activities, vessel damage, impairment in marine environments and damage to public health (Hardesty et al., 2015). Littered plastic shorelines also negatively impacts energy production, shipping, fishing and aquaculture resources (Cole et al., 2011; Sivan, 2011). A conservative estimate of the overall economic impact of plastics to marine ecosystems is approximately \$13 billion US/year (Raynaud, 2014), although the true environmental costs are difficult to monetarize. However, reported impacts

of marine plastic debris on marine life include over 700 species, from tiny zooplankton to the largest whales, including fish destined for human consumption (Xanthos & Walker, 2017).

**Plastic debris in the ocean: a multiplicity of sources and pathways**

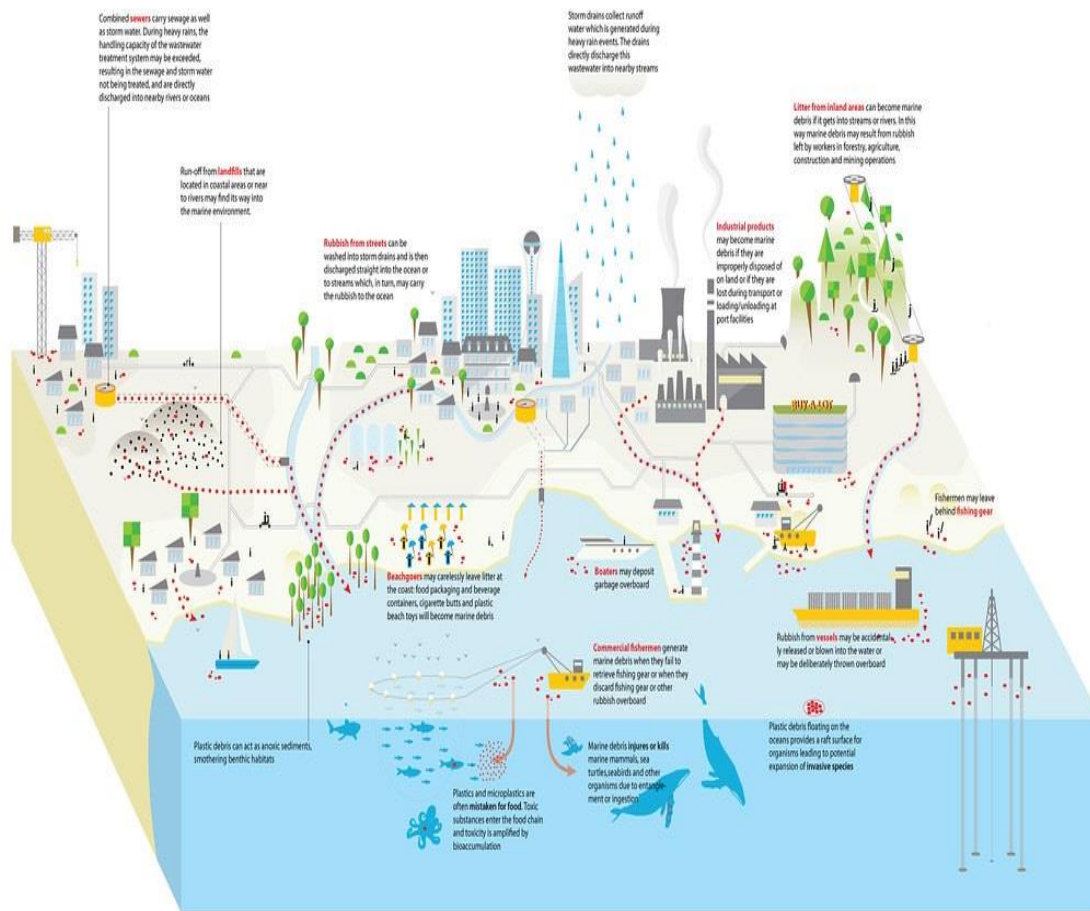


Figure 1: The multiple sources and pathways of plastic debris in the Ocean. Illustration obtained from: [www.flickr.com/photos/gridarendal/32241433611/sizes/h/](http://www.flickr.com/photos/gridarendal/32241433611/sizes/h/)

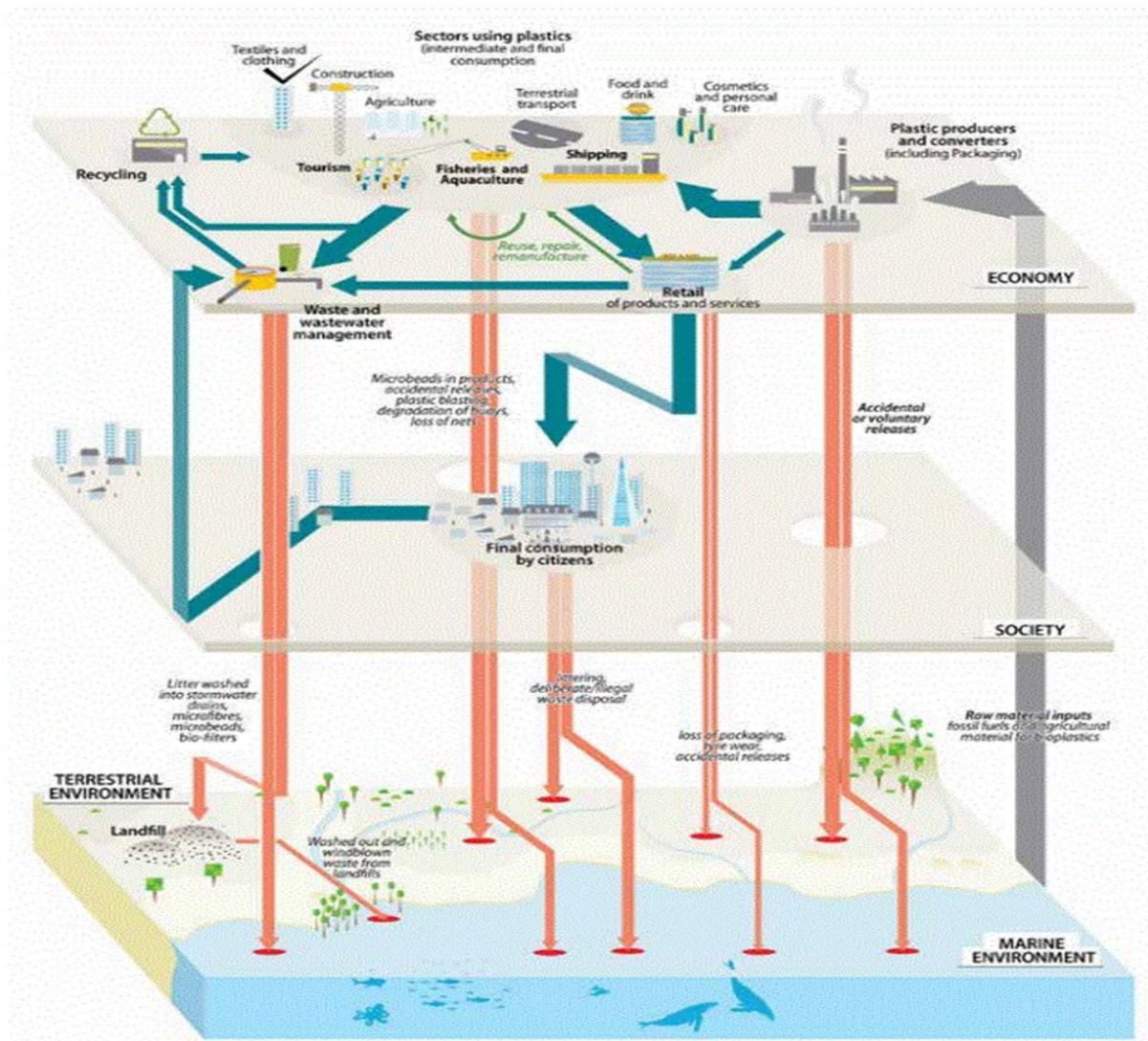


Figure 2: Sectors using plastics and their movement from the economy into the environment. Illustration obtained from GRID/Arendal by Maphoto, (2018).

Since microplastics originating from primary sources are identifiably manufactured, mitigation measures can be designed to reduce their input into the environment (GESAMP, 2015) as well as identifying other opportunities for changes, decisions and behaviors that might exist as shown in Figure 2.

## CHAPTER 2

### REVIEW OF LITERATURE

#### 2.1 Microplastics in Aquatic Environment

Marine litter results from the indiscriminate disposal of waste items that are either directly or indirectly transferred into the sea and oceans. Plastic waste are distributed throughout the ocean, occurring on shorelines, in surface water and seabed sediments from the Arctic to Antarctic. They may accumulate at remote locations such as the mid-ocean gyres, as well as close to population centers, shipping routes and other major sources.

Andrady (2011) attempted to address the fate of plastics and the mechanisms by which microplastics are derived in marine environment by broadly classifying plastics based on their chemical compositions as polyethylene, polypropylene, polystyrene, polyethylene terephthalate and polyvinyl chloride which are predominant form of plastics. These plastics, on exposure to solar UV radiation and other mechanical forces such as waves, tides, reduces their average molecular weight, thus causing the plastics to become brittle enough to fall apart into powdery fragments (microplastics). This process also enhances leeching of chemicals from plastics in water thereby increasing the toxicity of the marine environment.

The Mediterranean Sea is known to be the largest and deepest enclosed sea on earth as well as the busiest navigation crossroads and top tourist destinations in the world, surrounded by heavily populated and industrialized coast line, it is not surprising that the impact of human

activities is proportionally stronger. The global model prediction shows that the highest concentration of floating plastics in the world would occur in the Mediterranean Sea mainly owing to limited outflow of surface waters, a densely populated coastline and intensive fishing, shipping, touristic and industrial activities, thus substantial amounts of marine litter tend to accumulate in the Mediterranean basin, which according to simulations retain between 21 % to 54 % of all plastics (Suaria et al., 2016).

Suaria et al. (2016) investigated the abundance of plastic marine litters in the Mediterranean surface water using 74 samples collected with a neuston net of 200  $\mu\text{m}$  mesh size. Microplastics were found with mean abundance of 1.65 particles/ $\text{m}^2$  with an extrapolated abundance of  $3.1 \times 10^{12}$  microplastic particles in the entire Mediterranean. 93.3 % of these particles were classified as fragments and the rest consisting of pellets, films and foams. Also, 26 % of the counted particles was reported to be smaller than 300  $\mu\text{m}$  and 51% smaller than 500  $\mu\text{m}$ . 16 types of polymer were also identified using FT-IR spectroscopy with polyethylene (PE) being predominant.

