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**Microplastics occurrence and abundance in surface water in
Ecuador**

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Microplastics occurrence and abundance in surface water in Ecuador

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ABSTRACT

Plastic is a light, resistant, durable and low-cost material and has been widely used by industries over the years. When plastic is exposed to environmental conditions, or to human activities, it could degrade and break down into small pieces. Plastics that have a size smaller than 5mm and greater than 100 μm , are known as microplastics. So far, microplastics have been detected in surface waters such as rivers, lakes, and oceans, and even pristine environments. The increase of these particles in ecosystems present a potential threat to both aquatic organisms and humans. In this research, an analysis of surface water in different water bodies in Ecuador, was carried out. During this study, 10 sampling points from the coast, highlands and amazon region were selected. Sample points included high population density and pristine areas. Sediments recollected from superficial water were digested with a dissolution of 1M of NaOH, and filtrated for microplastic counting in a stereomicroscope. Concentration of microplastics ranging from 2-1164,38 particles m^3 was found within the sample points. Most common colors found were blue, black and red, and sizes ranges from 100-1000 μm . Positive correlation between ORP (oxidation-reduction potential) and microplastic concentration was determined; in addition, inversely proportional correlation between dissolved oxygen and temperature was also found. However, no correlation between population density and type of population was determined. Finally, a potential solution was proposed to reduce risk of microplastics intake, which its feasibility was evaluated through a series of interviews to potential customers and financial indicators.

Keywords: microplastics, health, environment, body of water, filter, membranes

RESUMEN

El plástico es un material ligero y resistente que ha sido ampliamente utilizado por las industrias a lo largo de los años. Cuando el plástico se expone a condiciones ambientales o actividades humanas, se puede degradar y romper en pequeños pedazos. Los plásticos que tienen un tamaño inferior a 5 mm y superior a 100 μm se conocen como microplásticos. Hasta el momento, se han detectado microplásticos en aguas superficiales como ríos, lagos y océanos, e incluso en ambientes prístino. El aumento de estas partículas en los ecosistemas presenta una amenaza potencial tanto para los organismos acuáticos como para los humanos. En esta investigación se realizó un análisis de aguas superficiales en diferentes cuerpos de agua del Ecuador. Durante este estudio, se seleccionaron 10 puntos de muestreo de la costa, sierra y región amazónica. Los puntos de muestreo incluyeron sitios con alta densidad de población y áreas remotas. Los sedimentos recogidos del agua superficial se digirieron con una disolución de 1 M de NaOH y se filtraron para el recuento de microplásticos en un estereomicroscopio. Se encontró una concentración de microplásticos que varía de 2 a 1164,38 partículas m^3 dentro de los puntos de muestra. Los colores más comunes encontrados fueron azul, negro y rojo, y los tamaños varían entre 100 y 1000 μm . Se determinó la correlación positiva entre el ORP (potencial de oxidación-reducción) y la concentración de microplásticos; además, también se encontró una correlación inversamente proporcional entre el oxígeno disuelto y la temperatura. Sin embargo, no se determinó ninguna correlación entre la densidad de población y el tipo de población. Finalmente, se propuso una posible solución para reducir el riesgo de ingesta de microplásticos, cuya viabilidad se evaluó a través de entrevistas a potenciales clientes e indicadores financieros.

Palabras claves: microplásticos, salud, ambiente, cuerpo de agua, filtro, membranas

CHAPTER I

1.1.Introduction

Contamination of the environment has become a growing problem over the years, since the massive production of plastics started around 1940s (Cole et al., 2011). Plastics are the most common material used because of its lightweight, durability and resistance to corrosion characteristics (Cole et al., 2011). This organic polymer is derived from the polymerization of monomers which are extracted from oil and gas (Cole et al., 2011). The inexpensive and efficient production techniques, which have been improved and optimized over time, are one of the main causes for its massive manufacture and consumption. Social benefits had reached far expectations, but this valuable commodity has become an important topic on environmental concern (Parker, 2019). Microplastics are defined as small fragments, fibers, and granules with a size smaller than 5 mm (M Cabrera, 2019). Microplastics can be categorized into two groups: primary and secondary microplastics. Primary microplastics are plastics that are produced directly in a microscopic size. This type of microplastics is mostly used in cosmetic and personal care products, but they can also be found in air-blasting media, in medicine as vectors for drugs and virgin plastic production pellets (Cole et al., 2011) . On the other hand, secondary microplastics are characterized by being small fragments derived from the partition of larger plastic debris (Vandermeersch et al., 2015). This fragmentation can be attributed to the use of materials like textiles, paint, car wheels or even plastic material which has been discharged in the environment (M Cabrera, 2019).

Microplastics occurrence in the environment has become more intense along decades. These contaminants have been detected in different aquatic environments like oceans, lakes, rivers, and estuaries (Lambert & Wagner, 2016). They are introduced to these environments by

multiple ways including storm water runoff, wind advection, atmospheric precipitation, and treated wastewater discharges (M Cabrera, 2019). Reports had published that microplastics can be fragmented into smaller pieces called “nanoplastics”. Nanoplastics environmental impacts are different from microplastics. Because of its smaller size, nanoplastics can go through the tissue and accumulate in an organism (Lambert & Wagner, 2016).

As mentioned before, microplastics presence in the environment has gained more importance in the past years, due to the potential threats that they represent to the ecosystem and society. Actual evidence shows that microplastics are present in marine ecosystems and are being introduced in the food chain (Hale et al., 2020). In addition, marine species are currently more exposed to microplastics, as there is an increasing quantity of microplastics in these ecosystems (Vandermeersch et al., 2015). Therefore, efforts on studying microplastic pollution should be strengthened toward the protection of aquatic ecosystems and health.

In Ecuador, microplastics pollution is a relatively new research topic of interest, which is gaining more attention. There are a few thesis projects and research papers that have investigated microplastic pollution in the country. These investigations include characterization of microplastic in the Guayllabamba basin (Donoso & Rios-Touma, 2020), number of particles in surface sea water, beaches and marine invertebrates in the Galapagos Island Reserve (Jones et al., 2021), and occurrence of microplastics in fish sold in a market (Mendoza & Mendoza, 2020). The aim of this objective was to investigate the occurrence and abundance of microplastics in surface water in 10 sampling points in Ecuador, across different regions and types of water bodies. The samples were taken in different water bodies like rivers, shorelines and paramo, with either high or low human intervention. Given the

pollution of the waterbody and human populated area nearness to them, the presence of microplastics vary in quantity.

1.1.1. Objectives

- Determine the occurrence and abundance of microplastics in the selected sampling points from different environments.
- Characterize microplastics found by type, color and range size present un surface waters.
- Identify the relationship of microplastics abundance with population density.
- Identify the relationship of microplastics abundance with the physical-chemical parameters of the waterbody like turbulence, electrical conductivity, dissolved oxygen, oxygen reduction potential and temperature.

1.2. Literature Review

1.2.1. Physical and chemical composition of plastic and microplastic

Plastic pollution is increasing in the world. High densities of plastics are found in marine environments due to anthropogenic activities (Eriksen et al., 2013). Plastics are a potential threat to species and human health, due to its composition and characteristics (Eriksen et al., 2013). It is imperative to understand its composition and diversity, to understand the fate of microplastics in the environment. (Hale et al., 2020). Plastics are classified by their chemical composition, size, shape, texture, and their fate after discard (Hale et al., 2020). Due to plastic versatility and attributes, these synthetic polymers have replaced other materials. Some of their uses include single-use food containers, beverage containers, furniture, toys, fabrics, medical devices, among others (Hale et al., 2020).

Plastic is composed by a long chain of organic polymers. The polymer chains that form plastics are generated by the combination of chemical monomers, into repeated units known as strands, which are derived generally from fossil fuels (Hale et al., 2020). The finished products can be homogenous in relation with the constituent polymer or various types of plastics can be blended to achieve desired characteristics (Vandermeersch et al., 2015). Density of plastic will change depending on the composition. Low-density polymers which are used commonly in single-use containers, are principally polyethylene and polypropylene, while other polymers like polycarbonate, polyvinyl chloride, terephthalate are denser (Hale et al., 2020). Depending on the characteristics mentioned, the fate of plastics in the environment will vary, so predictions on plastic transportation might be misleading (Hale et al., 2020).

Microplastics are categorized in primary and secondary microplastics. Primary microplastics, are the ones that are intentionally manufactured microplastics. It includes microbeads which are extensively used in personal care products for exfoliating, cleaning agents, paint, etc., and as industrial abrasives for delicate surfaces (Long et al., 2019). Secondary microplastic are more abundant and are generated from the partition or fragmentation of larger plastics during its usage or even after disposal (Hale et al., 2020). The half-life of plastics also depends on the polymer type and the ambient conditions they are exposed; it can vary from days to years or centuries (Hale et al., 2020). In addition, microplastics are classified by their shape and size. It is important to consider the size of the particles due to the close relation of the superficial area and the volume of the particle which lead to a high potential of leaching and absorption of chemical products (additives) (M Cabrera, 2019). The 5 categories of

microplastics based on their form are: flakes, fragments, fibers, films and spheres, as it is shown in Figure 1 (M Cabrera, 2019).

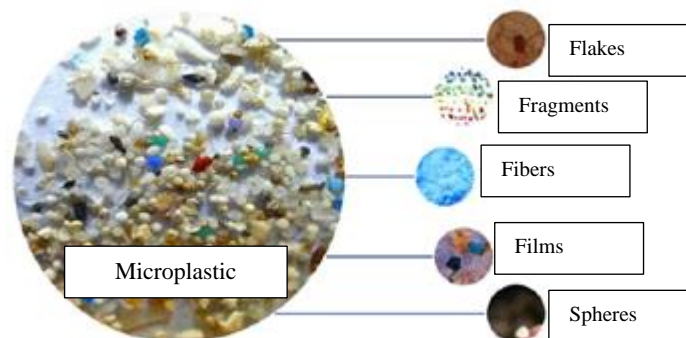


Figure 1. Categories of MPs depending on the shape.
Source: Retrieved from Cabrera (2019)

1.2.2. Microplastic sources and transportation

Microplastics in water environments are principally caused by the discharge of effluents after treatment (M Cabrera, 2019). Table 1 shows the most common microplastics found in wastewater treatment plants, according to the types and uses (Cabrera, 2019). Plastics partition or breakdown can be caused by a combination of environmental factors and conditions to which they are exposed, like photo degradation, oxidation and mechanical abrasion (Ryan et al., 2009). During the photo degradation phase, sunlight oxidizes the material, which leads to a bond cleavage causing a reduction of the molecular mass of this polymer, so the original plastic becomes brittle and can be disintegrated (M. A. Browne et al., 2007). Microplastics are generated in the terrestrial environment by the use and consumption of plastic products and the generation of waste (Koutnik et al., 2021). They are released from hotspots and conveyed by wind and water via surface runoff, canals and rivers, and are deposited on sediments, soils and surface waters (Koutnik et al., 2021). The presence of microplastic in the environment is caused due to the deterioration of discarded plastic

materials or products by physical, chemical and biological processes, or are even directly release into the environment from the consumers of products such as body scrubs via wastewater, fibers are commonly released during fabric washing and other sources (Koutnik et al., 2021).

Table 1. Common plastics mostly found in wastewater treating plants

Type	Use
PES	Textile fibers
PET	Microbeads for care products
PE	Bags, bottles
HDPE	Bottles, caps, domestic materials
PVC	Tubes, clothes, bags and fabrics
LDPE	Bags, food packaging
PP	Microbeads for care products, packaging films
PS	Plastic tableware
PA	Fabrics, toothbrush, threads
ABS	Cases
PC	Discs
PU	Fabrics
EAA	Resin

Source: Cabrera (2019)

Plastic accumulation at waterways and marine ecosystems is increasing due to the its easiness of transport. Plastics are lighter than other materials such as glass or metal (Ryan et al., 2009). Once the plastics are present at the marine ecosystems, fragmentation starts due to the combination of effects like wave action and the constant abrasion from other particles (M. A. Browne et al., 2007). Research shows the presence of microplastics of materials that were used for clothing, packaging and ropes (M. A. Browne et al., 2007).

Some of the microplastic sources include domestic and industrial products such as body cleansers and house cleaning products (Browne et al., 2007). Regarding cosmetic products, microbeads used in cosmetics are made of polyethylene and polystyrene particles which are

less than 1 mm in diameter (Browne et al., 2007). As it was mentioned before, these fragments are transported within wastewater and they remain after treatment which leads to the infiltration into aquatic ecosystems (Browne et al., 2007). Primary microplastics, can enter to waterways via domestic or industrial drainage systems (Cole et al., 2011). At wastewater plants, macroplastics are trapped and eliminated from the effluent with filters and some small plastics debris will be remaining within the oxidation ponds or sewage sludge, but an important quantity of microplastics will pass through the filtration systems and get into the aquatic ecosystems (Cole et al., 2011). Microplastics are characterize by their lightweight nature and its dispersion potential, wind act as a main factor for this transportation, the air currents contributed to the contamination by plastic on terrestrial environment and water bodies (A. A. Horton & Dixon, 2018).

1.2.2.1. Microplastic transportation

Transportation pathways from terrestrial territory to surface water and the behavior of microplastic at these ecosystems are complex topics to understand, given the different parameters that are involve in these processes. (A. A. Horton & Dixon, 2018). Plastics materials can enter freshwater environment by many ways like inadequate waste disposal, littering from landfill, and can also be transported from land by wind or surface runoff, so the presence of microplastics in the ocean is currently a topic of study since these aquatic ecosystems are considered the ultimate sink for all plastic (A. A. Horton & Dixon, 2018). Many models have studied microplastics transport mechanisms and fate to marine ecosystems, which can also help to understand the process that influence MPs transportation within freshwater environments (A. A. Horton & Dixon, 2018).

It has been recognized that far from being one of the main conveyor belts for plastic pollution, freshwaters and soils can also act like sinks as they are able to retain the microplastics that they receive (A. A. Horton & Dixon, 2018). Given the proximity and scale of plastic hotspot or inputs, some terrestrial areas and surface water can accumulate microplastics at higher concentration in comparison with oceans (A. A. Horton & Dixon, 2018). The accumulation and presence of microplastics in the environment depends on the source of origin, behavior and the transportation mechanism that takes them to that environment (A. A. Horton & Dixon, 2018).

The currents of winds can act like rapid ways of dispersion and allow microplastics to travel significant distances from their source or origin zone. This transportation pathway is more likely to lead and influence the widespread of MPs due to the less environmental boundaries that can act against this process (A. A. Horton & Dixon, 2018). Air movement is the main influence path to transportation in terrestrial areas and aquatic ecosystems (A. A. Horton & Dixon, 2018).

There is limited data related to the process that involves atmospheric transport and how it is connected or linked to terrestrial and aquatic plastic pollution (A. A. Horton & Dixon, 2018).

The shape and density of plastic play an important role on the transportation and retention in sediments. Many polymers have low densities so they are buoyant and will float, but when they have more denser characteristics they will sink once they get to a water body (A. A. Horton & Dixon, 2018). Size and shape influence accumulation of plastics and retention in the sediments, when particles have more irregular shapes they are more likely to present highly complex settling mechanisms, they will drown from the surface of the aquatic environment and can be easily retained underwater rather than returning to the surface in

comparison to the settling mechanisms of spherical particles which is less complex (A. A. Horton & Dixon, 2018).

Rivers are one of the principal destiny of microplastics, and once they reach this environment they will experience the same dispersion process which mobilize other sediments like sand and silt (A. A. Horton & Dixon, 2018). The velocity of the river flow influence the transportation, given that the greater the energy it has, it can mobilize a greater volume of particles, so they will be able to spread all plastics that were delivered to them (A. A. Horton & Dixon, 2018). On the other hand, when the river presents less energy, microplastics are more likely to settle and sink. Microplastics will be retained within the sediments (A. A. Horton & Dixon, 2018).

