Isolation and detection of Nanoplastics in Food, Beverages and Rainwater

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DECLARATION

I hereby declare that the data presented in this dissertation report entitled, "Isolation and detection of Nano plastics in Food, Beverages and Rainwater" is based on the results of investigations carried out by me in the Biotechnology Discipline at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Prof Sanjeev C. Ghadi and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "Isolation and detection of Nanoplastics in Food, Beverages and Rainwater" is a bonafide work carried out by Ms. Vaishnavi Vasant Parikar under my supervision in partial fulfilment of the requirements for the award of the degree of Master of Science in the Discipline of Biotechnology at the School of Biological Sciences and Biotechnology, Goa University.

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CONTENT

PREFACE

Research on nanoplastics is becoming essential since the effects of plastic pollution are becoming more and more obvious. The complex field of separating, identifying, and detecting nanoplastics from essential food, drink, and rainfall sources is explored in this dissertation. This thesis seeks to provide light on the ubiquitous existence of nanoplastics in our environment and their possible effects on ecological balance and human health through rigorous research and analysis.

This work aims to add to the expanding body of knowledge on nanoplastics by sharing the results and insights obtained from thorough research and promoting proactive steps to reduce their negative impacts. There are serious risks to both human health and the environment from plastic pollution. The study of nanoplastics found in consumables like food, drink, and rainwater is the main emphasis of this dissertation. This thesis seeks to determine the extent of nanoplastic contamination in our environment and the potential effects on human health and ecosystems through extensive testing and analysis. This effort attempts to advance our knowledge of nanoplastics by disseminating the findings of thorough research and advocating for well-informed choices and measures to mitigate their negative consequences.

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LIST OF ABBREVIATIONS

Entity	Abbreviation
Degree(s)Celsius	℃
Potassium hydroxide	KOH
Micrometer	µm
Microplastic	MP
Milk cream	Mc
Millimeter	Mm
Nanometer	Nm
Nanoplastic	NP
Nile Red	NR
Less than	<
Percentage	%
P-(acrylic acid)	PAA
Polyamide	PA
Polyethylene terephthalate	PET
Poly (ethylene-vinyl acetate)	PEVA
Polypropylene	PP
Polystyrene	PS
Poly (tetrafluoroethene)	PTFE
Polyurethane	PU
Polyvinyl Chloride	PVC
Rainwater	RW
Rhodamine B	RhB
Soft drink	SD

ABSTRACT

The contamination and possible effects of nanoplastics in food, drink, and rainfall are examined in this study. Sources, distribution, and possible health effects of nanoplastics contamination are investigated using a combination of experimental analysis and literature study. Spectroscopy and microscopy are two of the analytical methods used to identify and describe nanoplastics in samples taken from various sources. The results show a significant level of nanoplastic contamination in food items, drinks, and rainwater, underscoring the need for additional study and legislative actions to lessen the harmful impacts of nanoplastics on the environment and human health.

Keywords: Nanoplastics, Microplastics, Soft drink, Polyethylene, Polyethylene vinyl acetate, Rainwater, Raman spectroscopy, Milk cream.

CHAPTER 1:

INTRODUCTION

1.1 Background

Owing to their affordability and adaptability, plastics have diversified to become a part of every aspect of human activity and utility, displacing metal and wood. Since plastics are long-chain organic molecules, how they break down depends on how their physical characteristics alter and how they interact with other contaminants (Boda Ravi Kiran., 2022). Since plastics are long-chain organic compounds, changes in their shape, size, porosity, surface area, and crystallinity, as well as how they interact with other pollutants, determine how quickly they are degraded (Cai et al., 2017).

Approximately 368 million metric tons (MMT) of plastics were produced globally in 2019, with Asia accounting for half of this total. It is anticipated that these figures will rise yearly (Tiseo., 2021). According to reports, the packaging industry currently uses more than 2 million plastic bottles and bags daily. By 2030, plastic production is expected to have doubled (Khan., 2022).

Plastics degrade throughout their life cycle via a variety of processes, including mechanical, chemical, and biological degradation, and eventually break down into smaller pieces known as microplastics. Because of their extensive occurrence in a variety of ecosystems, including aquatic and terrestrial settings, microplastics have gained international attention along with nanoplastics being a potential threat (Lusher.,2015). The surface area to volume ratio of plastics increases with degradation, allowing additive chemicals to leak into the environment and endangering ecosystems (Teuten et al., 2009). Furthermore, microplastics give a variety of dangerous microbes and pathogens a stable site for attachment for an extended length of time, boosting the pathogens' bioavailability to lower trophic species like bivalves (Zhang et

al., 2022).