Just like the marine environment, freshwater environment is also prone to microplastic contamination. Eriksen et al. (2013) published the first open-water survey for plastic pollution within the Laurentian Great Lakes system. Samples were collected from 21 sites in three lakes (Lake Superior, Lake Huron and Lake Erie), and all samples were examined using scanning electron microscopy (SEM). All but one sample contained plastic. Using SEM, the average abundance was 43,157 particles/ $\text{km}^2$ . Traces of aluminum silicate particles were also found; Of 21 sites, eight were found to contain coal/fly ash with an average contribution of 20% (within



the 0.355–1 mm size classification). Lake Erie samples had the highest concentration of microplastics accounting for 85% of all microplastic particles collected in all samples combined, particle size ranges from 0.36 mm to 0.99 mm. Five microplastic type categories were identified with pellets and fragments the most abundant accounting for 81 % of total particles. The sources of microplastics, including the identity of the particles were linked to the eight states bordering the Great Lakes with over 144 coal-burning power plant which may release aluminum silicates into waterways through wastewater discharge. The multi-colored spherical particles were linked to consumer products such as facial cleansers, and other personal care products that float in freshwater systems.

Mani et al. (2015) reported the abundance and composition of microplastics at the surface of the Rhine River, one of the largest European rivers. Using 31 samples from 11 locations over a stretch of 820 km, they found microplastics present in all samples with varying concentrations across the river reflecting various sources and sinks such as waste water treatment plants, tributaries and weirs. The average abundance was 892,777 particles/km<sup>2</sup>. Opaque spherules constituted 45.2 % of microplastics by category followed by fragments with 37.5 %, transparent spherules with 13.2 %, fibres 2.5% and others with 1.1 %. Spherules are manufactured plastic products used in feedstock for the plastic industry, or as scrubbing granules and pellets in cosmetic products, as air-blasting agents or in industrial cleaner and other products (Fendall et al., 2009). The particle size ranges from 300 µm to 1000 µm with polystyrene (29.7%) as the dominant polymer, followed by polypropylene (16.9%), acrylate (9.3%), polyester (5.1%) and polyvinyl chloride (1.7%). Also, 86.4% of all particles analyzed were identified as being among the worldwide most produced polymers.

Faure et al. (2015) also conducted a study aimed at expanding the data set of microplastic pollution in Swiss surface water for better understanding of its distribution, behavior and impacts using 39 surface samples collected with a 300 µm mesh net. The samples were visually analyzed using a stereomicroscope and microplastics were found in all samples at concentration ranging from 11,000 particles/km<sup>2</sup> to 220,000 particles/km<sup>2</sup>. Particles were extracted by size and sorted in categories according to their appearance and characteristics. Fragments were predominant with foams and pellets representing a small fraction. The chemical composition of the particles analyzed through FT-IR ATR spectroscopy shows 62 % as polyethylene (used for packaging), 15 % as polypropylene (from fragments) and 12 % as polystyrene (used in buildings as insulating materials). This study further confirms the ubiquity and diversity of microplastics in fresh water ecosystem.

## 2.2 Microplastics in Human Consumables

Microplastics are not only found in the natural environment; from dust in our homes (Prata J., 2018); in organic fertilizer (Weithmann et al., 2018); but are also found in human consumables; table salt (Karami et al., 2017), in honey, sugar and beer (Bouwmeester et al., 2015) and most alarmingly, in bottles and tap water (Schymanski et al., 2017) (Kosuth et al., 2018).

Liebezeit et al. (2014) published a report on analysis conducted on 24 German beer brands obtained from local supermarket. Twelve of these were regular Pilsner type, five were wheat beer and seven were alcohol-free Pilsner. 0.33 L and 0.5 L by volume of samples were used for filtration and each rinsed with 0.8 µm filtered deionized water. 6ml of Rose Bengal was

used to cover the rinsed filter paper to stain all-natural particles. The non-stained particles during analysis under the dissecting microscope were regarded as microplastics. In all 24 beer samples, microplastic was found. Fragments were the most abundant while fiber and granular were least abundant, one alcohol free sample had the highest fragment count, most fibers were transparent but blue, black and green fibers were also present. A relative contribution ranging from 5 – 71 % for granular material, 14 – 87 % for fragments and 3 – 57 % for fiber was reported. The synthetic polymers found are particles of Polyvinyl polypyrrolidone (PVPP) which is used in granular form to clarify and fine beer prior to filtration. A complete insect belonging to the order Thysanoptera was also reported.

Similarly, Kosuth et al. (2018) analyzed 12 Beer samples for microplastics contamination. The beer samples were brewed from water sourced from Laurentian Great Lake as the prominence of plastic pollution within the Lake is widely known. Microplastics were found with concentration from 0 to 14.3 particles/L. Of all the 189 particles reported, the vast majority (98.4%) were classified as fibers with an average fiber length of 0.98 mm. The remaining particles were identified as fragments. Also in this study, 159 tap water samples collected from fourteen countries were analyzed using volume ranging from 447 – 603 ml and filtered through 2.5 µm cellulose filter. 539 anthropogenic particles at a concentration ranging from 0 to 61 particles/L averaging 5.45 particles/L were found with majority identified as fibers (98.3%) with size ranging from 0.1 mm – 5 mm. The remaining particles were identified as fragments or films. In addition, 12 commercially acquired sea salt samples were also analyzed and all sample were contaminated with a total of 461 anthropogenic particles at concentration ranging from

46.7 particles/kg to 806 particles/kg with most abundant morphology classified as fibers (99.3%) having an average length of 1.09mm.

Yang et al. (2015) tested 15 brands of sea salt, lake salt and rock salt samples for comparison in abundance, types and composition of the microplastics. The brands of table salts were collected from the supermarket with a weight ranging from 240 to 500 g and are analyzed for possible microplastic contamination. Three replica packages were used to compare different brands of the same type of salt while five replica brands were used for the comparison among the different types of salts. After sample analysis, sea salt contains 550-681 particles per kg; lake salt had 44 - 368 particles per kg and rock/well salt had 7 -204 particles per kg. In sea salt, fragment and fiber were the common type of particles and microplastics of less than 200  $\mu\text{m}$  represented the most particles accounting for 55% of the total microplastics. Polyethylene terephthalate was the most common type of microplastic followed by polyethylene and cellophane. The results from these overlying studies implies that sea products such as sea salts are contaminated by microplastics.

Mason et al. (2018) also conducted a research on synthetic polymer contamination in bottled water utilizing Nile Red method of particle identification. 259 bottles of water from 11 globally sourced brands purchased from 19 locations in 9 countries were used. The samples varied in volume ranging from 500 – 600 ml, 0.75 – 2 L and 750 ml. At random selection, the bottled water was indicated to be placed under a laminar flow fume hood and each injected with specific volume of Nile Red solution and filtered through a vacuum glass fiber filter of 1.5  $\mu\text{m}$  pore size. Filters were then examined under an optical microscope using a blue crime light

to elicit fluorescence and further analyzed using FTIR spectroscopy to confirm its polymeric identity. The result shown for particles > 100 µm had an average density of 4.15 particles/L with a range of 0 - 14 particles/L, while the smaller particles (6.5 – 100 µm) had an average density of 23.5 particles/L with a range of 7 – 47 particles/L. 17 bottles out of the 259 bottles analyzed (7 %) was reported to have no microscopic contamination in excess of possible laboratory influence indicating that 93 % of the bottled water tested showed some sign of microplastic contamination.

When averaged across all brands, 325 particles/L were found within the bottled water tested (broken down as an average of 10.4 particles/L occurring for particles >100 µm and 315 particles/L for particles within 6.5 – 100 µm). Polypropylene (polymer often used to make plastic bottle caps) was most common polymeric material with 54 %, nylon being the second most abundant with 16 % and polyethylene with 10 % of the particles analyzed. All microplastics > 100 µm were visually characterized according to their morphology; Fragments were found to be the most common type of particles with 66%, fibers with 13% and films with 12%. The significant variation in particle concentration in all these studies shows the heterogeneity and complexities of microplastic sources, the manufacturing process and the particle-fluid dynamics, among others making them challenging to study.

### 2.3 Microplastics and Potential Effects

Plastics may be easy and convenient for everyday use, but their negative impacts on our health cannot be overlooked due to its non-biodegradable nature, it keeps on piling in the environment and creating tons of trash of all shapes and sizes polluting the earth which

eventually, infiltrate the food chain and affecting the environment. The tremendous number of plastics found in the marine and fresh water environments have been linked to various animals ingesting them in the form of macro or microplastics with their food (Ivar do Sul & Costa, 2014).

Since the detection of microplastic in commercial seafood (Neves et al., 2015), the issue of potential contamination in food or human consumables has become increasingly important as the effects and toxicological risks associated from the intake of these particles covers the particles themselves, the polymer additives and absorbed contaminants as microplastics are considered hydrophobic and been known to absorb metals, bacteria and chemicals such as PCBs, PBDEs, and PAHs sometimes at a concentration many times higher than their immediate surroundings in which once ingested, some of these organic chemicals can desorb in the guts of animals making it detrimental to the environment. Single-use plastics and other plastics items except PET in particular have been a focus, because it has contributed to host of problems such as choked sewers, animal death and clogged soils (Verma et al., 2016).

The socio-ecological risk perspective and environmental implication of microplastics are linked to the unintended side effects associated to exposure from mode of operations leading to plastics consumption. For instance, the microbeads in cosmetic and personal care products that helps exfoliate our skin while keeping it radiant, packaging with plastics due to public demand for fresh food, and in the medical sector that guarantees aseptic medical products. Also, plastic bags been an easy way to transport our shopping (UNEP, 2016), all account for how plastics enters into our society contributing to environmental accumulation of plastic waste.

Chemical additives added during the manufacturing of commercial plastics in order to improve the strength, durability or grant the plastic specific characteristics have spring up many controversies associated with plastics (Elias, 2000). Additives such as antioxidants, flame retardants, plasticizers and stabilizers incorporated in cable insulations or electronic applications as well as in pesticides and insecticides all leach out chemicals as most of them are not chemically bound. As a consequence of plastics accumulation and fragmentation in oceans, plastic additives could represent an increasing ecotoxicological risk for marine organisms (Hermabessiere et al., 2017).