The principal point of origin or generation of microplastics are terrestrial environments and freshwater, the majority of studies that have acknowledge this topic had focused on marine environment, but currently more investigation towards this issue has been addressed in other environments. (Liedermann et al., 2018). Freshwater studies had been developing rapidly, but research related to spatial distribution of plastic debris in water columns has not been yet the main investigation topic (Liedermann et al., 2018).

Microplastic studies are focusing mainly on understanding occurrence of microplastics in the different environmental compartments such as terrestrial, waterbodies, marine and atmospheric, but not the links between them (A. A. Horton & Dixon, 2018). The interaction between these compartments can change due to weather and environmental conditions. Abundance and destiny of microplastics will depend on the degree of connectivity with the adjacent environment and can vary in time and space (A. A. Horton & Dixon, 2018). It is important to notice that microplastic transportation is not unidirectional, the dominant

transportation will go from terrestrial area to an aquatic ecosystem, but it also can go in the opposite way due to the conditions present in the environment that can help this dispersion.

The following graph illustrates the plastic cycle, the orange boxes represent plastic destiny or sink, blue boxes are the transport mechanisms, and the arrows show the pathways of MPs dispersion (A. A. Horton & Dixon, 2018).

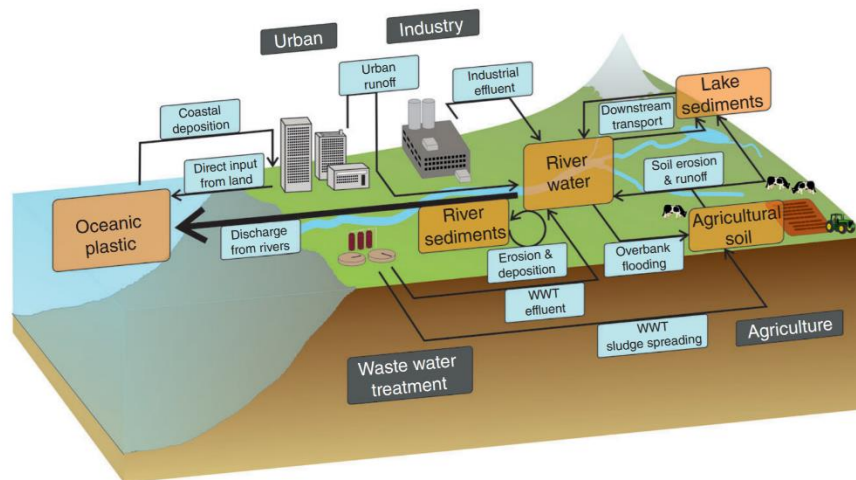


Figure 2. Plastic cycle (A. A. Horton & Dixon, 2018)

1.2.3. Occurrence of microplastic

1.2.3.1. In the environment

Microplastic pollution is a problem occurring all over the globe. There has been evidence of microplastic presence in high populated areas, as well as pristine environments (Mishra et al., 2021). In water environments, high microplastic concentration usually occurs in coastal areas with industrial activity (Sharma & Chatterjee, 2017). Furthermore, microplastic transportation in water bodies is influenced by different dynamics. After fragmentation, lower density microplastics would float in the water surface, meanwhile higher density

microplastics would sink and accumulate as sediment (Wright et al., 2013). The following table 2 presents some studies that have identified presence of microplastic in water bodies.

Table 2. Microplastic occurrence in water bodies around the world

Region	Location	Water body	Mean abundance of microplastics	Human interaction in the environment	Source
Antarctic	Antarctic Peninsula	Sea	1794 particles/km ²	Little to no interaction	(Lacerda et al., 2019)
North America	San Francisco	Bay	700000 particles/km ²	Urbanized area	(Sutton et al., 2016)
	Chicago metropolitan area Higgen's Cr.	River	0.57 particles/m ³	Urban area	(McCormick et al., 2016)
	Laurentian Great Lakes	Lake	450 – 450 000 particles/km ²	Urban and non-urban areas	(Eriksen et al., 2013)
South America	Galapagos Islands	Sea	0.16 ± 0.03 particles/m ³	Little Interaction	(Jones et al., 2021)
	Guayllabamba Basin	River	Between 0.73 – 1584.23 particles/m ³	Between urbanized and little urbanized areas	(Donoso & Rios-Touma, 2020)
Europe	Elbe River	River	5.57 particles/m ³	Mean concentration for mostly urbanize points	(Scherer et al., 2020)
Asia	Bonhai Sea	Bay	2200 particles/m ³	Urbanized area. Densely populated	(Dai et al., 2018)
	Malaysia Miri River	River	10700 – 14300 particles/m ³	Industrial and agricultural activities along the banks.	(Liong et al., 2021)

Other environmental matrix where microplastics are commonly found are sediments. Microplastic in sediments have been analyzed in several studies; they have been recorded in marine sediments since 1970 (Gregory, 1977). Usually, major concentration of microplastics

has been reported in beaches and shorelines. Klein et al., (2015) measured a microplastic concentration between 228 to 3 763 particles kg^{-1} in Rhine River in Germany. Claessens et al. (2011) found a concentration of 390 particles kg^{-1} in the Belgian coast. Some studies have reported higher microplastic concentration in sediments than water,, Liong et al., (2021) found higher occurrence of microplastics in sediment samples than in water samples, with a concentration of a range of 283.75 ± 15.9 to 456 ± 33.6 particles kg^{-1} for sediments and 10.7 to 14.3 particle L^{-1} in the Miri River.

1.2.3.2. In wastewater treatment plants

One of the main sources for microplastic pollution in water are wastewater treatment plans, as they collect influents known to contain microplastic (W. Liu et al., 2021). Therefore, treatment plants are the principal inland microplastic receptor before entering water bodies, treatment plants also convert primary microplastics to secondary microplastics (Sun et al., 2019). It is estimated that WWTP contribute up to 520 000 t per year of microplastics to rivers in Europe (A. Horton et al., 2017). Even though standard WWTPs are not designed for microplastic removal, some research has shown that advanced treatment could improve removal efficiency for this pollutant (Mintenig et al., 2017). The following table 3 shows some studies have measured microplastic concentration and daily discharge in WWTP.

Table 3. Microplastic concentration in influent and effluent of WWTPs

Location	Method of sampling	Influent concentration [particle/L]	Effluent concentration [particle/L]	Daily discharge [particles/day]	Source
Finland	Filtration device	430	9	3.7×10^4	(Talvitie et al., 2015)

China	Steel bucket	80	28	NA	(X. Liu et al., 2019)
Netherlands	Glass bottle	68-910	52	7.5×10^8	(Leslie et al., 2017)
China	Sampling device	6.6	0.6	6.5×10^6	(Long et al., 2019)
Canada	Glass	14	1	10.6×10^9	(Gies et al., 2018)
South Korea	NA	30	0.4	NA	(H. Lee & Kim, 2018)

Source: Extracted from Iyare et al. (2020)

1.2.3.3. In drinking water

Microplastic has also been detected in products consumed by humans, such as seafood, beer, oil, salts, and drinking water (Novotna et al., 2019). The source for microplastic contamination is still largely unknown; although some of its presence is being attributed to contamination of water bodies where drinking water is obtained (Eerkes-Medrano et al., 2019). Around the world, drinking water is estimated to have microplastic within a range of 0-0.057 particles L⁻¹ (Browne et al., 2011). However, some studies have reported larger concentrations. A study done by Pivokonsky et al., (2018) characterized microplastic concentration at inflow and outflow of three drinking water treatment plants, in Czech Republic. It was found concentration ranging from 0.338 ± 0.076 to 0.628 ± 0.028 particles m⁻³, where fragments and fibers being the most abundant microplastic (Pivokonsky et al., 2018). Other source of microplastic in drinking water is suspected to come from bottled

water, as the packaging materials are usually plastic. Oßmann et al., (2018) reporter values of 2.649 ± 2.857 particles m^{-3} in plastic bottles.

1.2.4. Effects of microplastics in the environment

One of the main concerns of microplastics in the environment is the transport of pollutants by adsorption and absorption mechanisms. Plastic has hydrophobic properties which resembles to organic pollutants in aquatic environments, and they can travel large distances through winds and ocean currents, carrying these pollutants even to remote environments (Barnes et al., 2009). Studies have found organic pollutants on the surface of microplastics such as PAHs, Polychlorinated biphenyls and organochloride pesticides (Hodson et al., 2017). However, the level of adsorption of a contaminant depends on the type of plastic and chemistry of the contaminant (Khalid et al., 2021). The presence of chemical compounds like benzene in polystyrene facilitates the diffusion into the polymer, therefore they are considered high sorptive polymers (Pascall et al., 2005).

Consequently, organisms who digest microplastic have a greater chance of being exposed to damaging pollutants. Microplastics are a potential threat at all levels of biological organizations, and as long its abundance increases, its bioavailability for organisms also increment (Auta et al., 2017). The bioavailability of MPs in the environment increases the opportunity of aquatic organisms to ingest them and to accumulate it on their tissues and organs. Accumulation of microplastic usually occurs within higher trophic levels such as seagulls, sea lions, seals, etc.(Romeo et al., 2015). Ingestion of microplastics by marine biota often occur because they are mistaken by food (Lönnerstedt & Eklöv, 2016). Ingestion can cause several chemical and physical damages. It has been documented that ingestion of

microplastic by marine biota can cause physical and mechanical effects such as fixation at the external surfaces, limiting mobility and blocking the digestive tract; on the other hand, chemical effects could be exerted like inflammation or hepatic stress (Setälä et al., 2016).

There is evidence that microplastic can act as an adequate surface for microorganism's biofilm development (Khalid et al., 2021). These biofilms are known as plastispheres, which are considered as a new ecological niche (Khalid et al., 2021). The microplastic abundance offers the opportunity for a variety of species and pathogens to spread to new environments because of the easiness of transport of microplastic. For example, Viršek et al. (2017) studied the occurrence of *Aeromonas salmonicida*, a bacterium responsible for sickening fishes, in microplastics in the North Adriatic Sea. However, the mechanism by which microplastics can strand other species is still not well understood (Khalid et al., 2021).

1.2.5. Effects of microplastic in human health

There is limited information about the toxicity of microplastic in human health (Vandermeersch et al., 2015). Nevertheless, it is considered that microplastic are a risk for humans health due to their physical and chemical effects on the organism (WHO, 2019). Physical characteristics are associated with shape and size of microplastic, while chemical factors relates with the type of pollutants found in these particles (Campanale et al., 2020).

Within the chemical characteristics, there is a widespread concern that microplastics release additives that were used during manufacturing. Special concerns occur with additives such as bisphenol A and phthalates, which are well known as endocrine disruptors and could bioaccumulation in the body (Oehlmann et al., 2009). Some researchers have proved the

presence of additives like bisphenol A, tetrabromobisphenol A, and phthalate in humans (Talsness et al., 2009). These substances are known for altering the homeostasis of the endocrine system and the way organs respond to hormonal signals. Some of the diseases linked to this issue are breast cancer, prostate cancer, metabolic disorder, asthma, among others (Campanale et al., 2020). However, it is possible that acidic pH found in the stomach, with the gastro-intestinal enzymes could remove adsorbed pollutants from the microplastic surface (Powell et al., 2010).

1.2.6. Additive exposure from plastic pollution

1.2.6.1. Bisphenol A

Bisphenol A is a synthetic organic compound which includes two 4-hydroxyphenyl groups in its chemical structure. This chemical has been used for hardening a wide range of plastics; it is a common plasticizer in the polycarbonate plastic production industry (Cariati et al., 2019). They can extend the shelf life for food or beverage, therefore their wide use for food packaging (Cariati et al., 2019). It reaches environmental matrixes when it leaches from plastic waste; the leachate rate varies depending on conditions and the type of product, for example, BPA leachates easier at higher temperatures (European Commission, 2011).

The General Court of the European Union has established that BPA is a substance of serious concern because of the hormonal-related issues in the human body (Munn et al., 2003). In addition, other diseases have been linked to BPA exposure such as obesity, cardiovascular problems and cancer (Cingotti & Jensen, 2019). BPA food contamination is responsible for approximately 12 404 cases of obesity and 33 863 cases for coronary heart disease in children in 2008 (Campanale et al., 2020). The European Union estimates that daily intake of bisphenol A varies between 0.02 and 59 $\mu\text{g kg}^{-1} \text{d}^{-1}$ in adults (Munn et al., 2003).

Regarding microplastic pollution, it is still under debate whether they are a relevant vector for uptake of BPA, since there are limited studies about the issue (Campanale et al., 2020).

1.2.6.2. Phthalates

Phthalates are esters of phthalic acid which have two carbon chains of different length. These chemical compounds are massively produced; it is estimated that their annual production is around 3 million tons (Wang et al., 2018). They are used as plasticizers to enhance properties such as flexibility, durability and elasticity to plastics (Peijnenburg, 2008), usually employed in PVC industry (Pérez-Andres et al., 2017). Other sources of phthalates include cosmetics, perfumes, lotions and varnishes (Meeker et al., 2009).

Studies have proven phthalates presence in various environmental matrices such as sediments, air, superficial water, soil and wastewater (Zhao et al., 2004). One of the most common phthalates found in environmental matrices is DBP (Zhao et al., 2004). This type of phthalate bioaccumulates in invertebrates, fishes and plants; it has been considered that exposure for these pollutants occurs by ingestion, probably by the high migration of phthalates contained in plastic containers (Pérez-Andres et al., 2017). Concentration range of DBP in water has been reported within a range of 1.0 – 13.5 $\mu\text{g L}^{-1}$ and 0.3-30.3 $\mu\text{g g}^{-1}$ respectively (Jin et al., 2015).

Phthalates esters usually leach out easily from plastic because their bond to the plastic matrix does not exist (Talsness et al., 2009). Phthalates such as DBP, DEHP and BBP cause adverse effects in sexual functions, fertility and development; therefore, they have been categorized by the European Chemical Agency (ECHA) as toxic chemicals for reproduction (Peijnenburg, 2008). Studies have shown a daily exposure of DEHP of 3 $\mu\text{g kg}^{-1} \text{d}^{-1}$, and 0.4 $\mu\text{g kg}^{-1} \text{d}^{-1}$ for DBP, with a daily ingestion of 0.7 and 0.1 $\mu\text{g kg}^{-1} \text{d}^{-1}$ for DEHP and DBP

respectively (Schechter et al., 2013). According to Wang et al. (2018), children are more susceptible to phthalate exposure, especially during early growth.

1.2.7. Microplastic research in Ecuador

In Ecuador, there has been few studies regarding microplastic pollution. A 10-year study performed by the International Organism of Atomic Energy (OIEA for its acronym in Spanish) analyzed the pollution of microplastics at the Eastern Tropical Pacific Ocean (Orayeva, 2020). The aim of this investigation was characterize microplastic presence in the Pacific Ocean (Leslie et al., 2017). Alongside with the support of the ESPOL and the National Fisheries Institute of Ecuador, the OIEA analyzed the compiled data of past expeditions and observation of plastic findings at four stations of the Ecuadorian coast near Esmeraldas, Puerto López, Salinas and Santa Clara (Orayeva, 2020). The microplastics in this investigation were classified into 3 types: fragments, fibers, and films. It was concluded that fibers were the most common particle of plastic present at open sea, and they can travel up to 10 000 km in the Pacific Ocean to reach remote areas like the Galapagos Island (Orayeva, 2020).