Microplastics and nanoplastics have become new pollutants that have harmed food security during the past ten years. Both microplastics and nanoplastics can pass through several environmental niches and trophic levels before entering the human body.

Nanoplastics, or NPs, are colloidal plastics with sizes ranging from 1 to 1000 nm, however, a maximum of 100 nm has also been proposed. The purpose of engineered nanoparticles (NPs) is to create particles with specific properties, like electronics, paints, and cosmetics. On the other hand, secondary NPs are the result of unintentional natural degradation of micro and macro plastic waste, and they are naturally more heterogeneous than engineered particles. Secondary NPs are negatively charged, extremely polydisperse, and have a variety of forms. (El-Hadri et al., 2020). The natural process that modifies the molecular structure of the polymer particle and alters its properties is referred to as plastic degradation. As a result, the rate of degradation was influenced by the type of polymer present, the temperature, the chemical structure, and any additional fillers or additives.

Six technologies are therefore categorized for the destruction of plastic particles: hydrolysis, physical degradation, thermal degradation, photodegradation, thermo-oxidative degradation, and biodegradation (Alprol., 2021). Moreover, additional chemicals including UV stabilizers, heat stabilizers, flame retardants, pigments, and antibacterial agents are added to plastic products throughout the production process. The surface area to volume ratio of plastics increases with degradation, allowing additional chemicals to leak into the environment and endangering ecosystems (Teuten et al., 2009).

Higher nanoplastics uptake by organisms and a greater ability to cross biological barriers, as well as the presentation of a larger surface-to-volume ratio that increases chemical and biological interactions and alters the uptake, transport, and even activity of molecules like

contaminating pollutants in the surrounding environment, can all lead to increased hazard (Gaylarde .et al, 2021). It has been demonstrated that plastic debris that is small enough to penetrate the nuclear membrane enclosing DNA can initiate mutagenic reactions that are thought to contribute to the cellular carcinogenesis process (Hernández et al., 2020).

According to studies, high temperatures can lead to the degradation and fracture of plastic polymers, which can release microplastics (MPs) into regular food and drink items (Astner et al., 2019). Plastic becomes brittle when frozen, suggesting a similar chance of MPs being released. Low temperatures, lots of microbubbles, high pressure, and an acidic environment - all of which are present in carbonated beverages—create the perfect environment for the release of contaminants from plastic bottles (Fu et al.,2015).

The market offers a wide range of packaged beverages, including alcoholic (beer, wine, whisky, etc.) and non-alcoholic (bottled drinking water, hot and cold tea, coffee, fruit squash, fruit drinks, energy drinks, soft drinks, etc.) options. These products have drawn a lot of attention because they are made with water as their primary ingredient and are created using various formulas and manufacturing techniques for immediate consumption (Alkaya and Demirer., 2015). Additionally, drinking products may have some risk because MNPs are transferred orally.

Opening and closing a drinking water bottle, which applies mechanical stress to HDPE bottle screw caps, has been found to release MP and has been recognized as a possible oral exposure source for ingestion (Weisser et al., 2021). It has also been established that heat-induced thermal stress releases microplastics (MPs) and nanoparticles (NPs) from food packaging. Examples of such situations include brewing tea in nylon and PET teabags (Cella et al., 2022; Hernandez et al., 2019), cooking rice in PE cooking bags

(Cella et al., 2022), holding hot liquids in paper cups coated in PE films (Ranjan et al., 2021), and using other plastic materials that hold hot liquids (Liu et al., 2022; Anna Winkler., 2022).

Rain and stormwater are thought to be a major way that microplastics (MPs) from terrestrial sources can enter the aquatic environment. This allows MPs to cycle between the aquatic, terrestrial, and atmospheric environments increasing the exposure of MPs to humans, animals, and plants. Notably, MPs can be re-suspended into the air from land and water surfaces, and they can settle in both aquatic and terrestrial environments because of wind, rain, and snow (Wei., 2023).