The physical impact of microplastics include the devastating injuries created to many forms of marine life from compaction of accumulated microplastics (plastic bezoars) in gills and intestines, thus interfering with feeding habits that unnaturally lead to death (Anderson et al., 2016) as well as plasticizers which have been linked to abnormal growth and reproductive problems as well as the endocrine disruption in multiple animal models (Kontrick, 2018). A 2016 UN report documented over 800 animal species contaminated with plastics via ingestion or entanglement; 69 % greater than that reported in a 1977 review, which estimated only 247 contaminated species (Andrady & Neal, 2009). Among these 800 species, 220 have been found to ingest microplastic debris in nature and many of these species are intended for human consumption which include invertebrates, crustaceans, and fish thus, seafood consumption specifically, represents one pathway for human microplastic exposure.

In human medicine, microplastics are used as carriers of medications into body tissues.

A report commissioned by the House of Commons Environmental Audit Committee of the UK

Parliament speculates that the additives and contaminants of concern, when adsorbed to marine microplastics, would act similarly to microplastics used in medical procedures, which transfer to human tissues, though there is insufficient data demonstrating this (Thomson et al., 2009).

Microplastics have a long life span because of its residence time within the aquatic environment, they have the ability to alter the quality of the water body due to their ability to transport pollutants and through the creation of plastic biofilms. The water quality standards furnished by the Environmental Protection Agency (EPA) in the United State approves the condition that a water body shall be kept to suit its designated uses and is the legal basis for controlling pollutants entering waterways. However, as a vessel, due to the synthetic material plastics are made from, most microplastics are hydrophobic in nature and have a large surface area to volume ratio; these two characteristics are quite favorable in that both algae and persistent organic pollutants (POPs) and fertilizers can easily absorb onto the microplastics (Kovac et al., 2016) and transported upstream into larger bodies of water which can increase the amount of nutrients leading to an increase in the algal growth, an increase in chlorophyll-a, and a decrease in the amount of dissolved oxygen when the algae dies.

Thus, when testing for water quality, quantities such as the amount of nutrients (phosphorus and nitrogen) present including the amount of chlorophyll-a, the amount of dissolved oxygen present as well as water pH and water clarity can be negatively impacted by the presence of microplastics as fertilizers and POPs interfere with the pH of the water body which changes the water quality making the water body unfit for recreation, drinking, fishing,



etc. The disruption in the pH can cause unsuitable conditions for the species that live there, upsetting both the food chain and the ecosystem as a whole (Steven B. 2019). So, the precise effect of microplastics on human health is difficult to determine and still very much contested as a lot of public uncertainty is linked toward variety of reasons as there are many different types of plastics, as well as different chemical additives that may or may not be present thus posing a question on 'How much is too much to be negligible'. The manufacturing practices for plastics have also changed over time making it even more difficult for scientists studying microplastics to determine exactly what materials and chemical additives may be present in samples.

Nonetheless, study conducted by Orb media and other researchers have found microplastic particles in drinking water (Mason et al., 2018), beer (Liebezeit et al., 2014), honey and salts (Kosuth et al., 2018) sourced from different locations around the world. However, these published studies and its media coverage have been contested by consumer protection agencies and food and beverage industries afraid of reputational effects but at the same time, have stirred public awareness concerning the possibility that microplastics could accumulate in the body and cause a variety of adverse health effects.

#### 2.4 Microplastic sampling, identification and qualification

In most open water studies, efficient identification method of microplastics is a serious challenge in quantifying its loads, especially with decreasing size. (Kovac et al., 2016) outline protocols for microplastics sampling on the sea surface with a sample analysis. The protocols which were in line with the recommendation for microplastic monitoring published by the

Marine Strategy Framework Directive (MSFD), a technical subgroup on marine litter describing the methodology for sampling, sample preparation, separation and chemical identification of microplastic particles.

Hidalgo-Ruz et al. (2012) published a report reviewing protocols of microplastic detection with a focus on the analytical methods, including sampling, processing and, especially, identification and quantification of microplastic in aquatic environments. It also addresses possible strategies to assess environmental risk associated from microplastic and prospects for minimizing its abundance in the aquatic ecosystems. For microplastic sampling and processing, the article specifies three sampling methods namely selective (applied to large particles samples), bulk (applied to sediment samples) and volume-reduction (applied to both water and sediment sample) methods. Sampling can either take place at the sea surface or in water columns using either manta trawls or neuston nets with high volumes of samples handled with ease and quickly however, smaller mesh sizes tend to clog easily. To separate plastics from other mixtures like sand or stones, density fractionation is necessary using liquids with higher density. Saturated sodium chloride (NaCl) solution is mostly recommended however, not all plastic types (PVC, POM) can be separated as they have higher densities than NaCl. The use of  $ZnCl_2$  with density of 1.6 kg/L was also suggested for separation with consideration for subsequent recycling and reuse which can help to combat environmental pollution was suggested even though, the MSFD Guidance still recommends the use of NaCl solution.

For microplastic identification and quantification, visual sorting is used in most studies which is suitable for particles larger than 1 mm however, the obtained values for microplastic occurrence strongly depend on the observer performing the separation, and identification especially for fibers (e.g., cellulose) using biological stain like Rose Bengal is required. For chemical compositions of microplastics, the FT-IR spectroscopy and Raman micro spectroscopy is required. The FT-IR has three different operating modes (transmittance, reflectance and attenuated total-reflectance - ATR). Larger particles  $> 500 \mu\text{m}$  can be analyzed with ATR-FTIR while for smaller particles, the combination of FT-IR and optical microscope must be applied. The Raman micro spectroscopy have also been applied in a few microplastic studies in marine and fresh water ecosystems, however, most of them analyzed only large particles or a small subsample. The smallest microplastic particles identified by RM in an environmental sample is around  $10 \mu\text{m}$  for both freshwater and marine systems. Raman micro spectrometer generally enables the chemical identification of microplastic particles independent of their morphology. These method of analysis are however, time and labor intensive.

Currently, analytical techniques available to detect microplastics of smaller sizes in aquatic environment remain very expensive thus the need to develop a method that is easy to use, inexpensive and precise is very important in analyzing microplastics as reliable result are crucial.

## 2.5 Research Goals and Objectives

This study is aimed at investigating the presence of microplastics in human consumable drinks, including tap and bottled water as well as soft drinks in North Dakota with objectives to:

- (I) Quantify the concentration of microplastics in drinking water /soft drinks and characterize them based on morphology and size.
- (II) Initiate method of microplastic monitoring that is inexpensive, easy to use and suitable for detecting smaller particle sizes ( $< 1$  mm) within the city of Grand Forks.

To my present knowledge, no survey of microplastics in consumable products has ever been documented in the state of North Dakota. For this study, I chose Grand Forks as the sample site.

## CHAPTER 3

### MATERIALS AND METHOD

Drinking water are obtained from rivers, lakes, streams, ponds, reservoirs, spring and wells. As water travel through these medium, it dissolves naturally occurring minerals and pick up substances from animal and anthropogenic sources resulting to water contamination and pollution. Drinking water in Grand Forks is surface water sourced from either the Red River, the Red Lake River or a blending of both.

#### 3.1. Sample Collection

Prior to the sample collection, the reusable glass bottles were washed and rinsed trice with distilled water and left inverted on a clean surface to dry before proceeding to sample collection. Hair was covered and powder free lab gloves were always worn throughout the laboratory procedures.

Tap water, bottled water and soft drinks were collected and purchased from the campus of University of North Dakota and from the local grocery store in Grand Forks on the 8<sup>th</sup> and 9<sup>th</sup> May, 2019.

A total of 24 drinking water samples and nine soft drink samples were used for this study; 15 of the drinking water samples are tap water both collected from public spaces (water fountains in residence halls and offices) and private residences (kitchen sinks in residential

apartments) all within the campus of UND while nine samples are bottled water purchased from a grocery store in Grand Forks.



Figure 3: Tap water samples collected in Fisherbrand glass bottles.

Tap water were each collected in a 1 L Fisherbrand reusable glass media bottles with caps (Figure 3). Collection was done by first running the tap continuously for one minute, followed by filling the bottles to the point of continuous overflow and dumping its contents for three consecutive times before collecting the final sample. This procedure is important as it helps to wash and rinse the bottles before sample collection. The samples were capped, labeled and preserved at 4°C until analysis.



Figure 4: Bottled water samples by brand used for analysis.

Bottled water samples of local and international brand were purchased from a local grocery store in Grand Forks area. The bottled water came in plastic containers excluding Voss water which came in a glass bottle (Figure 4) of volume 330 mL, 500 mL, 591 ml and 1000 mL respectively. 1 L of each brand of bottled water was used for analysis. The samples were preserved at 4°C.



Figure 5: Bottled soft drink samples by brand used for analysis.

Soft drink samples were all purchased in one location at a local grocery store in Grand Forks. The samples were bottled in plastic containers of 500 mL, 1 L and 2 L volume of contents (Figure 5). 1 L per sample of soft drink was used. Again, the samples were preserved at 4°C prior to analysis.

### 3.2 Sample processing and Filtration

Samples were processed using sterile petri dishes of diameter 60 mm with caps, GE healthcare Whatman binder- free glass microfiber filters of diameter 55 mm with pore size of 1.5  $\mu\text{m}$ , measuring cylinder, vacuum filtration apparatus, watch glass (Figure 6), desiccators, optical microscope, steel micrometer ruler, steel tweezers and steel spatula. Reagents and

solution used include Nile red fluorescent stain, acetone lab grade and distilled water. Nile Red solution was prepared in acetone to  $1 \text{ mg mL}^{-1}$  to yield a working concentration of  $10 \text{ } \mu\text{g mL}^{-1}$  (Mason et al., 2018).