Indeed, microplastic has been found in the Galapagos Island Reserve. Jones et al., (2021) analyzed the occurrence, composition, and environmental drives of microplastic contamination in the marine ecosystem, analyzing marine invertebrate's uptake. They found that surface sea water microplastic concentration was between 0.04-0.89 particles m^{-3} , with the highest concentration found at the harbor. Regarding marine invertebrates, it was confirmed uptake of microplastics by the analyze species, having a mean incidence of 52%. Giant barnacles (*Megabalanus peninsularis*) and Pencil Urchins (*Eucidaris galapagensis*)

presented the highest concentration of microplastics (Jones et al., 2021). Main source for microplastic pollution was assumed to come from the continent (southern part of Ecuador and northern part of Peru) (van Sebille et al., 2019), where Humbolt's current could be one of the major driver to plastic accumulation.

Another study quantified microplastics in fishes called "Peces Pelágicos", done in the city of Manta. These fishes are the most commercialized in the market at the city (Mendoza & Mendoza, 2020). Microplastics found in the intestinal tract were divided by size and color (Mendoza & Mendoza, 2020). Guidelines from Ministry of Environment and Rural Marine Environment of Spain were used for analyzing the intestinal tract as a methodology, finding a range of 213-338 microplastic particles in the studied species (Mendoza & Mendoza, 2020).

Donoso & Rios-Touma (2020) characterized microplastic presence in the Guayllabamba Basin, including San Pedro, Pita, Guayllabamba rivers in various locations. These locations were selected to retrieve information of rivers near high and low populated areas within the basin. Concentration values ranged from 0.73 particles m^{-3} at Pita River near Pintag (headwater to San Pedro River) to 1584.23 particles m^{-3} at Guayllabamba River. It was concluded that wastewater discharge to these rivers was one of the main sources for microplastic contamination.

Regarding drinking water contamination, there is limited research performed in Ecuador. A study performed by Paredes et al., (2020) determined the concentration of microplastics in drinking water in the city of Riobamba. 62 samples were analyzed along the city. Water system of this city is supported by 7 wells, located at 2 km from the city. Main findings of this study mentioned that 12% of the samples contained microplastic. Source of microplastic

was assumed to be from the storing tanks outside the wells, since they are made from LDPE (Paredes et al., 2020).

These studies have valuable information due to the few investigations of microplastic pollution in Ecuador; therefore, they can be used as baseline for further investigation toward this relevant and important topic.

1.3.Methodology

1.3.1. Sample collection

The sample collection was performed between January 09 to February 13 of 2019. The aquatic ecosystems selected for this investigation were chosen by USFQ experts considering either contaminated or pristine ecosystems. The manta net used for collecting the samples has a filtering size of 300um, which means that any particle bigger than this size will not be able to pass through it and will be retained. At the sampling site the conductivity, dissolved oxygen, redox potential, temperature and pH were measured and registered. The methodology used for collecting and managing of the samples is validated by the European Union (Marine Strategy Framework Directive, 2008/56/EC).

Table 4. Sampling locations

Area	Latitude	Longitude	Sites	Location	Population classification
Sea	-0.870	-80.550	Crucita	Coast region – Province: Manabí	Low
	0.980	-79.645	Esmeraldas	Coast region - Province: Esmeraldas	Medium

	0.996	-79.660	Las Palmas	Coast region - Province: Esmeraldas	Medium
River	-2.877	-78.957	Yanuncay	Highland region - Province: Azuay	Medium
	0.711	-79.691	Teaone	Coast region - Province: Esmeraldas	Rural
	-0.209	-78.427	Machángara	Highland region - Province: Pichincha	High
	-0.474	-76.981	Napo	Amazon region - Province: de Orellana	Low
	-0.208	-78.420	San Pedro	Highland region - Province: Pichincha	High
	-0.425	-76.988	Coca	Amazon region - Province: de Orellana	Low
	-2.844	-79.150	Páramo Cajas	Highland region - Province: Azuay	Remote
Paramo					

Note: The samples were taken by: M.Sc. Alejandra Valdés Uribe and Ing. Nicolás Saud.

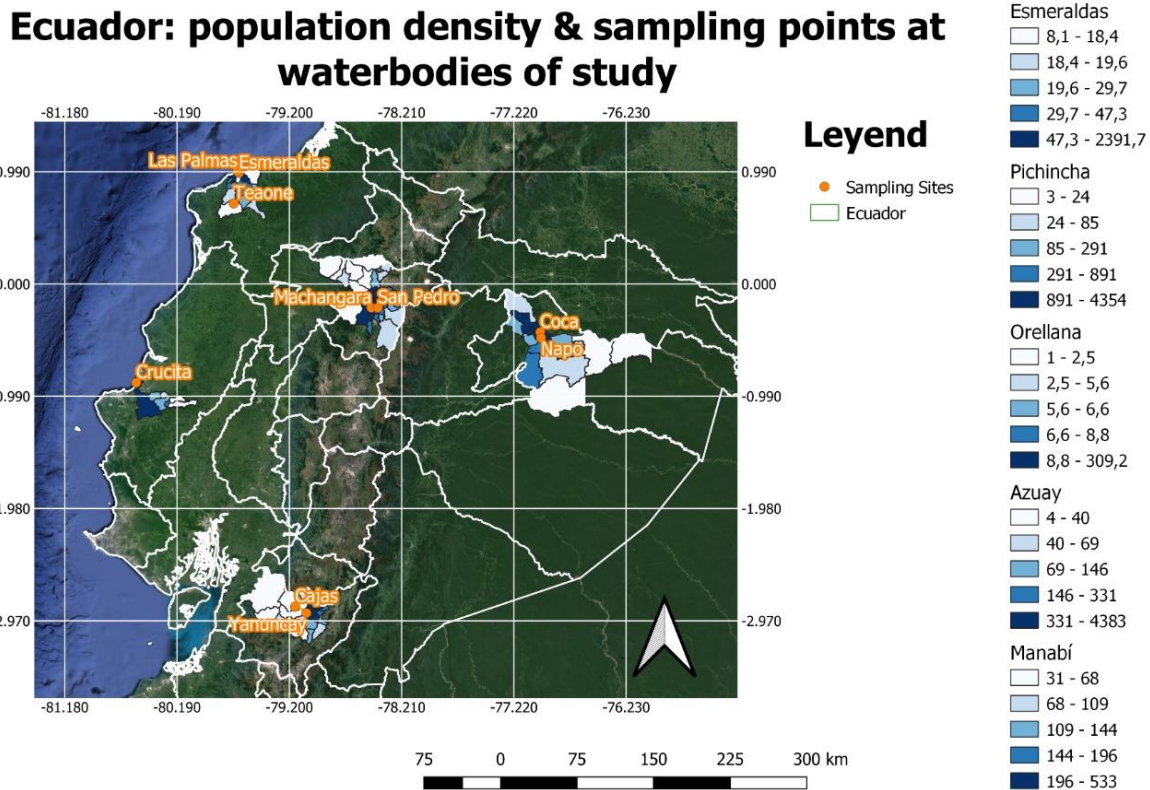


Figure 3. Sampling locations

The determined methodology for microplastics sampling and analysis required that the material used through the different processes do not contain any plastic, so materials such as aluminum, glass and cellulose filters were used instead. Which were carefully rinsed with ultra-pure distilled water before every process. The analysis was carried out at the (USFQ) Environmental Engineering Laboratory.

1.3.1.1. Sampling protocol in marine water

At marine ecosystems sample collection, a boat has used to hold and drag the manta net. The net was unfolded with the face part towards the direction of the movement. A rope was used to hold the net to the boat with a distance of 3 to 4 meters, to prevent contamination coming

from boat engine. The initial coordinates taken from the GPS were registered along with relevant data. A straight path parallel to the coast was followed from the initial point to the final point, and the return to the starting site took around 30 minutes with a 4-6 km h⁻¹ speed. At the end of the sampling, the net was taken off the ocean and was washed with a hosepipe to clean it up. The manipulation of the net represented some challenge, the net was not able to be washed with ocean water in the boat, so it was rinsed with ultrapure water.

After cleaning the net and collecting particles in the elbow, the elbow was removed. Accumulated material was sifted through a 250 µm sieve. The net and elbow was rinsed several times from the outside, in order to remove any adhering material on the net where the MPs can accumulate. The sediments were collected or accumulated in an aluminum tray, and it was kept under refrigeration. The water that fell through the sieve, was collected in a metal tray and was transferred to an amber glass bottle and they were kept in refrigeration during the process.

1.3.1.2. Sampling protocol in river water

For the river sample collection, the red manta (brand: WILDSCO) had 1 m of length and an aluminum frame of (30x45m). The net was positioned manually in the middle of the river and remained stationary with the open part facing the opposite flow of the river stream, without touching the bottom. The sampling time depend on the characteristics of the river and the organic & inorganic load. The minimum time per sample was 2 minutes, and the maximum time was 30 minutes, all the sampling was performed at least twice, and almost six times. The time of sampling for each site is described in Table 5.

Table 5. Total sampling time

Sites	Samples	Time (sec)
Crucita	3	60
Esmeraldas	4	1440
Las Palmas	3	60
Yanuncay	4	720
Teaone	3	1800
Machángara	5	600
Napo	1	900
San Pedro	5	900
Coca	1	900
Páramo Cajas	1	1800

The location of the net in the river was recorded using a GPS. The speed of the river at the entrance of the net was measured using a FLOWATCH/SOFTCS-41112501 equipment. The distance to submerge the net depended on the water body depth, to avoid the entrance of sediments in the bottom. For Esmeraldas River, a boat was used to get to the middle of the river. The boat stayed stationary in the selected point and the net was manually grabbed from the other side of the boat. The net was rinsed from the outside-in with river water, and the sediments and water were stored under refrigeration.

1.3.2. Sample preparation and analysis

The following section will describe the methodology used at each step through the process of preparation and analysis of the water samples. This analysis was carried in three stages: (1) isolation of the particles, (2) classification of the particles in the stereomicroscope.

1.3.2.1. Treatment of sediments and water for the identification of MPs

During the management of samples measurements were taken to avoid any external contamination that may influence the results. All the laboratory material used for this analysis were carefully rinsed three times with ultra-pure distilled water, and the samples were covered immediately to avoid any contamination. The control samples were used as reference to acknowledge the possible contamination that the analysed samples might present during the process previous to the accounting of microplastics in the stereomicroscope.

The organic and inorganic particles that were $> 5\text{mm}$ were removed with metallic tweezers and were washed with distilled water over a $250\ \mu\text{m}$ sieve. The collected particles were mixed again with the sediments. The water that was stored in the amber bottles and passed through an ultrasonication process and filtered through a $250\ \mu\text{m}$ sieve. The material retained on the sieve was mixed with the sediments.

To obtain the dry weight and use the same quantity of sample in the cellulose filters, the sediments were dried in an oven at $90\ ^\circ\text{C}$ for 24 hours. The dry samples were homogenized using a mortar and the dry weight of the sediments was recorded. Samples were labelled with the name of the monitoring place they belong and were stored in the lab for the next steps.

1.3.2.2. Digestion of the samples

The organic matter present in the samples can limit the determination and identification of microplastics. Therefore, a digestion process is needed to remove all organic materials. The digestion process was performed with sodium hydroxide solution 1M (NaOH). To prepare this solution, firstly all the laboratory material required for the process was rinsed three times with ultra-pure distilled water and then 39,997 g of sodium hydroxide was dissolved in 1 L of the same water.

The digestion of the samples was planned every week to avoid cross contamination due to the storage time. For each sampling site, the digestion was made in triplicate, using 0.2 g DW. Glass beakers were used for the digestion. The volume of NaOH solution added to the beaker was 20mL and they were placed in a sorbonne over a heating plate at 45°C for 24 hours.



Image 1. Sample digestion

1.3.2.3. Filtration of samples

Filtration system was assembled using a filtering flask, a Büchner funnel, pump, rubber tubing, cellulose filters, upper chamber, and a clamp. All the materials used in the filtration system were washed with ultra-pure distilled water before and during the process between every sample filtered.

Once the filtration system was assembled, the control sample was mounted. The filter was taken off and placed on the corresponding petri box. The samples that contain the sediment with the solution were dissolved and mixed with distilled water until it reached a volume of 200mL. For each triplicate the sample was divided in 4 filters.



Image 2. Filtration process

1.3.2.4. Counting of microplastics

The MPs analysis was carried out by a stereomicroscope which allows to see particles between the needed range (100 μ m – 5000 μ m). The whole area of the filter was analyzed and each microplastic was counted. Data about the color and sized was retrieved. The type of microplastic was also recorded, with the following classification: (1) fragments, (2) fibers, and (3), films, and the color of the microplastic. All the data was stored in an excel sheet and classified by the sampling site.

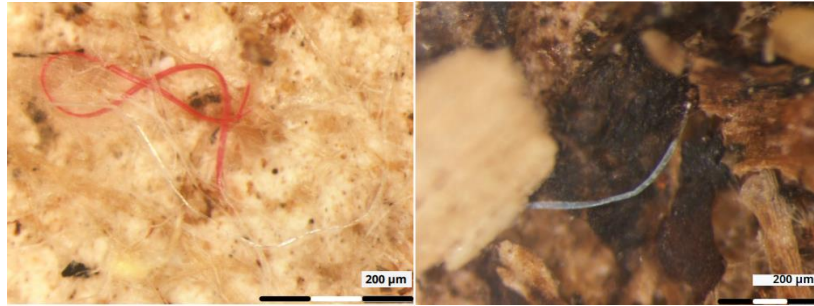


Image 3. Microplastics

1.3.3. Statistical analysis

In order to understand the relationship between microplastic abundance with environmental variables, nearby population characteristics, type of water body, Spearman rank correlation and p-value test was used. All statistical analysis was calculated employing the free software R Studio. For correlation between physical-chemical parameters and microplastic abundance, the Spearman rank correlation was used. Spearman rank correlation is employed to determine the degree of association between two variables; therefore, it can show if there is a statistical relationship between these variables (Hauke & Kossowski, 2011). In order to test correlation between microplastic abundance and characteristics of the nearby population (classification and population density), p-value test was used. P-value is expressed as the minimum non-arbitrary value that can be obtained in order to reject the null hypothesis (Hueso Kortekaas et al., 2021).

Sampling sites were classified according to its position to populated areas in: high populated (more than a million inhabitants), medium populated (between 1 million and 50 000 inhabitants), low populated (less than 50 000 more than 10 000), rural (less than 10 000 inhabitants) and remote (no nearby population). In Table 4, the classification of sites is shown. In Figure 4,

sampling point locations are situated with the distance to populated areas. For this analysis, an area of 1 km² of influence was considered.



Figure 4. Distance from closer populated area to sampling sites

1.4. Results and Discussion

Results microplastic characterization in superficial water from aquatic systems are summarized in Table 6. Table 6 shows that microplastic abundance in particles per m^3 is higher in Yanuncay, Machángara and San Pedro rivers. It is important to remark that in the case for Crucita, high value for standard deviation was obtain due to possible contamination of the samples. Unlike other sites, Crucita's counting of microplastic was resumed from sample 3, from filters done two years before. Therefore, it is assumed that possible contamination could have occurred, which explain differences between microplastic abundance in samples.

Using the dry weight as a basis, higher microplastic concentration was found in the Machángara, followed by San Pedro. Donoso & Rios-Touma (2020) found an abundance of 186,12 particles per m^3 in 7,59 km downstream San Pedro, after several populated areas. This value is lower than the calculated number in this study ($466,67 \pm 134,83$ particles m^{-3}) which could be explained by the location of the sampling point within this study. In Donoso & Rios-Touma (2020) San Pedro's sampling point was located further south, in a location upstream from Quito.