The most often used techniques are fluorescence microscopy, μFTIR spectroscopy, and Raman spectroscopy. Because fluorescent dyes adhere to the surfaces of plastic particles and glow when exposed to ultraviolet (UV) light, they are crucial tools for studying nanoplastics (Thomas, et al., 2019). Examples of these dyes include Rhodamine B and Nile Red (NR). This provides researchers with versatile tools for analyzing nanoplastics in a range of environmental settings and simplifies the identification and measurement of nanoplastics using fluorescence microscopy (Guo-Wen Xing 1. ,2023).

The most popular staining dye is Nile Red, which has become widely used because of its low cost and affinity for a variety of polymers (Prata et al., 2021). It is a solvatochromic, lipophilic dye whose emission spectrum is influenced by the staining medium's polarity. At specific wavelengths, Nile Red adsorbs on the polymer surfaces and causes them to fluoresce. Hydrophobicity, electrostatics, van der Waals forces, hydrogen bonding, and other interactions between the dye and the polymer are typically the cause of the adsorption (Aoki., 2022).

By dying white or transparent plastics purple or pink, the technique is essential for visual observation. It also causes the microplastics to fluoresce within common microscope fluorescence filter ranges which aids in the visualization of the various microplastic polymer types tested in lab settings, especially the transparent, white, and small-sized microplastics (Tong.,2020)

1.2 Aim and Objectives

Aim

Detection of nanoplastics in water, food samples and rainwater.

Objectives

1. Isolation of nanoplastic from water, food items and rainwater.
2. Detection of nanoplastic by Rhodamine B using epifluorescence microscope.
3. Identification of the polymer isolated using Raman spectroscopy.

1.3 Hypotheses/Research Question

The presence of nanoplastics in food and beverages is correlated with packaging materials and processing methods, leading to potential ingestion by humans and subsequent health effects.

1.3 Scope

Understanding the sources and pathways of nano plastic pollution is crucial for safeguarding the environment. This includes direct release from product breakdown, larger plastic debris, and wastewater discharge. It is imperative to investigate the distribution of nano plastics in various environmental conditions, such as air, water, soil, and biota. Furthermore, studying the behaviour of nanoplastics in the environment, including their transport, aggregation, degradation, and bioaccumulation processes, is essential. By developing innovative, cost-effective technologies and approaches for identifying nanoplastic pollution, we can take a proactive stance. We must also focus on devising techniques for the detection, quantification, and characterization of nanoplastics in samples, considering their small size. Finally, evaluating the potential human health implications of nanoplastics exposure, whether through ingestion, inhalation, or accumulation in tissues, is paramount for ensuring public well-being.

CHAPTER2:

LITERATURE REVIEW

The food chain, the environment, and packaging all contribute to the contamination of food and drink. When food is prepared, it is likely free of nanoplastics; however, during processing, transportation, packaging, and storage, it may become contaminated (Fadare et al., 2020). Since water is the main ingredient in packaged beverages, which are made using different formulas and manufacturing techniques for direct consumption, a lot of attention has been paid to them (Alkaya and Demirer., 2015). The most significant finding of the research on these particles is that the amount of nanoplastics in food varies based on the conditions surrounding food production, transportation, and storage, according to a study (Brachner et al., 2020).

According to Harush-Frenkel et al., (2010), recent research has shown that the surface charge of nanopolymers can change cationic particles, increasing their lung localization, cellular uptake, and pulmonary toxicity. Moreover, it was found that when endocytes translocate nano-polymer into cells, phagosomes follow along, causing particles to gather in lysosomes. This becomes a channel for the pathogens' cellular disintegration (Yihe Huang, 2022) a, Characteristics of nano-plastics in bottled drinking water, 2022. Research indicates that in human hematopoietic cells, exposure to NP can result in cellular oxidative stress and primary DNA damage (Rubio et al., 2020).

A study indicates that while NPs may comprise a significant fraction of a sample's mass, they may make up a large percentage of the sample's total particles. Since hetero-aggregation is an environmental process that is important at the nanoscale, it must also be considered. It is expected that this process will occur in a variety of environments, even though the efficiency of the process for NPs has not yet been measured. The process's efficiency will vary

depending on flow patterns, residence times, and the composition of natural colloids and suspended solids (Teuten et al., 2009).