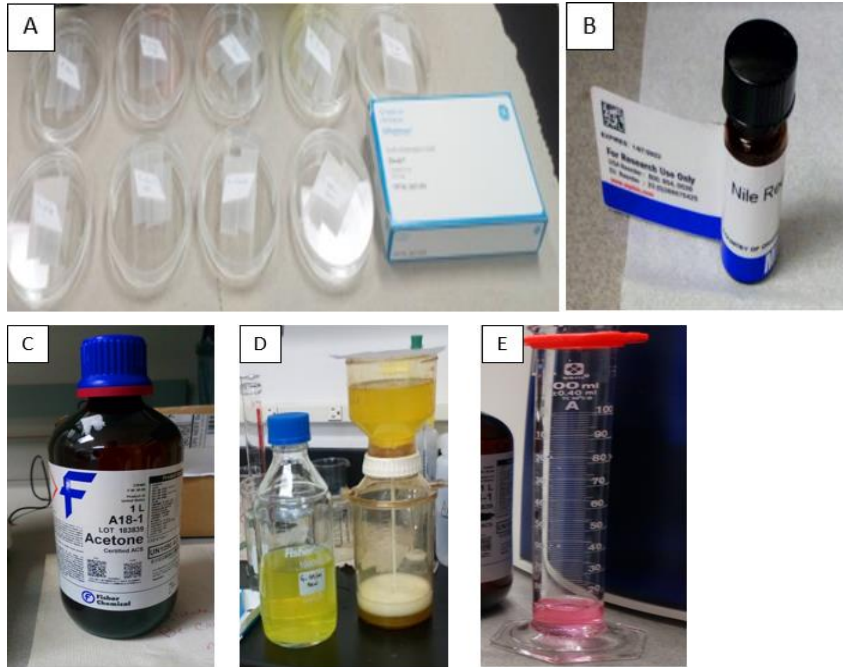


Figure 6: Examples of sample processing apparatus and reagents used for analysis; (a) Sterile petri dishes and glass microfiber filter paper; (b) Nile Red Dye; (c) Acetone – Lab grade; (d) Vacuum filtration set up with one of the sample; (e) 10 ml Nile Red fluorescent stain solution.

All the samples were placed under a laminar flow hood, opened and injected into it 10 ml of Nile Red solution (Figure 6e) and re-capped to incubate with the injected dye for at least 30 minutes. Nile Red was chosen due to its absorption affinity to plastics but not naturally occurring materials and allowing smaller particles to be detected which fluoresces under specific wavelengths of light (Erni-Cassola et al., 2017). Before filtration, filter papers were initially visualized under the microscope to verify any form of contamination prior to filtration.



The volume of samples used was recorded and the filter papers was marked into quadrants for easy and efficient counting and sorting of particles.

Vacuum filtration was done through a glass microfiber filter (GE healthcare Whatman filter paper, grade 934-AH, 55 mm in diameter and 1.5  $\mu\text{m}$  pore size) in multiple batches. For each batch, 1 L of distilled water was collected in the Lab and filtered and labeled as blank sample (negative control) accounting for background or laboratory contamination. During each filtration process, the empty sample bottles were rinsed trice into the filtration funnel with distilled water to ensure that all content in the samples was filtered. The filtration set up was then covered with a watch glass until filtration is over to avoid external contamination of samples. Each filtrate was re-filtered using a new filter paper, this is a precautionary step to capture any possible breakthrough of particles during the initial filtration. All particles found in the second filter was added to the initial sample during counting and identification. After filtration, the filter papers were kept inside the sterile petri dish, capped and left in the desiccator to dry for 48 hours before proceeding to particle counting and identification.

### 3.3 Microscopy

Before particle identification, care was taken making sure that the machine and its surrounding platforms were dust free. The samples (dry filter papers) were all processed in a dark room using the Nikon Eclipse 80i (Upright) fluorescence microscope with a 5-megapixel integrated camera. Through an orange light shielding plate, the samples were each placed in an uncovered petri dish on the stage of the microscope. Using an objective lens of 4x and 10x magnification (image field of 15.7 mm and 4 mm), microplastics were visually identified and

characterized by manipulating the fluorescence particles (enhanced by the Nile Red dye) for resiliency with a sharp pointed tweezer. Particles that appeared tough to breakage were counted, sorted classified (morphology) and photographed while enhancing the images using a spotlight application software and analyzed for particle size using ImageJ software.

For credible result, the particle count obtained from the blank samples for all batch per sample type was subtracted from the total number of microplastic counts in that samples type. All sorted particles were capped sterile petri dish and preserved at 4°C for future analysis.

## CHAPTER 4

### RESULTS

Microplastics in all sample was characterized based on concentration, size distribution and morphology. The concentration of microplastics was measured as the number of particles per liter of each sample. The size of microplastics were measured using Image J software. The morphology of microplastic particles were categorized into five types based on toughness and shape: Fragments are rigid, thick with sharp crooked edges and irregular shapes; Filaments can be short or long with different thicknesses; Films also appear in irregular shapes, but in comparison with fragments, they are very thin and flexible and usually transparent; Fibres have irregular shapes, and are usually thin, flat and lengthy while Pellets are soft and spherical in shape (Kovac et al., 2016). During observation, microplastics appeared red if samples were dyed with Nile Red as shown in Figure 8, 11 and 14. As distilled water was used for cleaning and preparing samples, we also measured the microplastics in distilled water as a blank to be subtracted from the results of samples. On average 2 mpp/L was found in the blank samples and these microplastics appeared as fibres in morphology.

#### 4.1 Microplastic Characterization in Tap Water

15 tap water samples were collected from the buildings on the campus of the University of North Dakota. The laboratory analysis was done in three batches on separate days. The concentrations of microplastics are summarized in Table 1 and Figure 7.

Table 1. Microplastic particles in Tap water

Sample Type	Sample Name	Concentration (mpp/L)
Tap Water (Private Residence)	State Apartment (110)	463
	Manitoba Apartment(3605)	337
	Campus Road (3600)	472
	Hamline Apartment (1100)	218
	530 Tulane Dr	402
Tap Water (Public Space)	Clifford Hall	93
	Police Department	101
	Twamley Hall	72
	Wilkerson Common	77
	Chester Library	83
	Student Wellness Center	74
	Robin residence Hall	132
	School of Medicine	67
	Gamble Hall	78
	Biomedical Research facility	66
	Distilled Water (Batch)	2

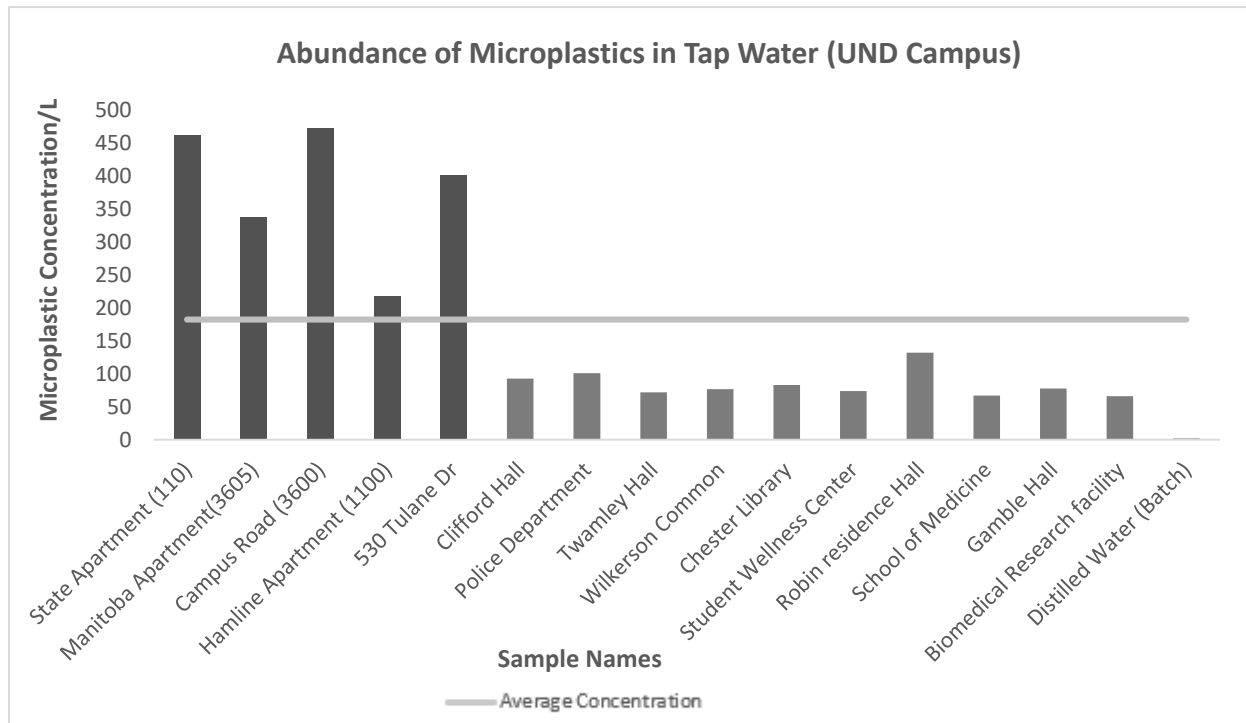


Figure 7: The abundance of microplastic particles found in Tap water collected within the campus of University of North Dakota.

Microplastics particles were found in all samples with concentrations ranging from 66 mpp/L to 472 mpp/L and an average of 182 mpp/L. Higher concentrations was found on samples collected from private residences than those from public spaces. On average, tap water collected from kitchen sinks in the private residences contained 378 mpp/L while tap water from public spaces contained 84 mpp/L. In general, Campus Road (3600) sample had the highest number of particles at 472 mpp/L followed by State Apt (110) with 463 mpp/L and 530 Tulane Dr with 402 mpp/L. Biomedical Research facility, School of Medicine and Twamley Hall had the lowest concentration of microplastic particles at 66 mpp/L, 67 mpp/L and 72 mpp/L.

The types and sizes of microplastics varies (Figure 8). The sizes of microplastic particles ranged from  $> 2.5 \mu\text{m}$  to 3 mm however, particles of smaller sizes ( $2.5 \mu\text{m} - 100 \mu\text{m}$ ) were most abundant, averaging 153 mpp/L and accounting for 84% of total microplastic analyzed. Particles of sizes greater than  $100 \mu\text{m}$  averaged 29 mpp/L.