Regarding other studies of microplastic abundance in surface water, values change across the globe, depending on location, sampling season, percentage of wastewater discharge and solid waste management. In Brazil, microplastic abundance was measured in Guanabara Bay, in the metropolitan region of Rio de Janeiro by Olivatto et al., (2019), where this zone receives raw sewage, industrial effluents and petroleum residues. Average concentration found for Guanabara Bay was of 1,40 – 21, 3 particles m^{-3} . In the presented study, ranges of microplastic in coastal ($6,03 - 22,78$ particles m^{-3}) resemble those of Olivatto et al., (2019)

for Guanabara Bay. However, Rio de Janeiro has a population of approximately 6,75 million habitants, while Esmeraldas and Crucita counts with 161 688 and 14 050 habitants respectively and a concentration of $22,78 \pm 3$ and $15,16 \pm 13$ particles per m^{-3} respectively. In Galapagos, average concentration of surface seawater was $0,16$ particles m^{-3} . Nevertheless, Galapagos values are low comparing with other results across other beaches (Jones et al., 2021). In a study done in Macaronesia islands in the North Atlantic, microplastic concentration range was from 21 to 89 particles m^{-3} (Herrera et al., 2020).

Table 6. Microplastic content in shorelines, rivers and paramo of Ecuador

Water body	Sample	Microplastic abundance [particles/g]	Standard Deviation	Microplastic abundance [particles/m³]	Standard Deviation
Sea	Crucita	160,0	136,11	15,16	13
	Esmeraldas	278,3	37,86	22,78	3
	Las Palmas	301,7	37,53	6,03	0,75
River	Yanuncay	193,3	12,58	1164,38	76
	Teaone	128,3	55,30	3,01	1
	Machángara	1853,3	179,54	686,42	67
	Napo	125,0	45	7,97	3
	San Pedro	1800,0	520	466,67	134
	Coca	100,0	15	134,57	21
Páramo	Cajas	53,3	10	2,00	0,39

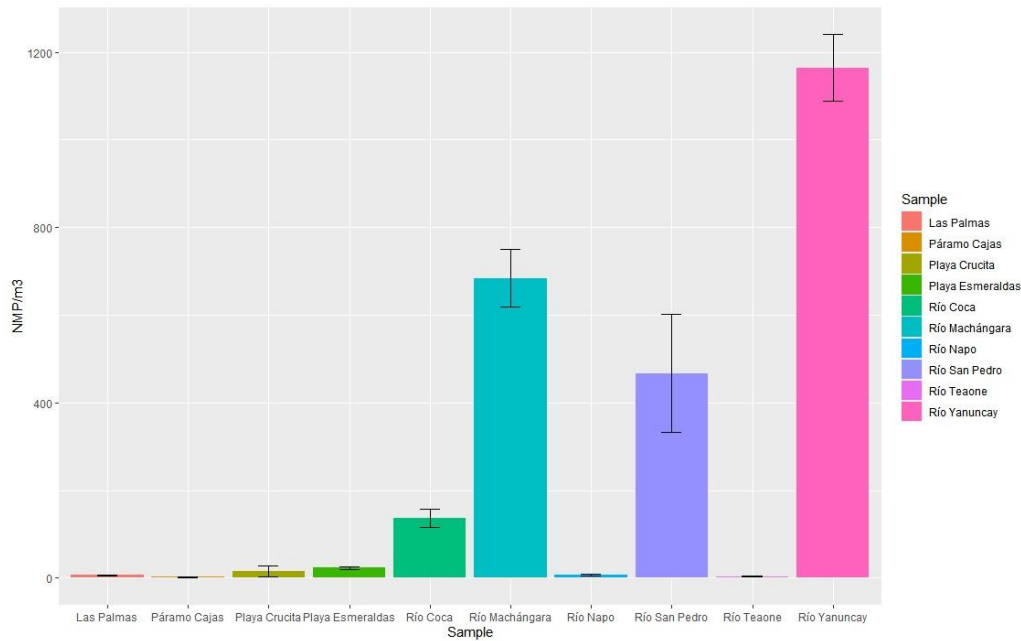


Figure 5. Abundance of microplastic in particles m^{-3}

Lower microplastic abundance was found in Cajas Páramo and Teaone River. In the case for Teaone, nearest populated area was Huele, a small town of 9483 habitants, but located at 509 meters of the sampling point, and at the same level of this town. Therefore, it was a point of low influence of anthropogenic activities, which may explain the lower concentration of microplastic.

In the case of Cajas, microplastic presence was not expected to be very high. As it is seen in Table 6, Cajas Paramo has a microplastic abundance of 2 particles m^{-3} . Cajas sample was taken upstream of a low populated area (9,11 km to Sayausí, a small town of 8474 habitants), and far from Cuenca (17,5 km). In other pristine areas, microplastic presence have fallen into these values. In the Arctic Ocean, South and Southwest of Svalbard, 0-1,32 particles m^{-3} were found in superficial waters (Lusher et al., 2015). Nevertheless, in high mountain ecosystem, lower concentration for microplastic has been found. For example, Cabrera et al., (2021)

found an average concentration of $0,131 \pm 0,02$ particles m^{-3} in Antisana glacier. In a remote mountain Lake in Switzerland (Lake Sassolo), an average concentration of 0,0026 to 0,0044 particles m^{-3} was identified (Negrete Velasco et al., 2020). Parolini et al., (2021) found a concentration of microplastic in snow between $0,39 \pm 0,39$ particles L^{-1} to $4,9 \pm 2,48$ particles per L^{-1} .

For Cajas Paramo, direct pollution from wastewater could not be considered as source of microplastic contamination. It can be inferred that this contamination may be the result of environmental transportation from other sources. In water, transportation of microplastics in the surface depends on the distance, physical characteristics like density and dispersion paths that influence accumulation on water (Scherer et al., 2020); however, in the area of analysis there were not population sites upstream that could involve water contamination.

For high remote areas, winds could be a source for dispersion of microplastic, as it allows them to travel large distance from their origin point (A. A. Horton & Dixon, 2018). Some studies have shown that in fact wind can transport microplastic and deposit them in high mountain ecosystems (Melanie et al., 2021), therefore, atmospheric transport might be the main pathway for microplastic pollution. Microplastic are more easily transported by wind thanks to its size and low density (0.65 to 1.8 $g\ cm^{-3}$) (Brahney et al., 2021). Nevertheless, influence of microplastic abundance by direction and origin of air masses still remains an open question (Marcela Cabrera et al., 2021).

This first analysis shows that a relationship between populated areas and abundance of microplastic could be occurring, but it is not the only source for microplastics in an aquatic system. Cities are one of the main contributors to microplastics abundance of the environment, due to the activities that cause the release or generation of this particles

including the disposal of packaging, use and washing textiles, construction, wastewater treatment, transportation (Moruzzi et al., 2020). Some recent studies have shown that tire and road wear particles might be one of the main sources of microplastics dispersion in the environment, and as the other plastic debris they can be introduced in the marine environment by many paths like air, storm water, road runoff, etc. (Järlskog et al., 2020). However, there were statistically significant differences in microplastic abundance (particles per m^{-3}) with classification of populated areas ($p = 0.029$); therefore, there is no correlation between microplastic abundance within high or low populated area. In the case of population density within the area of influence, again there were statistically significant differences ($p = 0.012$), showing no correlation between population density and microplastic abundance.

Other variable to consider regarding microplastic abundance is wastewater treatment and solid waste management. One of the major sources of microplastic in the environment occur from the inappropriate waste management (Barnes et al., 2009). Furthermore, limited services for collection and operation can lead to population throwing their waste into the environment (Lestari & Trihadiningrum, 2019). Poor management of landfill operation could be a source for microplastics; leachates can contain microplastics and it could filtrate to groundwater and nearby water bodies (He et al., 2019). Nevertheless, other factors should also be taken into account when analyzing source for microplastic pollution. For example, the city of Cuenca is currently treating 95% of their sewage, however, it has the highest microplastic concentration of this study ($1164,38 + 76$ particles m^{-3}).

Regarding colors, predominantly color in most of the surface water was blue, followed by red and black. Blue microplastics represented between 22,07-68,22% of samples; red microplastic between 6,67- 31,89% and black microplastics 5,89 – 25,64%.

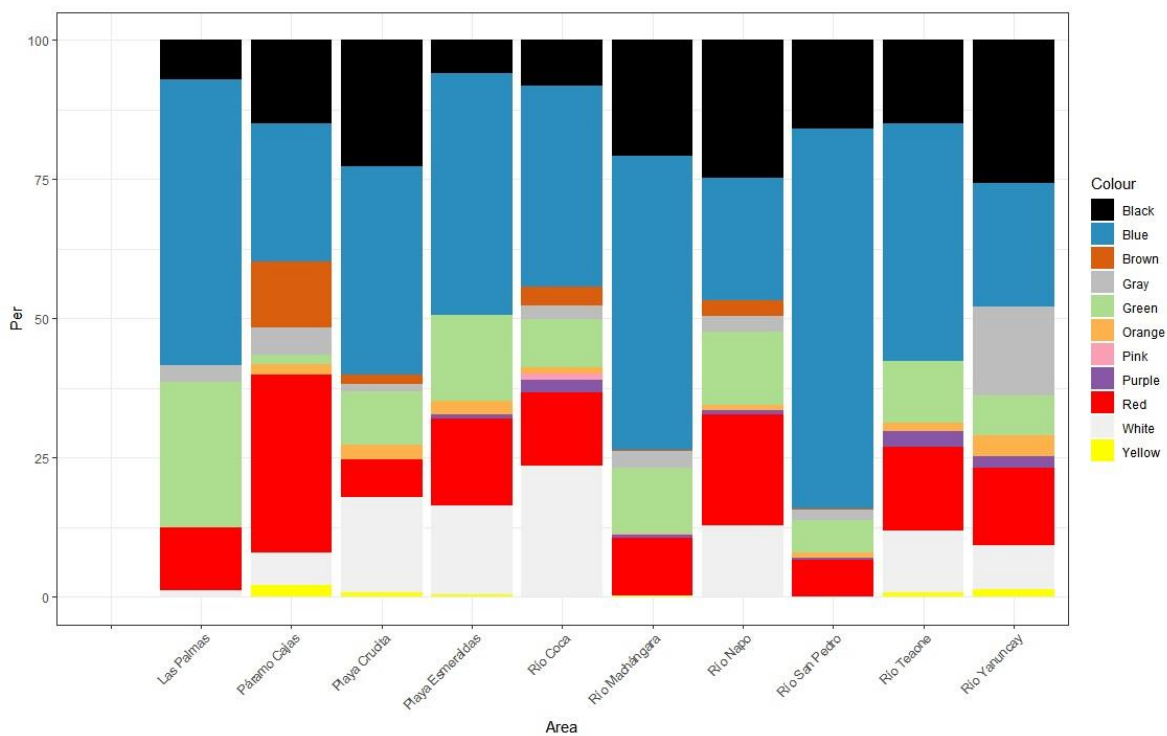


Figure 6. Abundance of microplastic by color

Regarding type of microplastic found, as it is seen in Figure 7, fibers are the most common type of microplastic identified. This result was expected for this analysis, as various studies showed that fibers are the most common type of microplastic found in superficial waters (Browne et al., 2011; Donoso & Rios-Touma, 2020a; Jones et al., 2021). Size and shape is a determining factor for microplastic spatial distribution in aquatic environments, as denser particle may sink, while lighter ones usually float and are easily transported (A. A. Horton & Dixon, 2018). Also, fibers were the most common type of microplastic in high mountain ecosystems as glaciers, such as Tibetan, Alpine and Everest (Marcela Cabrera et al., 2021). Most common type of microplastic identified was fiber for all types of aquatic systems. There was not a statistically significant difference between fiber and water body ($p = 0.07$), which means that there is correlation about fiber frequency in aquatic systems. By analyzing further

this relationship, no statistically significant difference was found between river ecosystems and sea ($p=0.468$); therefore, there could be a correlation between fiber frequency between sea and river environments. However, these correlations must be further analyzed.

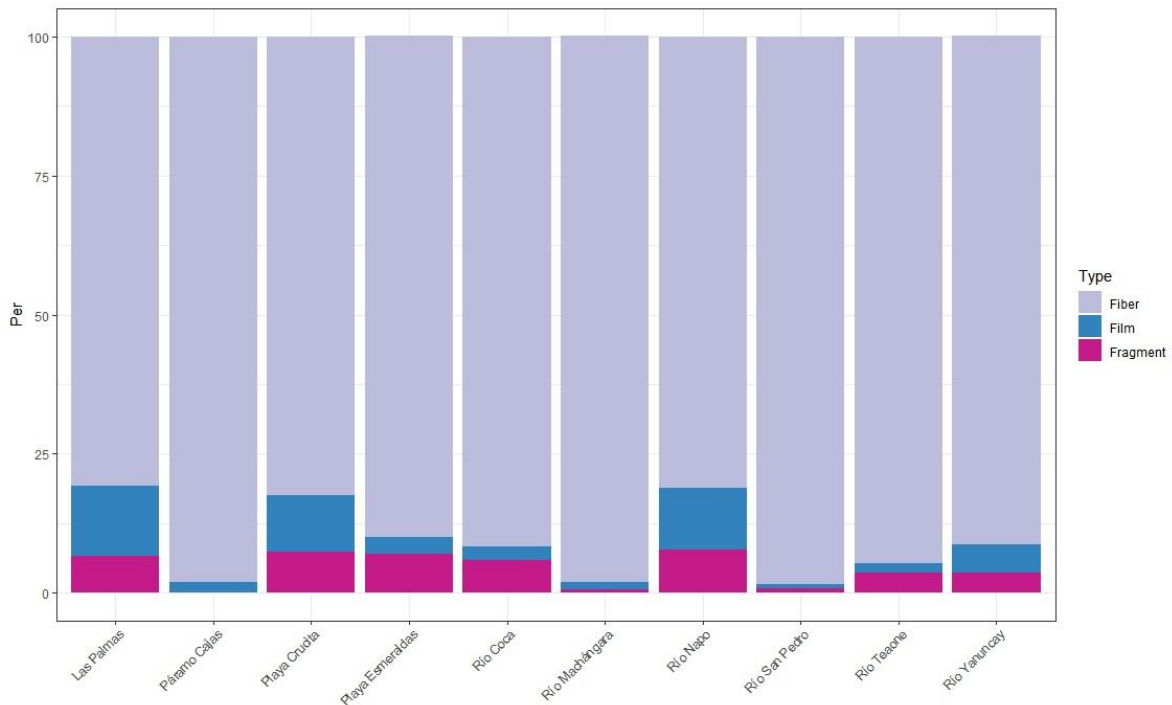


Figure 7. Abundance of microplastic by type

In Figure 8, a heatmap of Spearman Rank correlations was made to show relationship between physical-chemical characteristics and amount of microplastics. In the case of conductivity and turbidity, no significant relation was found. On the other hand, results show that dissolved oxygen and temperature had a significantly inversely proportional correlation while the oxidation reduction potential (ORP) shows a positive correlation with this parameter. In an aquatic system, low dissolved oxygen levels are an indicator of water contamination and water quality (Bozorg-Haddad et al., 2021). Oxidation reduction potential

is a measure that determine the oxidizing or reducing potential of water; positive values indicate oxidizing conditions whereas negative values indicate reducing conditions. It is an indirect measure to determine oxygen levels in aquatic systems; high levels of ORP often means higher concentration of oxygen (Myers, 2019). Therefore, source for microplastic abundance in the analyzed areas could be explained by general wastewater contamination from nearby localities. Nevertheless, by results obtain previously, population distance and density does not directly influence microplastic abundance, as transportation mechanisms should also be included.

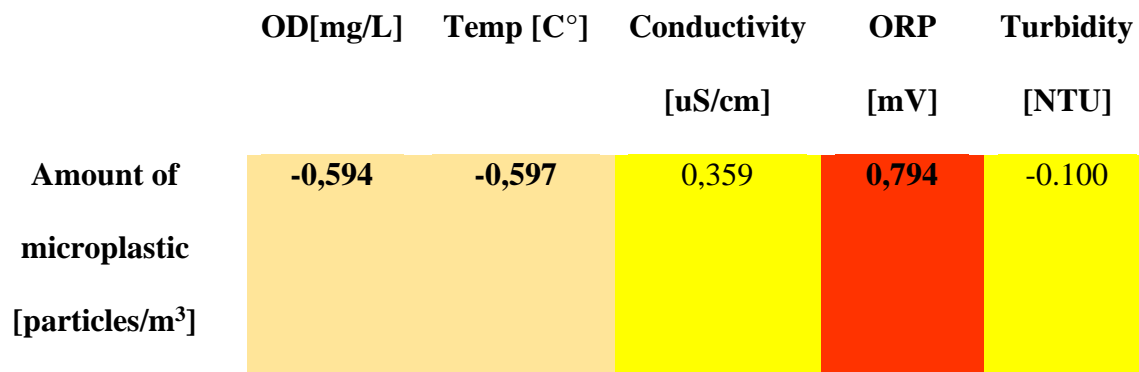


Figure 8. Heatmap of Spearman Rank correlation value of the abundance of microplastic with physico-chemical parameters of water

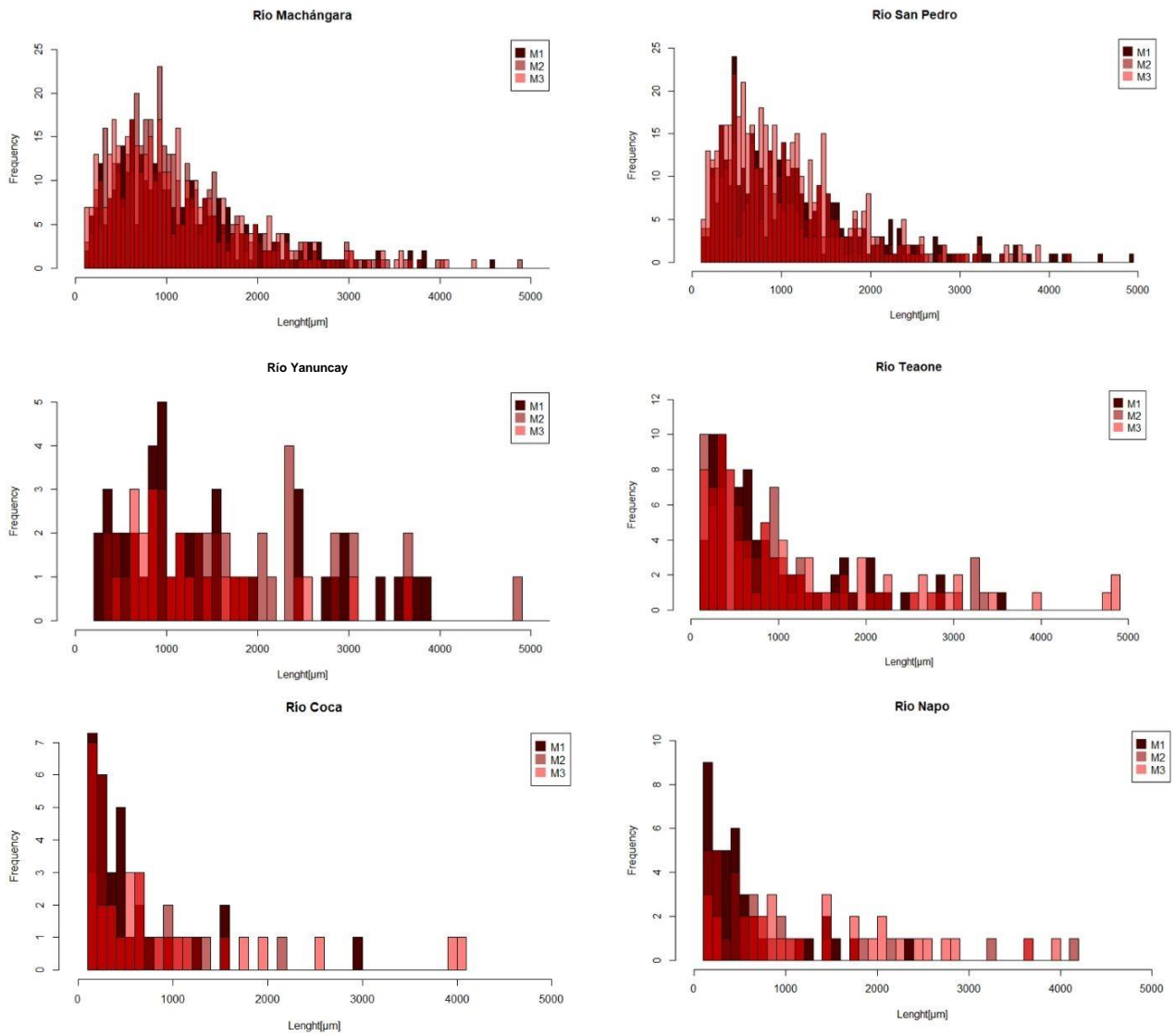


Figure 9. Size distribution of microplastic in river ecosystems

Regarding size distribution, most common range for all the analyzed water ecosystems follows into the 100 – 1000 µm category. In river ecosystems, it is possible to notice that rivers located within high and medium urbanized areas, have higher presence of microplastic within the 500- 1000 µm range. It is inferred that microplastic found have not yet passed through a rough abrasion process, as they are near discharge point for wastewater and

pollution from the population centers. Therefore, it is possible to find larger microplastics. Plastic degradation can occur by ultraviolet radiation, mechanical-physical degradation and weathering (Vermaire et al., 2017), which could have occurred for rivers in low populated areas and rural sites, as microplastic found in these sites probably come from far sources. For coastal areas, a similar situation as rivers could be occurring, as shown in Figure 10.

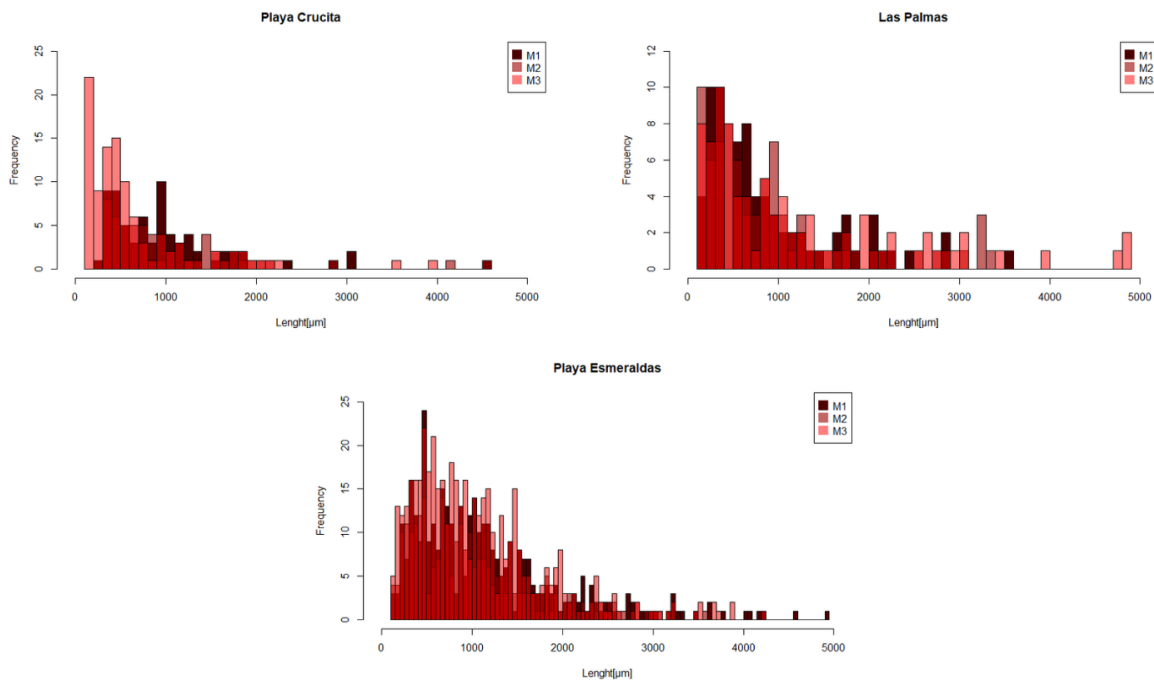


Figure 10. Size distribution of microplastic in ocean ecosystem

A peculiar characteristic that was identified during analysis of Machángara and San Pedro River were a unknown transparent fibers, which were tied up in all over the filter surface. It was not identified as plastics in the first instance, so it was taken to a further analysis to find out its chemical profile. The sample was taken to the SEM (field-emission scanning electron microscope) which analyzed the sample by obtaining its chemical profile and having a better idea of its origin. In figure 12, the chemical profile of these fibers is observed.

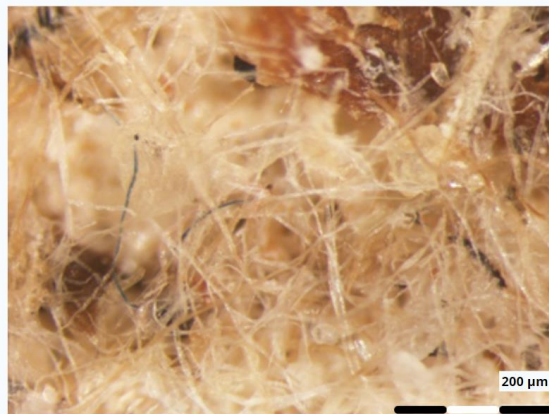


Figure 11. Machangara sample filters

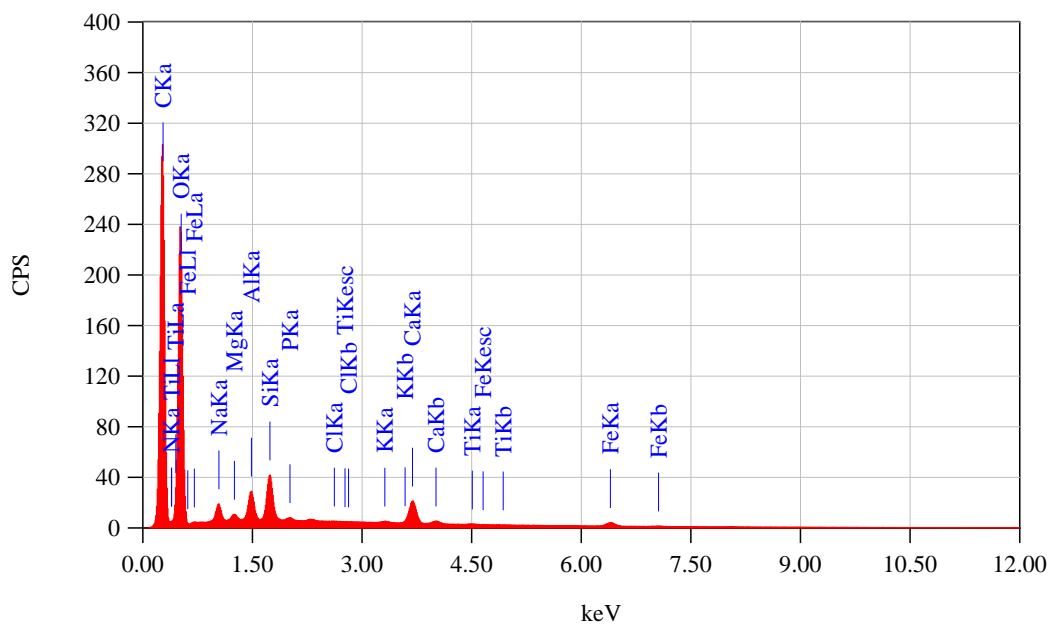


Figure 12. Fiber chemical profile

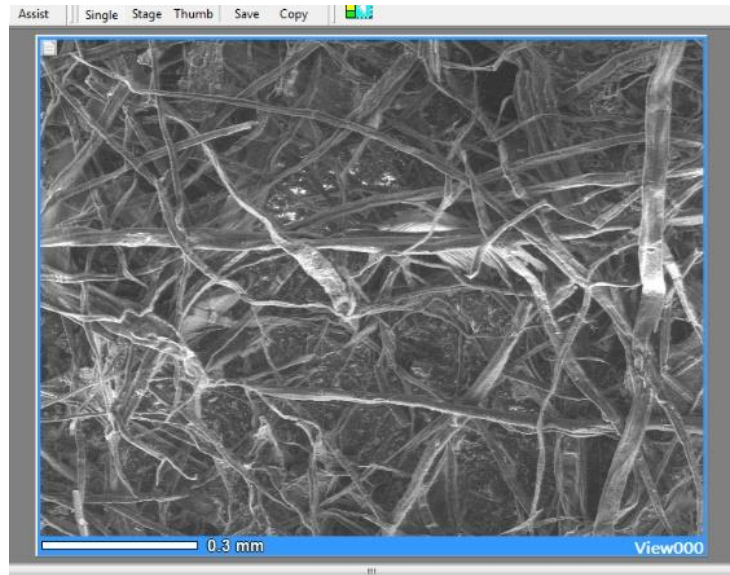


Figure 13. Machángara sample fibers under SEM

In figure 13, the strange fibers can be seen more closely in the photograph taken by the SEM. This figure is useful to compare to other fibers like natural fibers which are described in other papers. With the figure and the chemical profile, it is assumed that this might be a natural fiber which are composed by cellulose and lignin (Saba et al., 2015). Some natural fibers hemp, flax, kenaf and jute (Saba et al., 2015).

Natural fiber reinforced composites are considered a good alternative for replacing materials which depends on petroleum sources. This material is characterized by its low price and availability. They are characterized by better formability, they are renewable, abundant, cost effective and are safer towards health (Saba et al., 2015). Its uses are reflected in several auto-industrial applications, construction, fabrics, ropes and customary products (yarns). SEM images of Kenaf are showed below, showing similarities with the unidentified fiber.

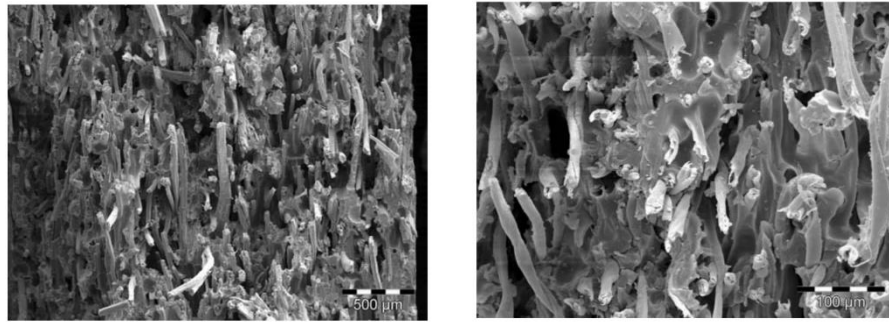
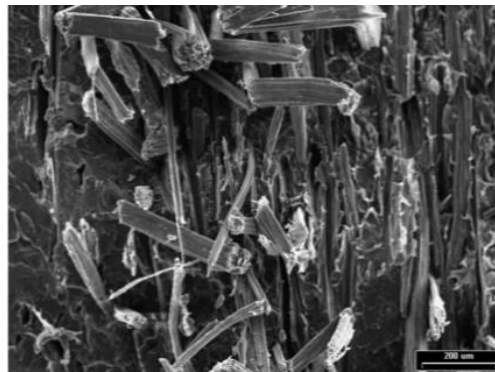


Figure 14. SEM photograph of a fracture surface of kenaf/PLA and cotton/PLA + lignin composites (Graupner, 2008)

In a study done by Lee et al. (2009), they also analyze the natural fiber/polypropylene (PP) composite in the SEM. In this case they used jute with PP (B.-H. Lee et al., 2009). The images that were reported are similar to the ones described before.