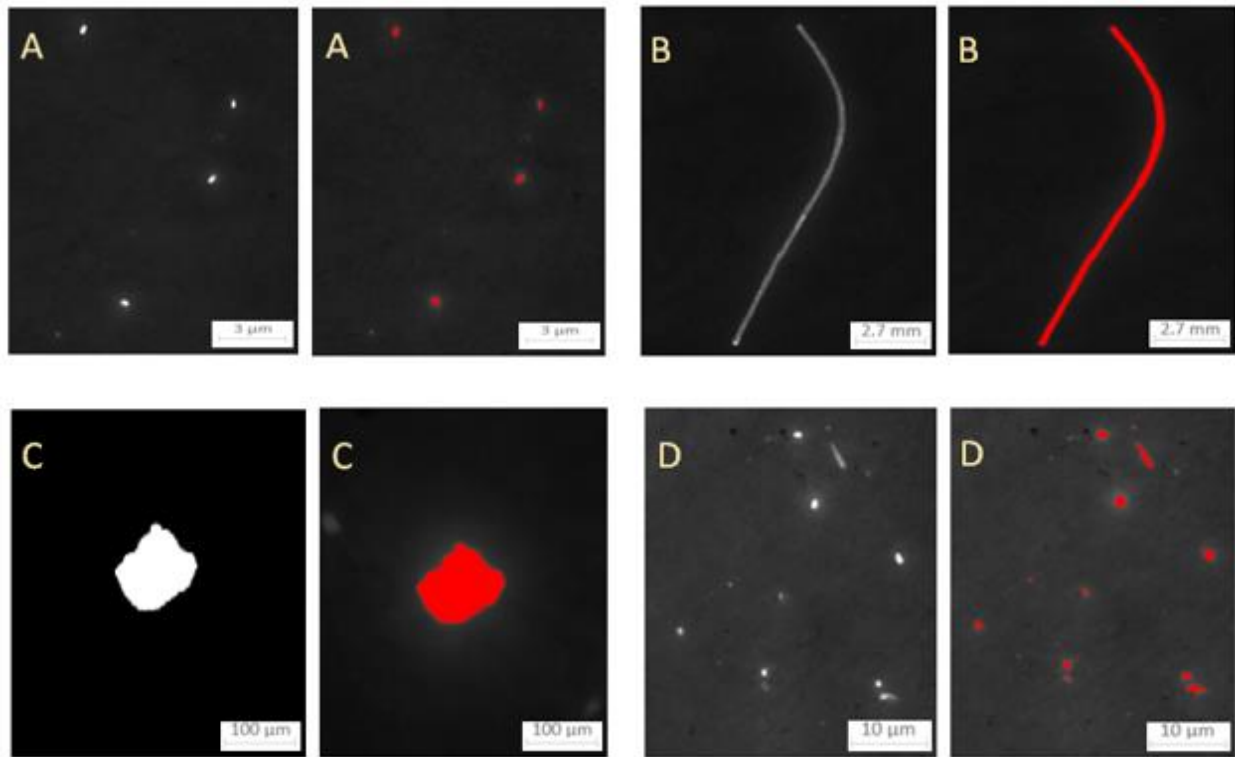


Figure 8: Examples of microplastic particles having different morphology found in Tap water samples. Microplastics appear as red when the sample was dyed with Nile Red. The morphology is classified as pellets in A, fibres in B and fragments in C and D.

Four categories of microplastic types plus an unknown category were identified and the results are summarized in Table 2. 48.6 % of microplastics was identified as fibres, followed by fragments at 41.8 %, pellets 5.7 %, filaments 3.7 % and unknown particles of 0.2 % (Figure 9). Note that 2 fibre particles were identified in the blank samples and was subtracted from the fiber composition before computation.

Table 2. Microplastic morphology in tap water

Sample Name	Fragments	Fibres	Filaments	Pellets	Unknown
State Apt (110)	262	167	7	25	2
Manitoba Apt(3605)	227	103	0	7	0
Campus Rd (3600)	174	201	47	50	0
Hamline Apt (1100)	68	127	7	16	0
530 Tulane Dr	42	316	8	33	3
Clifford Hall	65	21	0	7	0
Police Dept.	35	62	4	0	0
Twamley Hall	33	29	6	4	0
Wilkerson Common	5	72	0	0	0
Chester Library	21	54	3	5	0
Wellness Center	19	47	8	0	0
Robin Res. Hall	47	82	3	0	0
School of Medicine	42	22	1	0	1
Gamble Hall	43	16	0	8	0
Biomedical Res. Fac.	61	11	6	0	0

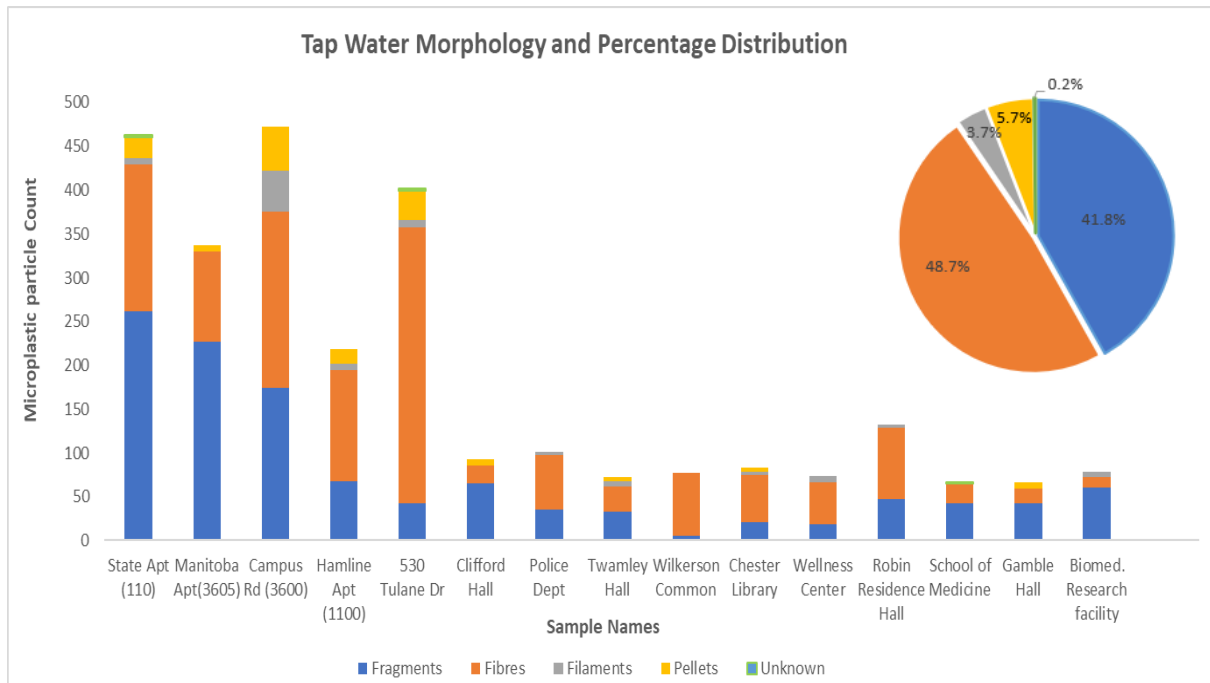


Figure 9: The percentage distribution of particles by morphology in tap water samples

#### 4.2 Microplastic Characterization in Bottled Water

9 bottled water samples were purchased from the grocery store in Grand Forks.

Laboratory analysis was done in 2 batches on separate days. The concentration of microplastics are summarized in Table 3 and Figure 10.



Table 3. Microplastic particles in Bottled water

<b>Sample Names</b>	<b>Concentration (mpp/L)</b>
Dasani Purified Water	121
Voss Artesian water	49
Fiji Natural Artesian Water	52
Great Value Purified water	166
Aquafina Pure water	114
Essentia Overachieving H2O	98
LIFE WTR Purified water	102
Nestle Purelife	127
GlaceauSmart water	79
Distilled Water (Batch)	2

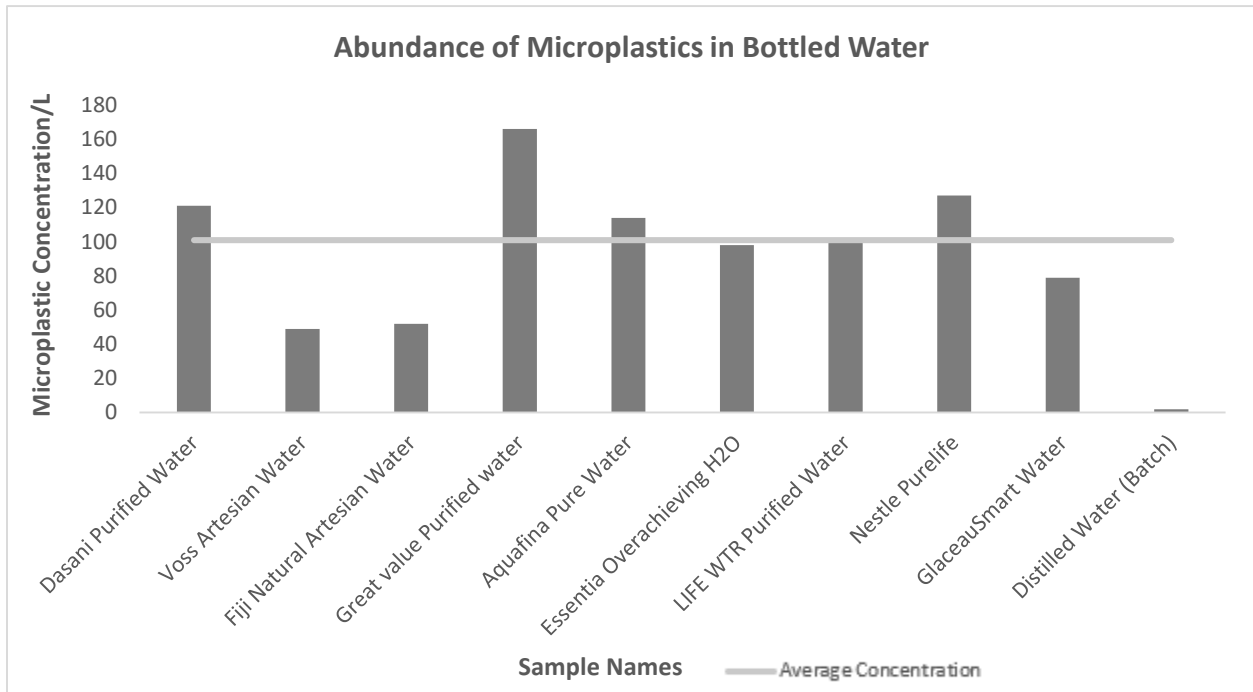


Figure 10: The abundance of microplastic particles found in bottled water purchased from a grocery store in Grand Forks, ND.

Microplastic particles were found in all samples with concentration ranging from 49 mpp/L to 166 mpp/L and average of 101 mpp/L. Great Value purified water had the highest number of particles with 166 mpp/L, followed by Nestle purelife with 127 mpp/L and Dasani purified water with 121 mpp/L. Voss artesian water and Fiji natural artesian water had the lowest concentration of microplastics at 49 mpp/L and 52 mpp/L. The types and sizes of microplastic particles varies (Figure 11). The size of microplastic particles ranged from 5  $\mu\text{m}$  to 1.4 mm. Again, particle sizes < 100  $\mu\text{m}$  were most abundant averaging 93 mpp/L and accounting for 92 % of total microplastics analyzed. Particles of sizes greater than 100  $\mu\text{m}$  averaged 7 mpp/L.