(a)

Figure 15. SEM micrographs of the fracture surface of the jute fiber reinforced bio composite with nominal fiber fractions.

1.5. Conclusions and further research

Plastics are the most used material in our society due to its versatility of use, low-cost production durability and resistance. It can be found in different objects in our daily activities. The massive presence of this material becomes a potential threat to the environment if it is not well managed, since plastics do not degrade but fragment into smaller pieces, generating microplastics. These fragments can be generated by the partition of bigger plastics or because of manufacture requirements.

Microplastics represent a problem of pollution in many environments. This material travels into the waterways originated by the bad management of plastic waste. Microplastics can cause important problems in the ecosystems and the species that will scale into potential threat humans' health, such as bioaccumulation and transportation of other pollutants such as heavy metals. This problematic has gained more importance over the years. More investigations are carried out, with researchers confirming its presence in various species intestinal tracts, marine ecosystems and pristine environments.

Indeed, microplastic pollution has been identified in aquatic systems in the country, with values ranging from 2,00 particles m^{-3} at remote sites (Cajas Paramo) and 1164,38 particles m^{-3} at populated areas (Cuenca). Microplastic research around the globe has shown that abundance of microplastic varies because of many variables that influence microplastic concentration. Closeness to population areas, atmospheric transport, aquatic transport, solid and wastewater management are some of the parameters that could influence microplastic abundance within aquatic systems. In this study, population density and closeness alone did not influence alone microplastic abundance, as statistical analysis showed. In order to completely understand microplastic concentration in aquatic systems, all the variables should

be analyzed, specially transport mechanisms. Microplastic transportation in the environment is still an area of uncertainty, which needs more research.

Furthermore, recognition of which type of plastics are found within the sample could be important in order to understand their source. It is important to mention that microplastic identification with the stereomicroscope could fall into subjectivity of the observer, by under or overestimate actual concentration of microplastics. Therefore, it is important that further analysis include other identification techniques such as FTIR spectroscopy and/or Pyrolysis GCMS. FTIR spectroscopy has been widely used for polymer identification, being one of the most common techniques for microplastic identification (Chen et al., 2020). Nevertheless, spectroscopic techniques cannot identify additive presence (Cavagnino & Ladak, 2021). Pyrolysis an GCMS provides a mass quantity identification, determining type of polymers and different concentration over various matrices (Cavagnino & Ladak, 2021).

This first approach to characterize microplastic pollution in the Ecuador could be further analyzed to complement previous studies. It would be important to measure changes in microplastic abundance within seasonal changes, to understand how environmental conditions over year affect concentration. In addition, analysis over the whole water column in various ecosystems and sampling point within hydrographic systems would be a key parameter to understand transport of microplastics in aquatic systems. Furthermore, recognition of which type of plastics are found within the sample could be important in order to understand their source.

CHAPTER II: I-CORPS Product Feasibility

2.1. Introduction

Microplastic pollution is indeed an environmental problem, which is gaining more attention. Therefore, it is important to develop more solutions about microplastic problem. Current efforts to mitigate this problem include the development of filtration systems in wastewater treatment plants, as they are one of the main microplastic sources of water environments (Scherer et al., 2020). In addition, microplastics can have several harmful effects on living organisms including humans, due to their capability of carrying many toxins and persistent contaminants (Li et al., 2018). Therefore, water filtration systems have been discussed as a possibility for avoiding possible microplastic ingestion in some research (Eerkes-Medrano et al., 2019).

With an idea of the current microplastic issue in the country and worldwide, evaluation of possible solutions was analyzed through the National Science Foundation's Innovation Corps (I-Corps) program. This program provides researchers with tools to get insights into entrepreneurship to start a business. Their curriculum integrates scientific investigation and industrial discovery in a data-driven culture, regarding evidence and relevance. Through I-Corps program, researchers could reduce time to translate a lab-size idea into a promising market product (National Science Foundation, 2021).

The course was structure in different modules that covered main themes for a business model canvas, industries insights, pitching the idea, and funding resources. Through 8 weeks, and following a pivoting model, each idea was evaluated around customer needs and problems, by performing 20 interviews to the potential customer segment. The developed business model was done around the feasibility for creating a home filter application, with its

preliminary parameter designs. This filter will remove contaminants including microplastics that could come in potable water from the distribution and storing systems of the country.

2.1.1. Objectives

- Define possible solutions to reduce microplastics risk of ingestion.
- Develop a business model for a filtering system, which can be able to remove microplastics and other contaminants in water.
- Determine financial feasibility for the water filter preliminary prototype.

2.2. Problem definition

Water scarcity is an urgent topic for humans, as it is imperative to provide an adequate supply of clean water to everyone. Water availability and pollution of water sources have become more challenging over the years (Orlove & Caton, 2010), as urbanization processes continue to increase. Plastic presence in the environment (terrestrial, freshwater and marine environments) is a problematic that has been an increasing in the last decades due to poor management of plastic waste (Li et al., 2018). When plastics are exposed to ambient conditions like sunlight, wind, and physical and chemical effects, they are likely to breakdown into microscopic size, which can lead to an easily dispersion (Li et al., 2018).

Ingestion of microplastic occurs mainly through ingestion and inhalation (Campanale et al., 2020). Their biological effects have not been widely studied. (Li et al., 2018). Nevertheless, some research has identified the possibility that microplastic in food could prevent proper digestion of nutrients. In addition, once microplastic are inside the body, they have the capacity to translocate through circulatory system, therefore they could be accessing to any part of the body. They could also cause oxidative stress, which leads to inflammation of tissues (Hueso Kortekaas et al., 2021). Regarding fauna, there is evidence on the significant

consumption of plastic by marine species and they may be retaining in the gut causing physical and chemical effects on the aquatic life (Liedermann et al., 2018). Studies have shown that in aquatic species like fish, the toxins are bio-accumulated and is a potential threat on causing intestinal problems and influence on the metabolic profiles (Li et al., 2018).

Besides health complication associated to microplastic ingestion, microplastic pollution and ingestion could represent losses of economic resources. Some of the areas affected by this issue included fishing industry, marine tourism, oil and gas industries, health and safety organisms (J. Lee, 2015). Nevertheless, according to available research, is not yet possible to quantify precisely annual decline of ecosystems services relation to microplastic debris (Beaumont et al., 2019). In general, marine plastic debris has been estimated to cost between \$3300 to \$33000 per ton of marine plastic per year (Beaumont et al., 2019).

By having previously investigated microplastic issue, the main problem was decomposed to understand its relationship with society, economics and environmental conditions. In this preliminary analysis, 4 different aspects were considered, which are shown on the Fishbone diagram on Appendix A. With all these issues, the main problematic was synthesized into a more specific description with relevant ideas proposed.

- 1. Production and Responsible Consumption:** it depends on manufacturing costs and high demand of plastics due to its versatility.
- 2. Environmental Conditions:** transportation and partition of the microplastics will be influenced by the environmental matrices.
- 3. Treatment of effluents:** complexity of the recovery and treatment of microplastics at the treatment plants due to the non-availability of a specific methodology for treating this material at the facilities.

- 4. Education:** there is lack of knowledge over this issue, massive consumption of plastic material in our society, lack of investigation available on these recent topics.

2.3. Business Model Development

The business model development passed through a several steps of modifications during the progress of the program. The pivoting of the initial idea helps to modify and changed the proposal through the development of the interviews, so it took a different course from the beginning to the final idea.

- The first proposal was the development of a microplastic filtering system that can be added to the final step in wastewater treatment plants in terms to retain any microplastic that may be present on the effluent and avoid its dispersion on surface water.
- The second proposal was the development of a filtering system for food industries affluent. The purpose of this filter was to remove MPs and other contaminants from the water that is used at the facilities through their production processes.
- The final proposal is the development of a filter system that removes microplastics and other contaminants that may be present in the potable water that is used at homes. This filter can easily be added to the sink at home and will also help to balance the pH of the water and the flavor of it.

The business model development followed an iterative method that allowed the pivoting of the main idea. This pivoting was based on a series of interviews performed to potential customers, that allowed the identification of their problems and needs. With the interviews performed, one important pivot was made along the line, which it will be explained in the following sections.

2.3.1. Proposal 1: Wastewater microplastic filters

Hypothesis 1

Companies that own wastewater treatment plants will have to filter microplastics because treated water that is recirculated on its processes must obey certain quality standards.

Hypothesis 2

Food industries are concerned of the quality of their products regarding MPs contamination that may come from drinking water sources or recirculated water which are used in daily processes.

In this stage, interviews were performed to companies that have wastewater treatment plant for their operations. On the other hand, interviews were made to food industries, in order to know if they have an actual concern about the quality of drinking water used for their operations. Question made to this customer segment can be found on Appendix B.

The result for the interviews made for proposal 1 are summarized next:

- Microplastic pollution in wastewater is not a problem they have considered before
- Wastewater treatment is very efficient, and it is not likely it will be replaced in short term.
- Drinking water used obey to quality parameters.
- Microplastic pollution in recirculated water is not seen as a potential threat for industry processes.

The interviews showed that food industries had not much interest about neither a microplastic filter for treating drinking water and nor wastewater in their treatment plant. Industries that were interviewed knew only a few information of this topic and had never made a microplastic characterization of their influents and effluents. They considered that the current treatment process is capable of microplastic removal. The interview results from the proposal 1 influence in the main idea, so it was pivoted into a second proposal, which modifies the filter purpose and the customer segment, as described in the next section.

2.3.2. Proposal 2: Drinking water microplastic filters

Hypothesis

Parents and caregivers are concerned about the drinking water that their children and elders are consuming; therefore, they are interested in finding solutions to get better water.

Survey

For the evaluation of the proposal 2, a survey was performed in order to extend our customer segment and evaluate the interest of the people over this proposal. The survey included people from different regions of the country also embracing cities that do not have a good quality of potable water. It has several questions that help to filter the potential customers into different categories like the family conformation to identify if they lived with children or elderly people and their appreciation to water quality. Questions performed in this survey are detailed in Appendix D.

A total of 187 people answered the survey, from which 126 are currently living with children or elder people, and therefore part of our targeted customer segment. The importance of water

quality was measured with a value of 3 or higher in the rank between 1-5. The sample rate showed that all of the people that completed the survey consider important to have a good water quality at home. Figure 15 shows that the 33% of the people currently have a filtration system at home, this value could indicate possible new customers for the filter.

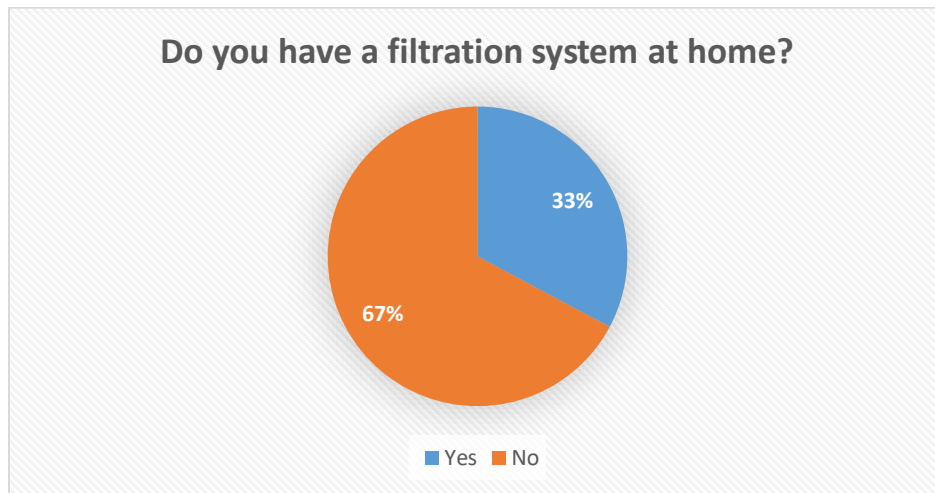


Figure 16. Answer for question 5 of the survey

From those who currently have a filtration system at home, about 40% of the people are not completely satisfied with their current system, which could also represent new potential customers for the business.

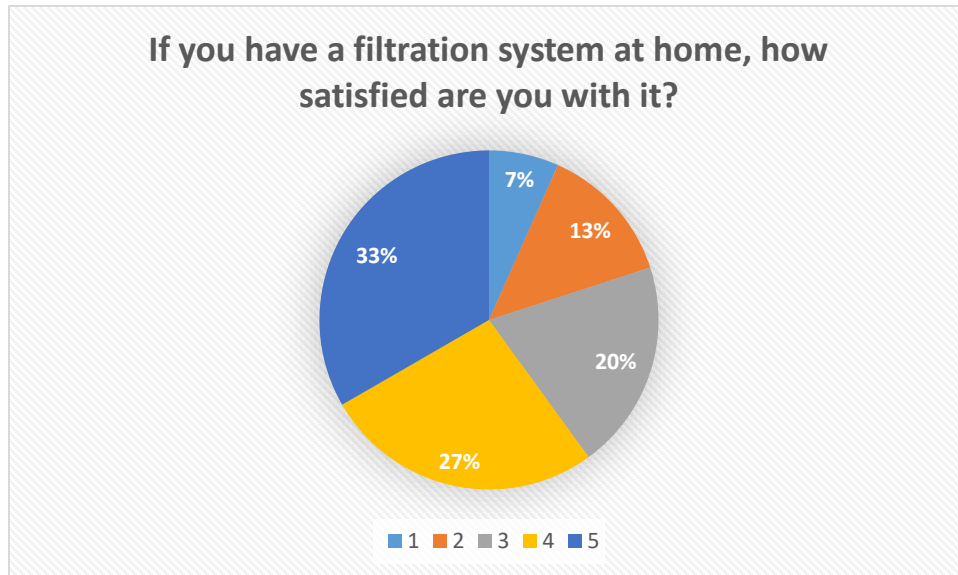


Figure 17. Answer for question 6 of the survey

From those who do not count with a filtration system, Figure 17 shows that about 43% of them are actually planning to acquire one, and 53% of them are considering it. The results obtained from the survey indicate a good overview of potential customers, given that some of them are not happy with their current filtering system and are planning to acquire another one.

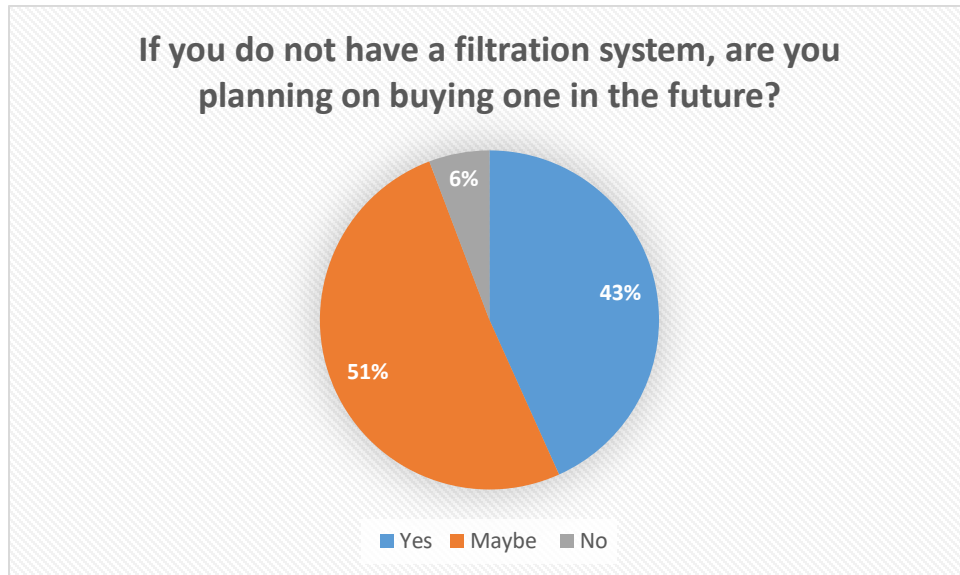


Figure 18. Answer for question 7 of the survey

Another important aspect that was considered, was the knowledge of microplastics presence and pollution, since this was one of the main problems discussed upon the proposal 1. In Figure 18, it was confirmed that slightly half of this sample knew about microplastic and in Figure 19, that more than half of them is worried about microplastic presence in drinking water.