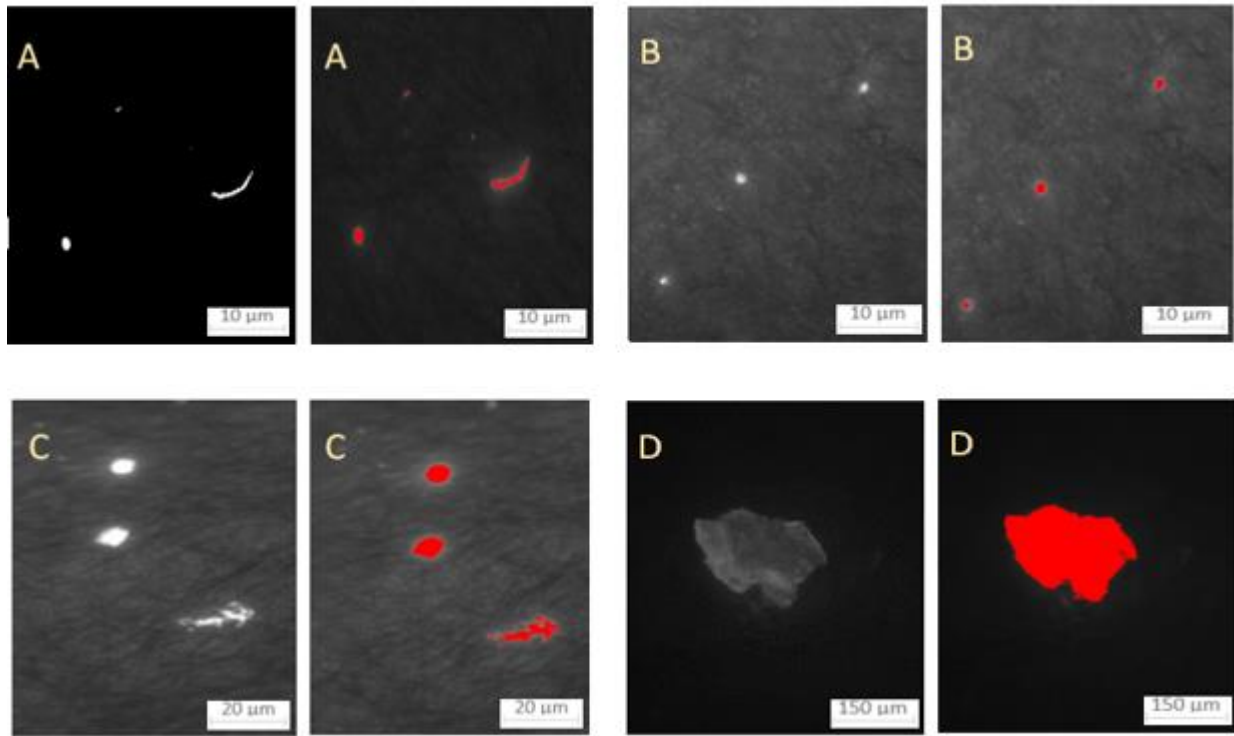


Figure 11: Examples of microplastic particles having different morphology found in bottled water samples. The morphology is classified as fibres in A, pellets in B and fragments in C and D.

Four categories of microplastic types were identified and summarized in Table 4. The percentage distribution of particles (Figure 12) identify fragments as the most abundant with 51.7 %, followed by fibres with 38.1 %, filament 9.1% and pellets 1.1 %. Also, two fibre particles were identified in the blank sample and was subtracted from the fibre composition before computation.

Table 4. Microplastic morphology in bottled water samples

Sample Names	Fragments	Fibres	Filaments	Pellets
Dasani Purified Water	88	17	15	1
Voss Artesian water	7	42	0	0
Fiji Natural Artesian Water	11	38	3	0
Great Value Purified water	82	56	25	3
Aquafina Pure water	30	77	5	2
Essentia Overachieving H2O	64	26	8	0
LIFE WTR Purified water	54	41	7	0
Nestle Purelife	76	35	12	4
GlacéauSmart water	57	14	8	0

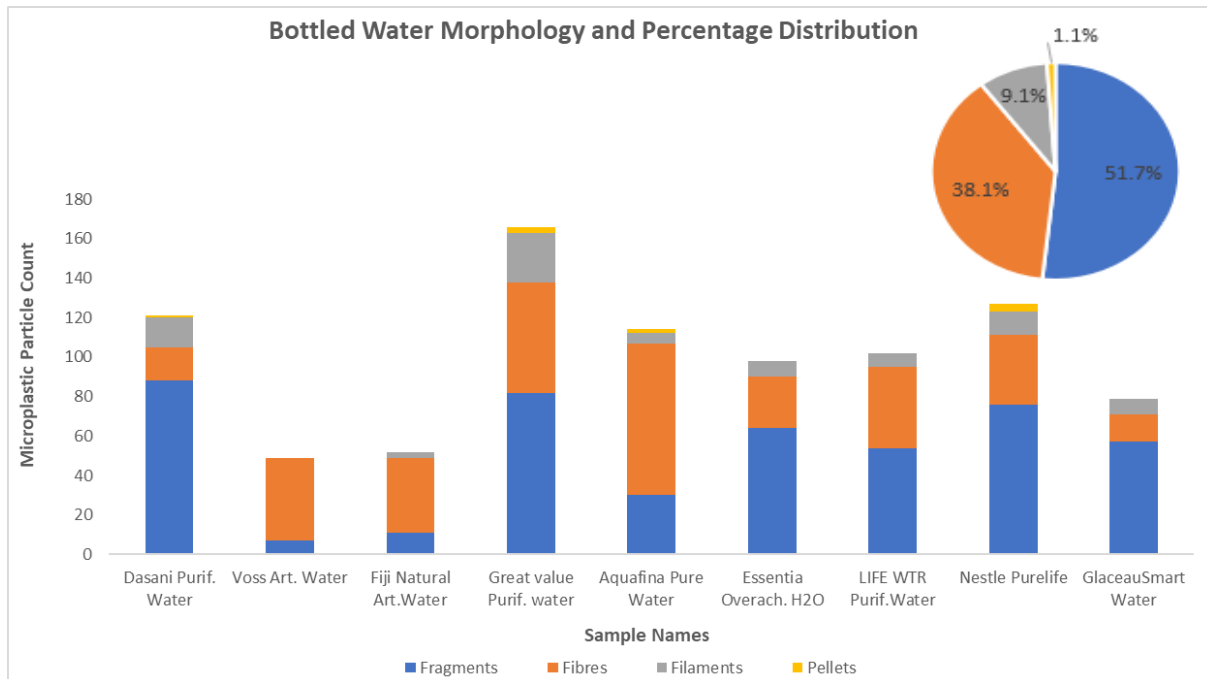


Figure 12: The percentage distribution of particles by morphology in bottled water samples.

### 4.3 Microplastic Characterization in Soft Drinks

Nine popular soft drink brands were purchased from a grocery store in Grand forks and laboratory analysis was done in 2 batches on separate days. The concentration of microplastics are summarized in Table 5 and Figure 13.

Table 5: Microplastic particles in Soft Drinks

<b>Sample Names</b>	<b>Concentration (mpp/L)</b>
Sprite	176
Pepsi	247
Coca-Cola	142
Orange Crush	183
Fanta	81
Dr Pepper	256
Mtn Dew	77
7-Up	169
Diet Coke	102
Distilled Water (Batch)	0

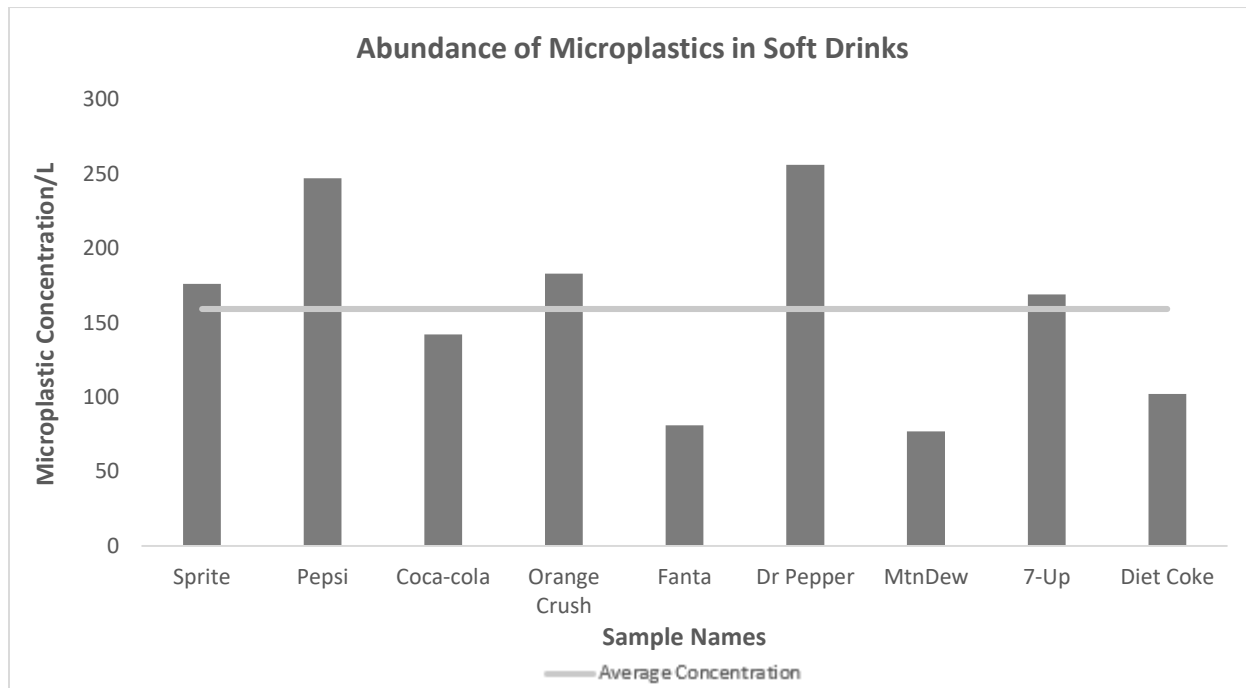


Figure 13: The abundance of microplastic particles found in Soft drinks purchased in Grand Forks, ND

Microplastic particles were found in all brand samples with concentration ranging from 77 mpp/L to 256 mpp/L and an average of 159 mpp/L. Dr Pepper sample had the highest number of particles at 256 mpp/L followed by Pepsi with 247 mpp/L and Orange crush with 183 mpp/L. MtnDew and Fanta samples had the lowest concentration with 77 mpp/L and 81 mpp/L. The types and sizes of microplastic particles varies (Figure 14). The sizes of microplastic particles ranged from 3  $\mu\text{m}$  to 1.2 mm. Particles of smaller sizes (> 3  $\mu\text{m}$  - 100  $\mu\text{m}$ ) were most abundant, averaging 113 mpp/L and accounting for 71 % of total microplastics analyzed while particles of sizes greater than 100  $\mu\text{m}$  averaged 46 mpp/L.