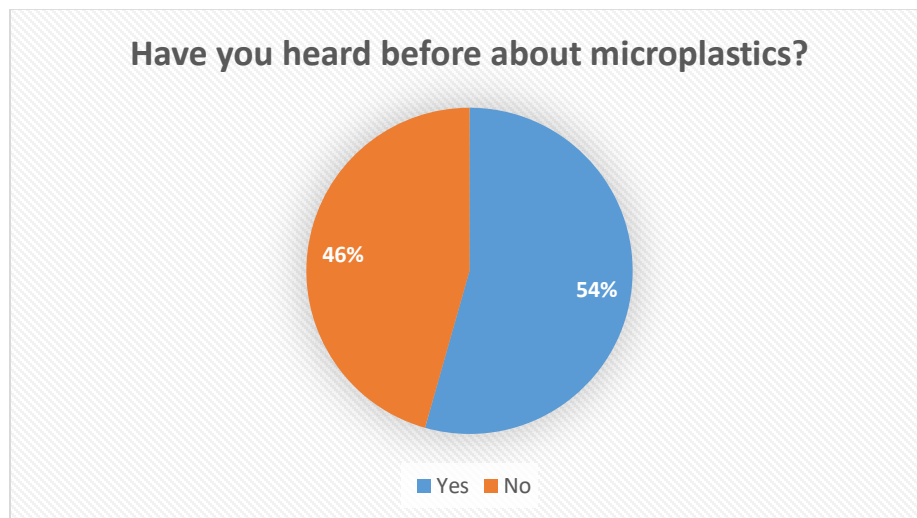


Figure 19. Answer for question 9 of the survey

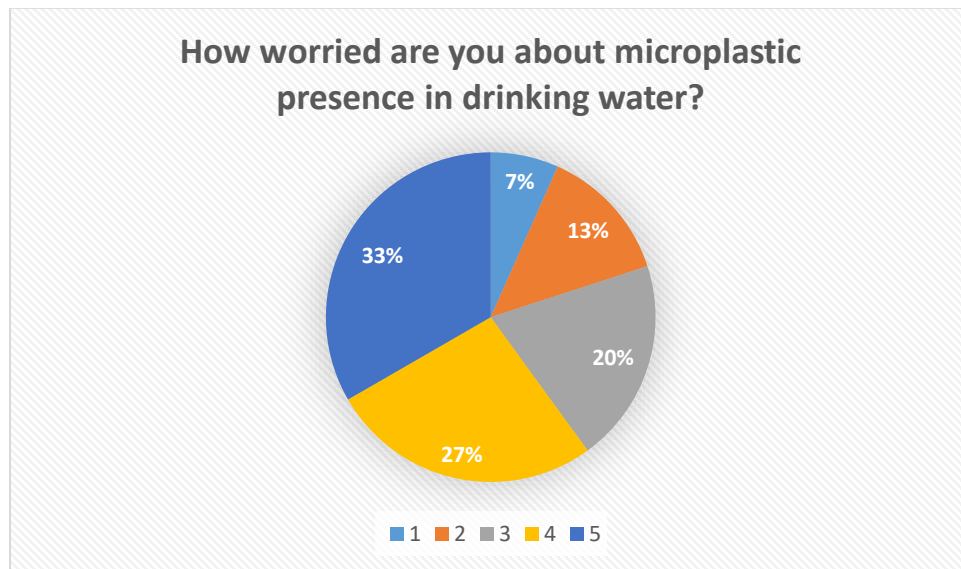


Figure 20. Answer for question 10 of the survey

From data collected in the survey, interviews with potential customers were arranged. People were selected according to their location (preferably outside the capital), their age (older than 25 years old was preferred). Questions performed to this customer segment are detailed in Appendix C. Some important results were retrieved from these series of interviews. The general results for interviews performed to parents and caregivers are described next:

- There is still lack of knowledge about microplastics.
- Parents and caregivers are in fact worried about water quality; however, this is more common outside the capital.
- This customer segment is more worried about other common pollutants of water (coliforms, excess chlorine).

- Some of them considered that it could be possible that microplastic pollution in drinking water will be regulated.
- Value of the filters is one important factor whether to change or not their current filtration system.

From these results, it was concluded that the filter had to include other features for removal of other water contaminants. In addition, it was determined that a cost-effective filter is an important aspects customers consider as valuable for acquiring the product. Finally, since microplastic occurrence in drinking water is still a new issue, it is important that the business model includes awareness of this problem to the customers.

2.4. Ethical and professional aspects of the proposed solution

Access to good quality water is a fundamental human right. Therefore, it is important that users are aware of water quality they are drinking and using for their daily activities. Water security for the community by mitigating potential threats should be the main driver for creation of a water filtration system. As microplastic pollution may be a potential threat to human health, a water filter that gets rid of them and other water pollutants is important for society. Water filter manufacturing process should guarantee the advertised results for its product. Hence, tests must be made in order to verify water quality for the proposed filtration system. In addition, it is important that proposed prices are aligned with actual manufacturing processes and materials.

2.5. Filter preliminary design

As a solution for the microplastic contamination in water bodies, some studies have proposed filters as a final stage in water treatment. F. Liu et al. (2020) proposed a pilot-scale biofilter for treated wastewater from secondary treatment which included biological nutrient removal and clarifiers, in Denmark. The biofilter was a 1 m³ stainless steel column packed with layers of stone wool (1 m), filterlite (0.4 m) and gravel (0.1 m). Results indicated that this biofilm removed 79%-89% of microplastics (F. Liu et al., 2020). Wolff et al. (2021) analyzed the efficiency of a sand filter for WWTP in Germany. The sand filter consisted of a dual filter bed with a first layer of anthracite (1.050 m with a grain size of 0.71-1.4mm) and a second layer of quartz (0.6 m, with a grain size of 0.71-1.25 mm) (Wolff et al., 2021). Removal achieved a 99.2% -99.9% efficiency (Wolff et al., 2021).

Other types of microplastic filters have been produced to filter water for drinking purposes, which was the main focus and results for the business model analyzed in the previous section. This type of filters corresponds to a type denominated point-of-use (POU) water filter. POU water filters are design to remove pollutants at the end of the water system, for example at the faucet or a shower (SU et al., 2009). Despite of their small size, POU water filters designs include the best available technology for water filtration, such as activated carbon in granular or powdered form, for microorganism removal and other pollutants (Synder et al., 1995).

Most of drinking water filters have activated carbon as their main material for filtration. For example, Epic Water Filters USA have developed a filter of activated coconut carbon that removes 99.62% of microplastics (Epic Water Filters USA, 2021). Currently, this filter is used in a wide range of portable water objects. As it was seen in the previous section, microplastic filter is feasible within parents and caregivers, as long as it includes the removal of other water pollutants. In this section, design parameters are going to be discussed more in depth, including information about the chosen filter materials. It is important to remark that it is a preliminary design, which has not yet been tested.

This type of water filtration has some advantages and disadvantages. On one hand, POU filtration is easy to install, usually with straightforward installation steps to add-on to the faucet where it is more needed (EPA, 2006). In addition, they are a more affordable option compared to large water filtration systems. Regarding negative aspects, POU filters are only on-point specific, therefore, are not suitable for the whole water system. In addition, usually they do not achieve VOC removal because of their volatile nature (EPA, 2006).

2.5.1. Preliminary design

By analyzing the best available technologies in the market, it was decided that the preliminary filter design will be formed by a microfilter membrane (for microplastic filtration) and a carbon block. More information about these two technologies will be explained in the following section.

2.5.1.1. Microfiltration

Microfiltration is a separation process driven by pressure (Scott, 1995), usually within ranges of 30-240 kPa (0,3 – 2,4 bar) (Tchobanoglous et al., 2003). Membrane for microfiltration are most commonly used for turbidity reduction, suspended soil reduction and disinfection; it is also considered as a pre-step for reverse osmosis (Tchobanoglous et al., 2003). Microfiltration membranes usually have pore size between 0.1 and 10 μm (EPA, 2006). It is a good way to remove suspended particle, small colloids, some bacteria, ions, algae and sediment; however, it may not be very efficient in removing most viruses. Microfiltration works by size-exclusion, where larger particles are retained in the filter, and liquid or smaller particles pass through. Eventually, accumulation of particles will cause the stopping or restriction of flow, so the filter has to be cleaned or replaced (Scott, 1995).

Some preliminary calculations were done in order to characterize microfiltration design parameters. Equations employed were retrieved from Tchobanoglous et al., (2003). For calculations. Water pressure in faucet systems were consider within a range 300 kPa (3 bars), which falls within the range for houses not over four stores (Steel & McGhee, 2001). In addition, a flowrate of 3 L min^{-1} was used for a household faucet. Type of operational mode configuration chosen was dead-end mode. In this configuration, all of the water applied passes through the membrane, with particles retained over the surface. Usually this types of

configurations are used for low pollutant concentration (Tchobanoglous et al., 2003). Microplastic concentration was set in 4,34 particles L⁻¹ (Novotna et al., 2019). In the following table 7, highlighted rows are the calculated values. Rejection and recovery rates were determined by the designers. Transmembrane pressure gradient, transmembrane water flux rate were taken from Tchobanoglous et al., (2003).

Table 7. Some design parameters for the microplastic membrane

Variable	Definition	Unit	Value
P _{tm}	Transmembrane pressure gradient	bar	2.4
P _f	Inlet pressure of the feed water	bar	3
P _p	Pressure of the permeate	bar	0.6
Q _p	Permeate flowrate	m ³ /h	0.18
F _w	Transmembrane water flux rate	(m ³ /m ² *h)	0.09
A	membrane area	m ²	2
r	Recovery	%	98
R	Rejection	%	85
C _f	Feed water concentration	particles/L	4.34
C _p	Permeate concentration	particles/L	0.65

Membrane area needed for the design (2 m²) should be taken into account when finding or building the microfilter membrane. Permeate concentration (microplastic concentration) should be check when doing test of water quality for the proposed filter.

For calculating P_p ,

$$P_p = P_f - P_{tm} \quad (1)$$

For calculation permeate concentration,

$$C_p = \left(1 - \frac{R \%}{100}\right) * C_f \quad (2)$$

Finally, for membrane area,

$$A = \frac{Q_p}{F_w} \quad (3)$$

It is important to mention that further designs should include fouling processes, implementing iterative models to observe changes in pressure needed.

2.5.1.2. Activated carbon: carbon block

Activated carbon is a material derived from an organic material such as coal, wood, coconut that is subjected to pyrolysis followed by contact with oxidizing gas at high temperatures, having as a result a porous material and large surface area (Tchobanoglous et al., 2003). They are commonly use in water treatment. It is usually employed for organic compounds and free chlorine removal, in addition to odors and taste. They usually require line pressures of at least 210 kPa in order to have good results (Wu et al., 2017). There are two types of activated carbon: granular activated carbon (GAC) and activated carbon block (ACB). For the designed preliminary filter, ACB was chosen as a material for chlorine and microorganism removal.

2.6. Business Model Canvas

The business model is summarized in the business model canvas in the Appendix E. Below are described all the elements developed.

2.6.1. Value proposition

This section describes product characteristics that are attractive to customers, how the product solve their problems and needs, and therefore, stand out from similar products (Osterwalder & Pigneur, 2009). Micro-Filters EC will provide a water filter for pollutants that may not be removed during municipal treatment, such as excess of chlorine, heavy metals, and microplastics, with an accessible cost. This filter will enhance family's health but assuring a good water quality and preventing any health risk associated with pollutants in water. It has an easy installation, facilitating its implementation in home water systems.

2.6.2. Customer Segment

This section describes the main group of customers to whom the product will be addressed to (Osterwalder & Pigneur, 2009). Based on the interviews performed, the primarily customer segment involves parents and caregivers of kids and elders, since they are usually worried about their family's health. Therefore, they are concerned about the quality of water they are drinking and using for their daily life.

2.6.3. Customer Relationships

This sections describes the type of relationships the company will have with its customer's segments (Osterwalder & Pigneur, 2009). Micro-Filters EC will manage a personal assistance relationship by offering guide through filter installation if needed. In addition, our

company will create awareness of microplastic problem through our web page and social media, thus building a strong community around this problematic.

2.6.4. Channels

Channels describes how the company will reach its customer segment in order to deliver its value proposition (Osterwalder & Pigneur, 2009). Micro-Filters EC will create awareness of the product in social media. In addition, paid search will be implemented for people looking to improve their current water quality. The distribution channel will be physical, with a direct sales channel through a web page and indirect sales channel in hardware and zero waste stores. Finally, relationships with the customers will be enhanced by a satisfaction survey after one year of the product use.

2.6.5. Key Activities

This sections mentions the most important things the company has to do in order to make the business model work (Osterwalder & Pigneur, 2009). For the proposed business model, production activities for the filter assembling are one of the most important activities for the business. Besides filter manufacturing, the company will continue to get updates of the best technologies available for filtration operations. In addition, awareness campaigns will be an important activity toward community consciousness about microplastic problem.

2.6.6. Key Resources

Key Resources describes things necessary to develop the product, which can be physical, digital, human, financial, etc. (Osterwalder & Pigneur, 2009). The materials needed to develop and manufacture the filter are the physical resources, while installation technicians and filter manufactures are considered the human resources. Intellectual resources involve the final filter design. Financial resources include crowdfunding's from initial investors.

2.6.7. Key Partnerships

Key Partners include people, organization or companies that would allow the business model to work, by optimizing the model and reducing risks (Osterwalder & Pigneur, 2009). In this business model, the main Key Partners include raw material suppliers, from whom the company will get accessible prices for the materials. In addition, NGOs will be an important partner since awareness of microplastic problem is a key issue for our company to keep growing.

2.6.8. Cost Structures

Cost structures refers to all expenses involved in the operation of the business model (Osterwalder & Pigneur, 2009). In Micro-Filters EC, fixed cost includes salaries for workers, social media advertising and the webpage fees. Rent for the manufacturing facilities will be a large cost in the operation of the business. In addition, since the filter will be sold in retail stores, a reseller fee is also expected. Variable costs include raw materials, since it will depend on the projected demand of the filter.

2.6.9. Revenue Streams

This section includes the cash flow that the company will generate from the customer segment (Osterwalder & Pigneur, 2009), removing the costs included in the previous section. The company will get their revenue from asset sales of the filters, by a competitive value pricing, since it will take into account the price of the competitors.

2.7. Feasibility analysis

In order to know the feasibility and utilities of Micro-Filters EC, financial projections were made for the next 10 years. Some of the considerations and expenses taken into account for

the financial analysis include rent of facilities, furniture, equipment, office materials, salaries, production costs and projected sales with the appropriate increase on the filter price. The sections described above are further detailed in the next section.

2.7.1. Budget A

Budget A considers the cost of rent of a small manufacturing plant and offices for administration activities. Manufacturing activities would take place in a one-story house of 380 m², with 68 m² for administrative offices. The detailed price is shown on Appendix F.

2.7.2. Budget B

Budget B includes three main sections: office materials, furniture, industrial and office equipment. For the industrial equipment, devaluation of 10% was taken into account. This budget was done considering a small team and minimum equipment necessary to run the business. In addition, some administrative cost to run the business are included. The detailed budget is detailed on Appendix G.