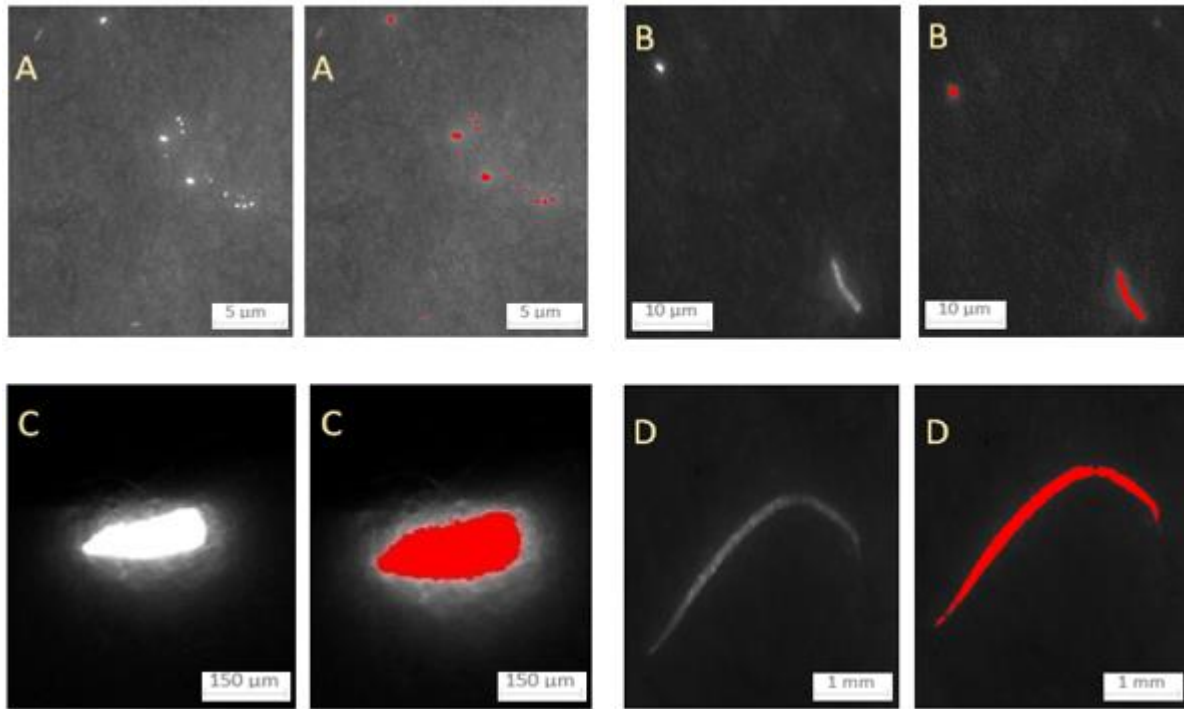


Figure 14: Example of microplastic particles having different morphology found in Soft drink samples. Morphology is classified as Fragments in A through C and fibre in D.

Due to broken light reflector on the microscope, only 29 % of microplastics of sizes > 100 μm were analyzed by morphology. 4 categories of microplastic types were identified with an unknown category summarized in Table 6. The percentage distribution of particles (Figure 15) identify fragments as most abundant with 58.7 %, followed by fibres with 32.2 %, filament with 6.2%, films 1.9 % and 1 % of unknown particles. No particle was found in the blank samples.

Table 6: Microplastic morphology in soft drinks

Sample Names	Fragments	Fibres	Filaments	Films	Unknown
Sprite	11	7	0	0	0
Pepsi	23	35	3	1	0
Coca-Cola	39	3	5	2	0
Orange Crush	11	14	0	0	0
Fanta	32	4	4	0	0
Dr Pepper	46	28	4	2	2
MtnDew	10	37	3	3	0
7-Up	33	3	6	0	0
Diet Coke	41	4	1	0	2

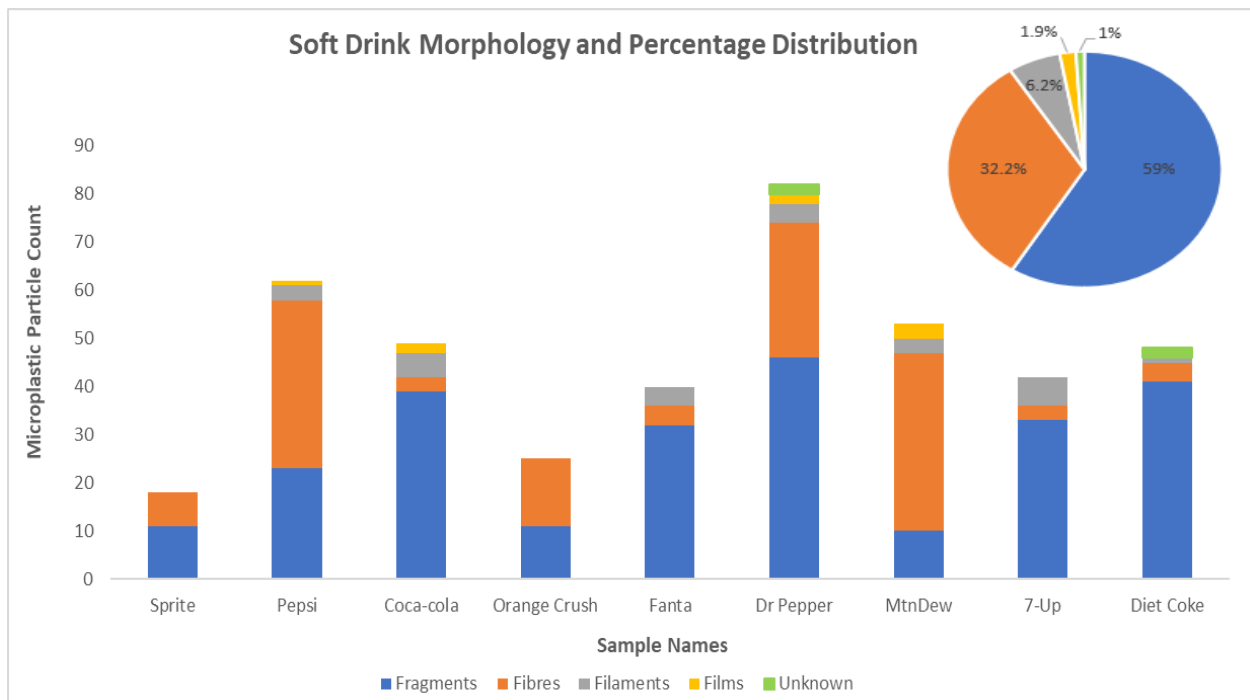


Figure 15: The percentage distribution of particles by morphology in Soft drinks



## CHAPTER 5

### DISCUSSION

Few studies have been documented on microplastic contamination in human consumables. Kosuth et al. (2018), Mason et al. (2018), Pivokonsky et al. (2018), Schymanski et al. (2018) and Salvagente, (2018). In addition to analyzing drinking water samples, this will be the first documented study conducted to characterize microplastics in soft drinks consumed in US. This study act towards acknowledging the need for efficient, in-expensive and reliable method of microplastic analysis with diverse size range. Here, fifteen samples of tap water and nine samples each of bottled water and soft drinks were analyzed for presence and characterization of microplastics using Nile Red dye method of identification. Investigation was done on particles down to 2.5  $\mu\text{m}$  following the procedures and methods used in Mason et al. (2018).

Tap water had the highest average concentration of microplastics with 182 mpp/L (range 66 – 472 mpp/L), followed by soft drinks with 159 mpp/L (range 77 – 257 mpp/L) and bottled water 101 mpp/L (range 52 -166 mpp/L). Also, the concentration of microplastics in tap water collected from private residences (Kitchen sinks) averaging 378 mpp/L were higher than those samples collected from public spaces with 84 mpp/L which is lower than the overall average as shown in Figure 7. This outcome might be as a result of the filtration systems (Elkay's drinking water fountain stations) installed in public spaces within the campus which uses carbon block filter media with spun polypropylene prefilter mesh that helps eliminate

chemicals and particles from incoming water through the system. This indicates that filtration systems are effective in reducing contamination by microplastics in tap water.

The sizes of microplastics falls within  $>2.5 \mu\text{m} - 3 \text{ mm}$  for tap water,  $5 \mu\text{m} - 1.4 \text{ mm}$  for bottled water and  $3 \mu\text{m} - 1.2 \text{ mm}$  for soft drinks. However, microplastics of smaller sizes  $< 100 \mu\text{m}$  were most abundant in all samples occurring at 84 % in tap water, 92 % in bottled water and 71 % in soft drink samples. This results shows the occurrence and abundant distribution of microplastics of smaller sizes in human consumable products and in the environment and therefore, cannot be underestimated. Characterization of microplastic particles (Figure 9, 12 & 15) found fragments and fibres as most abundant in all samples. Fragments accounted for 41.8 %, 51.7 % and 58.7 % in tap water, bottled water and soft drinks while fibres accounted for 48.6 %, 38.1 % and 32.2%. Other categories include filaments at 3.7%, 9.1%, 6.2% in tap water, bottled water and soft drinks; pellets at 5.7% and 1.1% in tap and bottled water; films at 1.9 % in soft drinks and unknown particles of 0.2 % and 0.9 % in tap water and soft drinks.

This results are indicative of the different potential sources of microplastic contamination. Tap water in Grand Forks are sourced from Red River and Red Lake River which cut across communities, golf courses and parks with recreational activities such as fishing, camping etc. Majority of soft drinks and bottled water used in this study were sourced from public water systems (from municipal water supplies to sourcing directly from tap water) in big cities across the US as well as Artesian Wells in the case of Voss and Fiji bottled water (both accounted for the lowest concentration of particles in all samples). Surface run-offs, wastewater effluent (both treated and untreated), combined sewer overflows, industrial

effluent, degraded plastic waste (from littering), atmospheric deposition i.e. emission (could include contamination from machinery, fiber shedding by everyday wear and tear) as well as plastic bottles and caps that are used in bottled water and soft drinks may also be sources of microplastics contamination in the samples.

It is however, worth mentioning that Voss Artesian water and Fiji Natural Artesian water packaged in glass and plastic bottles recorded the lowest concentration of microplastics in bottled water analysis at 49 mpp/L and 52 mpp/L. This results do not justify the contamination of microplastics generated or influenced by bottling types but rather the source of water.

In Table 7, we compared our results with those published by Pivokonsky et al. (2018), Kosuth et al. (2018), Schymanski et al. (2018), Mason, et al. (2018), and Salvagente, (2018). These studies used diverse and wide range of samples; Pivokonsky et al. (2018) analyzed 27 liters of treated water from water treatment plants, Kosuth et al. (2018) analyzed 159 tap water samples from different countries, Mason et al. (2018) also analyzed 259 bottled water from different countries, Salvagente, (2018) analyzed 15 branded soft drinks from Italian grocery stores and Schymanski et al. (2018) recently tested 22 different packaged mineral water from returnable plastic bottles, single-use bottles and glass bottles. On the other hand, our study analyzed 15 tap water samples collected within the campus of UND, nine bottled water and nine soft drinks of popular brands purchased in Grand Forks, ND.

Table 7: Comparison of this study on microplastic abundance with other studies

<b>Tittles</b>	<b>Other Studies</b>	<b>This Study</b>
Sample Source	Water Treatment Plant: Treated water	Tap water
Microplastic Average/L	338	182
Particle Size/ Range	> 1 $\mu\text{m}$	2.5 $\mu\text{m}$ – 3 mm
Dominant Morphology	Fragments and fibres	Fibres
Reference	Pivokonsky et al. (2018)	This Study
Samples Source	Tap water	Tap water
Microplastic Average/L	5.45	182
Particle Size/ Range	100 $\mu\text{m}$ – 5 mm	2.5 $\mu\text{m}$ – 3 mm
Dominant Morphology	Fibres	Fibres
Reference	Kosuth et al. (2018)	This Study
Samples Source	Mineral water from single-use bottles	Bottled water
Microplastic Average/L	14	101
Particle Size/ Range	>5 $\mu\text{m}$	5 $\mu\text{m}$ – 1.4 mm
Dominant Morphology	Fragments	Fragments
Reference	Schymanski et al. (2018)	This Study
Samples Source	Bottled water	Bottled water
Microplastic Average/L	315; 10.4	93; 7
Particle Size/ Range	<100 $\mu\text{m}$ ; >100 $\mu\text{m}$	<100 $\mu\text{m}$ ; >100 $\mu\text{m}$
Dominant Morphology	Fragments	Fragments
Reference	Mason et al. (2018)	This Study
Sample Source	Soft drinks	Soft drinks
Microplastic Average/L	9.5	46
Particle Size/ Range	<5 mm	>100 $\mu\text{m}$
Dominant Morphology	Fragments and fibres	Fibres
Reference	Salvagente, 2018	This Study

The average concentration of microplastics found in this study for tap water (182 mpp/L) is lower than to those reported by Pivokonsky et al. (2018) with 338 particles/L. The difference in concentrations are due to the high number of samples analyzed using small filter pore size (0.2  $\mu\text{m}$ ) during filtration in Pivokonsky et al. (2018) which are capable of capturing more particles especially the smaller sizes (down to 1  $\mu\text{m}$ ) than the 1.5  $\mu\text{m}$  pore size filter used having the

smallest particle size of 2.5  $\mu\text{m}$ . Fragments and fibres were abundant in Pivokonsky et al. (2018) while fibres were predominant in our tap water analysis.

Kosuth et al. (2018) found an average concentration of microplastics at 5.45 mpp/L in tap water with particle size ranging from 100  $\mu\text{m}$  to 5 mm. This concentration is lower than that found in our study with 182 mpp/L and particle size ranging from 2.5  $\mu\text{m}$  to 3 mm, fibres were predominant in both study. In bottled water analysis presented by Schymanski et al. (2018) for single-use bottles had an average concentration of 14 mpp/L with particles size > 5  $\mu\text{m}$  which is lower than that found in our bottled water analysis averaging 101 mpp/L with particle size ranging from 5  $\mu\text{m}$  – 1.4 mm. Fragments were predominant in these studies. The discrepancies in concentration might be partly explainable due to different brands analyzed or the differences in methodology employed in these studies.

The method employed by (Kosuth et al. (2018) uses Rose Bengal stain for particle identification under a dissecting microscope while Schymanski et al. (2018) used micro Raman-spectroscopy, an expensive method capable of detecting and chemically quantifying microplastics and we used Nile Red dye for microplastic detection via fluorescence under an optical microscope. The difference between using Nile Red dye and Rose Bengal is associated to its relationship with plastics. While Nile Red has strong absorption affinity to plastics irrespective of polymer types and size making it efficient in microplastic identification, Rose Bengal only stains organic materials in the sample leaving synthetic materials such as plastics unstained for identification however, fibre particles are underestimated using the latter method as they tend to absorb the stain making them resistance to identification (Erni-Cassola

et al., 2017). This, could further explain the wide differences in the concentration of particles reported by these studies.

Our method of analysis and that presented by Mason et al. (2018) are same but differs with other studies. However, in the order of magnitude, the number of samples (259 bottled water) analyzed in Mason et al. (2018) with average concentration of 315 mpp/L and 10.4 mpp/L are higher than our nine bottled water samples with concentration of 93 mpp/L and 7 mpp/L for particles  $< 100 \mu\text{m}$  and  $> 100 \mu\text{m}$  in size. This variation in number of samples could significantly explain the wide difference in concentrations. Again, fragments were predominant in both studies.

For soft drink analysis, the average concentration we reported (46 mpp/L) is a representation for particles  $> 100 \mu\text{m}$  in size which is higher when compared to those found in Salvagente, (2018) with 9.5 mpp/L. Fragments and fibres were abundant in both studies. The methodology employed in Salvagente (2018) was not reported hence comparison was only limited to the average concentration of microplastics found.

Regardless of the differences between our studies, some similarities do exist; Microplastics were found in all samples analyzed, for all studies smaller particles provide a larger contribution to the total number of particles across all samples and also, fragments and fibres were predominant across all sample types. Our study and others (Kosuth et al. (2018), Pivokonsky et al. (2018), Mason et al. (2018), Schymanski et al. (2018) and Salvagente, 2018) have acknowledged the status quo by presenting valuable analysis supporting the presence of

microplastics in human consumable products (drinking water, salt, soft drinks and other beverages).

## CHAPTER 6

### CONCLUSIONS

We successfully applied the Nile Red stain method of microplastic identification in analyzing 33 samples comprising of tap water, bottled water and soft drinks, as well as in seven blank samples. Microplastics were found in all samples excluding two batch of blank samples used as negative control during soft drink sample analysis. After identification, counting and sorting of particles, tap water samples had the highest concentration of microplastics with average of 182 mpp/L followed by soft drinks with average concentration of 159 mpp/L then bottled water samples with the lowest concentration of 101 mpp/L. Primarily, fragments and fibres were predominant in all samples as well as the prevalence of smaller size particles < 100  $\mu\text{m}$  elucidated by adsorption of Nile Red to confer microplastic identity. Investigation was done on particles down to 2.5  $\mu\text{m}$  in size.

The presence of microplastics in human consumables is troubling especially the high proportion from drinking water. While soft drinks can be reduced or avoided, drinking water cannot be estimated or restricted, yet tap water was the most prominent identifier to microplastic contamination among the three consumables analyzed. Based on the type of microplastics found, it is imperative to identify proposed ways of mitigation.

Upon investigation, water sources for tap, bottled and soft drinks used in this analysis hails from freshwater through municipal and public water supply, making surface run-off,



wastewater effluents and industrial processes (packaging products) the three main sources of microplastics in the samples. Therefore, municipal and drinking water treatment systems should be optimized to effectively remove particles of sizes as microplastics. Municipal wastewater treatment can effectively remove more than 90% of microplastics from wastewater with the highest removals from tertiary treatment such as filtration, conventional treatment when optimized to produce treated water of low turbidity, can remove particles smaller than a micrometer. Advanced treatment can remove even smaller particle; for example, nanofiltration can remove particles  $> 0.001 \mu\text{m}$  while ultrafiltration can remove particles  $> 0.01 \mu\text{m}$  (WHO Information sheet, 2019).

Also, drinking water filtration systems should be encouraged for home owners to considerably minimize exposure to microplastics. The impact of filtration was clearly identified in this study as shown in Figure 7 were the concentration of microplastics found in tap water samples collected from public spaces (with filtration systems) were lower than tap water samples collected from kitchen sinks in private residences.

As observed, over 70 % of microplastic particles are  $< 100 \mu\text{m}$ . Considering toxicological risks for humans after the oral intake of microplastics, especially small particles are of particular concern as they are able to translocate into the body tissue and cause harm (Browne et al., 2008) by penetrating deeply into organs (EFSA, 2016). For these reasons, more research should be undertaken with targeted, well-designed and quality-controlled prospects to better understand the occurrence of microplastics in the water cycle and in drinking-water throughout

the water supply chain by identifying the sources of microplastic pollution and the uptake, fate and health effects under relevant exposure scenarios.

## ABBREVIATIONS

EPA	Environmental Protection Agency
FT-IR	Fourier-Transform Infrared Spectroscopy
HDPE	High Density Polyethylene
LDPE	Low Density Polyethylene
mpp/L	Microplastic particles per liter
MSFD	Marine Strategy Framework Directive
NaCl	Sodium Chloride
NOAA	National Oceanic and Atmospheric Administration
PAHs	Polycyclic Aromatic Hydrocarbons
PBDEs	Polybrominated Diphenyl Ester
PCBs	Polychlorinated Biphenyls
PET	Polyethylene Terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinylchloride

PVPP	Polyvinyl Polypyrrolidone
POM	Polyoxymethylene
POPs	Persistent Organic Pollutants
RM	Raman Spectroscopy
SEM	Scanning Electron Microscopy
UN	United Nations
UNEP	United Nations Environment Programme
WHO	World Health Organization

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