2.7.3. Budget C

Budget C includes salaries for direct and indirect workers, with their corresponding social benefits such as IESS payment, vacations, reserve fund and other by law benefits. Finally, the variable cost for filter making or manufacture process was also included. Detailed prices and salaries are detailed on Appendix H.

With all these costs taken into account, an initial investment of \$24 241.66 would be needed to start.

2.7.4. Revenue

As it was mentioned before, revenue will come from asset sales of the filter. The initial price for the filter will be of \$11.00, with an increase of 4.11% of its price each year, for the next 10 years. In order to calculate the estimated sales for the first year, it was considered that there are 3 810 548 families in Ecuador, from which 83.6% have access to potable water, having a total of 3 185 618 families as the total available market as the filter is designed to filter a pre-treated water. According to INEC (2016), 35,6% of population considers that water is not suitable for consumption, while an additional 42.4% boils water before consumption, the serviceable available market was calculated to be 2 484 782 families. For the serviceable obtainable market, it was considered that people who will buy the filter correspond to a medium- high social stratum, 13.1% of these families were considered, as this represents Social Economic Stratum A and B. This gives a total of 325 506 families. Nevertheless, it considered that the product will have a market penetration of 2% on its first year and an expansion of 10%; these values were taken from a similar filter company in Ecuador (Jaramillo & Mendoza, 2019). This gives a market of 6510 families. In Appendix I the projected income is detailed for the next 10 year

2.7.5. Cash flow and sensitivity analysis

Table 8. Cash flow for Micro-Filters EC for the next 10 year

DESCRIPTION	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9	Year 10
Investment	24 241.66	-	-	-	-	-	-	-	-	-
Income	0	71 610.00	82 008.48	93 907.91	107 533.95	123 125.30	140 963.70	161 371.01	184 714.94	211 415.49
Cost and operation costs	0	71 026.50	73 945.69	76 977.466	8 0133.54	83 411.00	86 814.17	90 347.51	94 015.61	97 823.25
Devaluation (-)	0	1 233.71	12 33.71	1 233.71	1 198.65	11 98.65	11 98.65	1 198.65	1198.65	1 198.65
Profit before taxes	0	(24 891.87)	(23 539.00)	(13 020.84)	10 316.33	38 515.64	52 950.87	69 824.84	89 500.67	112 393.58
Taxes	0	5 476.21	5 178.58	2 864.58	2 269.59	84 73.44	11 649.19	15 361.46	19 690.14	24 726.58
Cash flow	(24 241.66)	(30 368.07)	(28 717.58)	(15 885.43)	8 046.73	30 042.20	41 301.67	54 463.38	69 810.52	87 666.99

From the values detailed in the previous section, in Table 8, the cash flow was calculated. Beside the cash flow, some financial indicators were calculated in order to analyze the feasibility of the project. Net present value for this project for the next 10 years has a total of \$27 036,15, which is higher than the initial investment. The internal rate return shows a value of 26,12 % and a payback period of 4.61 years. With these values it can be concluded that the business model is feasible to perform.

2.8. Conclusions

Microplastic pollution is a growing issue that has called the attention of researchers over the last years. Current research upon water bodies and the occurrence of microplastics will be a helpful tool to identify the sources of the problem and the possible solution towards it. Potential threats that microplastic represents to humans' health is an aspect that should be include for further research. Some studies have reported presence of microplastics in water bodies, which some are source for potable water systems. Even though there is current treatment for potable water, pollution for storage and distribution system could affect water quality that is arriving to families' homes. In addition, some remote places might be drinking directly polluted water without appropriate treatment. Therefore, finding solutions for avoiding water contaminants, including microplastics, is an imperative activity.

Programs like I-Corps was very interesting and useful, due to the ideas that emerge from students and people trying to solve problems. With the information described before about microplastics contamination and potential health issues, the team started with the development of the idea for the construction of the business model in I-Corps program. Many hypotheses took place over the process and the ideas were pivoted until one solution idea was the chosen one given all the background, studies and activities realized. The business model canvas describes the elements needed for the product development such as: value proposition, customer segment, customer relationships, channels, key activities, key resources, key partnerships, cost structures, revenue streams, and feasibility analysis.

During the test stage, interviews were done to the customer markets that were defined in the hypothesis. Results from the interviews help to mold and construct the business model and the main idea for the solution of the problem. Along these steps, it was acknowledged that

the microplastic pollution and presence on waterbodies, and even on potable water systems, was not a well-known subject that people were aware, but they showed interest about the issue. When the customer segment and the problem were identified the design for the preliminary filter was developed. A literature review about filter materials and requirements was performed to identify possible materials for the design of the microplastic filter. By analyzing the best available technologies in the market, it was decided that the filter will use a microfilter membrane (for microplastic filtration) and a carbon block.

Changes made during test stages helped to define the business idea model for developing the product. The work done through the interviews and surveys was important to identify the problem and the need of people about secure water. It is relevant to mention that further research is needed to determine the risk that microplastics pose to human's health. This proposal starts from a niche market; however, as this problem is growing in time and more people are getting aware of it, new technologies could be developed for generating other solutions to the microplastic problem, and to larger customer segments.

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APPENDIX

Appendix A: Problem deconstruction

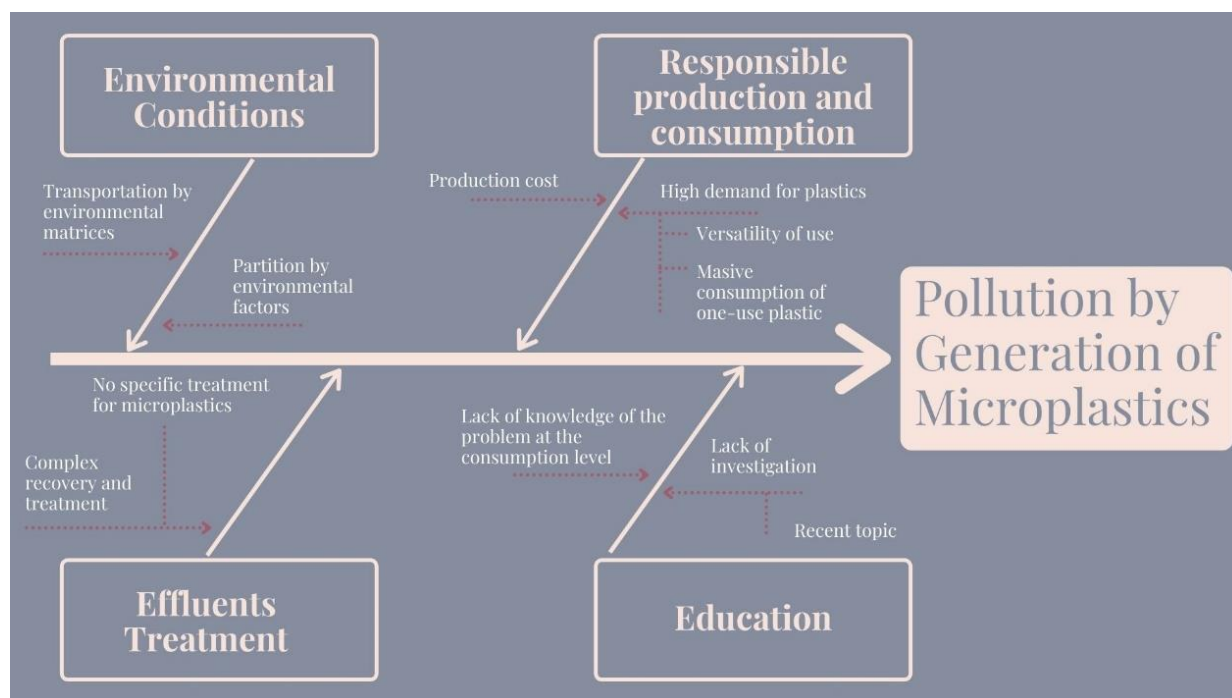


Figure 21. Fishbone diagram

Appendix B: Interviews questions for proposal 1

Customer segment: Industries generating wastewater

1. Why does your company own a wastewater treatment plant?
2. With the water treatment, which contaminants are removed?
3. For the utilization of water in your process, do you count with any filters?

4. What is the purpose of the recirculated water at the industry processes?
5. Are there any other uses for recirculated water? (Eg. Sanitary use, using for the garden)
6. Which are the quality parameters for recirculated effluents for their optimal performance?
7. Did the construction and implementation of the treatment plant represent a significant investment for your company?
8. Have you planned any improvement or upgrade in the near future to the actual process used for treating water in your industry?
9. Which are the parameters that will potentially influence in the decision of improving the current treatment?
10. Do you have an estimated cost for treating 1 m³ of water?
11. Have you ever made a characterization of your effluents?
12. What did you currently know about microplastic pollution? What about microplastic occurrence in wastewater?
13. What do you think will be the tendency of the industry upon the use of plastic?

Customer segment: Food industries

1. How do you use potable water in the processes? What is water being used for?
1. Do you have any current treatment for potable water used in your processes?
2. Have you characterized the drinking water that you use?
3. Do you have any quality standards for the water used in your processes?

4. Have you planned any improvement or upgrade in the near future to the actual process used for treating water in your industry?
5. Which products require strict water quality parameters to be fulfilled?
6. Have you identified any quality relationship between drinking water you use with the quality of the final products?
7. Have you made a characterization of MPs in your products?
8. With your current knowledge on MPs, do you think it would be an issue in the food industry?
9. Why does your company own a wastewater treatment plant?
10. With the water treatment, which contaminants are removed?
11. For the utilization of water in your process, do you count with any filters?
12. Do you have an estimated cost for treating 1 m³ of water?
13. What do you think will be the tendency of the industry upon the use of plastic?

Appendix C: Interviews questions for proposal 2

Customer segment: Parents and caregivers

1. How would you grade the water quality of the drinking water you are getting at home?
2. Do you consider that water quality has a direct effect with your family's health?
3. Have you had any issue with potable water at home?
4. What are the contaminants you are more concerned about?
5. Do you have a filtration system at home?
6. What kind of filter are you currently using?

7. How did you come to know your current filtration system?
8. How much did you pay for it?
9. Who made the decision of buying or not buying a filtration system at your home?
10. What are the main characteristics you look up into a filter system?
11. Have you planned in acquiring a new or better water filtration system? Why or why not?
12. Would you be interested in acquiring a filter that removes additional contaminants that the ones that are being already removed?
13. What do you know or have you heard about microplastics?
14. Do you think that microplastic presence in drinking water would be a potential health problem eventually?

Appendix D: Survey questions

1. Name, age, personal email, occupation, city of residence.
2. Do you live with children or elder persons?
3. How important do you consider water quality? Rank it from 1-5, 1 being not important and 5 very important.
4. Grade your home's water quality. Rank it from 1-5, 1 being not important and 5 very important.
5. Do you have any filtration system at home?
6. If you currently have a filtration system at home, how satisfied are you with it? Rank it from 1-5, 1 being not satisfied and 5 very satisfied.

7. If you do not have a filtration system, are you considering acquiring one in the future?
8. What is your motivation to have now or later a drinking water filtration system?
9. Have you heard about microplastic?
10. Grade how worried are you about microplastic presence in water. Rank it from 1-5, 1 being Not at all and 5 Very worried.

Appendix E: Business Model Canvas

Business Model Canvas











 <p>Key Partners</p> <ul style="list-style-type: none"> Raw material suppliers NGOs 	 <p>Key Activities</p> <ul style="list-style-type: none"> Production activities. Up-to-date technology research. Awareness campaigns 	 <p>Value Propositions</p> <ul style="list-style-type: none"> Filtration of microplastics that are not currently removed, besides other contaminants. Takes care of family health. Prevents any health risk associated with bad quality water. 	 <p>Customer Relationships</p> <ul style="list-style-type: none"> Personal assistance: Guide through filters installation with our technicians. Communities: awareness of microplastic problems 	 <p>Customer Segments</p> <ul style="list-style-type: none"> Parents and caregivers with kids and elders
	 <p>Key Resources</p> <ul style="list-style-type: none"> Filters materials. Filter designs Installation technicians. Filter manufactures Research and development team. Crowdfundings 		 <p>Channels</p> <ul style="list-style-type: none"> Digital marketing channel: social media and paid search Distribution Channel: physical Direct Sales Channel: through our webpage Indirect Sales Channel: Hardware stores and zero waste stores. Service Channel: satisfaction survey (after 1 year) 	
 <p>Cost Structure</p> <ul style="list-style-type: none"> Cost-driven: affordable materials Fixed cost: salaries, social media advertising, physical manufacturing facilities, reseller fees, webpage fees. Variable cost: raw materials 		 <p>Revenue Streams</p> <ul style="list-style-type: none"> Asset Sales Competitive and value pricing 		

Figure 22. Business Model Canvas

Appendix F: Budget A

Table 9. Renting annual costs for Micro-Filters EC operations

Detail	Qty.	Unit value	Total
Renting 380 m ²	1	\$ 1 000	\$ 12 000

Source: Estimated cost from web page plusvalia.com

Appendix G: Budget B

Table 10. Materials, equipment and administrative costs for Micro-Filters EC operations

Office materials			
Detail	Qty.	Unitary value	Total
Paper ream	1	\$3.79	\$3.79
Printing ink (4 packs)	3	\$ 41.5	\$ 124.50
Markers	5	\$0.53	\$2,65
Stapler	1	\$4.06	\$4.06
Staples box	2	\$0.48	\$0.96
Pen	10	\$0.33	\$3.30
Notebook	6	\$1.50	\$9.00
Post-it	10	\$0.87	\$8.70
Furniture			
Desk	3	\$24.41	\$73.23
Ergonomic Chair	3	\$71.41	\$214.23
Sofa	1	\$299.00	\$299.00
Equipment			
Industrial equipment	1	\$10 000	\$10 0000
Computer	3	\$703.79	\$2111.37
Printer	1	\$285.7	\$285.7

Administrative cost			
Notary	1	286.94	\$286.94
Rate to Registro Mercantil	1	25	\$25.00
Designation for managers and presidents	1	50	\$50.00
Total			\$13 457.37

Source: Prices retrieved from Computron and Pycca

Appendix H: Budget C

Table 11. Salaries for direct and indirect workers, fixed and variable costs.

Direct workers			
Detail	Qty.	Unitary value	Total
Installation technician	1	402	\$ 402.00
Factory workers	2	402	\$ 804.00
Indirect workers			
Maintenance	1	402	\$ 402.00
Manager	1	1000	\$ 1 000.00
Marketing and Sales	2	530	\$ 1 060.00
Social benefits			
IESS Payment 11.15%			\$ 4 907.78
Decimo tercero			\$ 3 668.00
Decimo cuarto			\$ 3 668.00

Vacations			\$ 1 834.00
Fund of reserve			\$ 3 668.00
Raw material			
Polymer mesh (microfiltration membrane)	542	0.08	\$ 43.36
Carbon block	542	0.5	\$ 271.00
Plastic mold	542	0.15	\$ 81.30
Fixed costs			
Water	1	50	\$ 50.00
Electricity	1	300	\$ 300.00
Internet	1	26.4	\$ 26.40
Web Hosting	1	120	\$ 120.00
Dominion	1	12	\$ 12.00
Maintenance	1	12	\$ 12.00
Total			\$71 026.50

Appendix I: Projected income

Table 12. Projected income for the next 10 years

Description	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9	Year 10
Projected market size	6510	7161	7877	8665	9531	10484	11533	12686	13955	15350
Projected prize	\$ 11.00	\$ 11.45	\$ 11.92	\$ 12.41	\$ 12.92	\$ 13.45	\$ 13.99	\$ 14.56	\$ 15.15	\$ 15.76

Total income	\$	\$	\$	\$	\$	\$	\$	\$	\$	\$
	71,610.00	82,008.49	93,907.92	107,533.96	123,125.31	140,963.70	161,371.02	184,714.95	211,415.49	241,952.35