Because NPs are primarily manufactured using a variety of formulas and manufacturing techniques for direct consumption, it is possible that they have traveled through various industrial processes and packaging before being discovered in packaged food products, as recent studies have shown (Alkaya and Demirer, 2015). Recent research indicates that the main cause of Micro and nanoplastic contamination in water bodies is the discharge of treated industrial effluent (Wang et al., 2019).

Plastic particulates found on soil are transferred from land to water bodies by a variety of environmental factors, such as animal movements, water turbulence, and climatic changes (Kutralam-Muniasamy et al., 2019). eventually participating in agricultural irrigation techniques and thereby entering the food chain (Boyle and Ormeci, 2020).

Studies by (Markic et al., 2020) reveal that NPs have been detected in a wide range of foods and drinks, such as honey, beer, chicken, salt, sugar, tea bags, milk, salmon, seaweed, shrimp, and bivalves. The average annual intake of MP/NPs by humans is between 39,000 and 52,000; individuals who drink bottled water unintentionally swallow an additional 90,000 particles (Mason et al., 2018).

In drinking water bottles, MP/NP contamination resulted in 40.1 mg kg−1 body-weight day−1 for adults and 87.8 mg kg−1 body-weight day−1 for children, according to Zuccarello et al., (2019).

Investigation carried out for the same brand of water was tested with various packaging materials (glass, plastic) to examine the impact of the materials on MPs pollution. The findings demonstrated that the average number of microplastics (MPs) in glass bottled water (204 particles/L) was significantly lower than the average number of MPs in plastic packaged water (1410 particles/L). Additionally, according to Schymanski et al., (2018), the packaging materials were separated into four categories: glass bottles, beverage cartons, returnable plastic bottles, and one-way plastic bottles. Compared to water from single-use plastic bottles (average: 14 particles/L) and water from beverage cartons (average: 11 particles/L), the amount of plastic particles in water from returnable bottles (average: 118 particles/L) was significantly higher. But water from glass bottles also contained a significant number of MPs (average number: 50 particles/L).

To the study's findings, drinking a litre of soft drink exposes consumers to approximately nine microplastic particles, which is a moderate amount in comparison to exposure levels found in other studies. Eighty percent of the soft drink samples showed signs of significant microplastic contamination. Brands packaged in Tetra pack were found to have the highest concentration of microplastics. Tetra Pak and PET had pollution loads of 6.9 and 10.25, respectively (Altunışık., 2023). However, Zhou et al., (2021) reported that roughly 40% of the MPs found in China's drinking water were found to be between 25 and 100 μm in size.

According to research, iced carbonated beverage bottles can release MPs/NPs at levels between 68 and 4.66×10^8 particles/L, which could be harmful to people's health. Compared to PET and polyethylene PE bottles, PP bottles released more MPs and NPs (Chen., 2023). The extreme quantity of MPs larger than 1.5 μm found in thoroughly cleaned vegetables and peeled fruit, as reported by Conti et al., (2020).

In accordance to a number of studies, high temperatures can cause plastic polymers to break down and degrade, releasing microplastics (MPs) into common food and drink items (Astner et al., 2019; Ranjan et al., 2021). For example, plastic degrades in the presence of freezing (Fu et al., 2015), providing a comparable risk of MP release. Carbon dioxide (CO2) is more soluble at lower temperatures (Liger-Belair, 2019). When you shake after drinking carbonated beverages, a lot of CO2 microbubbles are released. Despite the tiny size of these bubbles, strong forces could be produced if they burst. Kadivar et al., (2021) state that cavitation erosion caused by bubble rupture can seriously corrode or mechanically damage submerged objects such as ship propellers and rudders.

Furthermore, our study discovered that multiple freeze-thaw cycles (from -18°C to 25°C) significantly increased the release of MPs and NPs. This can be attributed to the combined effects of pressure, the ice-rich environment, and CO2 bubbles. Therefore, in order to reduce the number of MPs and NPs they consume when drinking carbonated beverages, it is advised that consumers limit the number of freeze-thaw cycles. Furthermore, given the prevalence of freeze-thaw cycles on Earth's surface, this phenomenon could hasten the aging and environmental degradation of plastics, potentially endangering the security of ecosystems (Yalin, 2023)

Moreover, MPs have been discovered in milk samples. A total of 150 MPs particles were counted from the 23 milk samples (22 adults and 1 child) that were tested from five international and three national brands in Mexico, with an average concentration of 6.5 ± 2.3 particles L−1 (Kutralam-Muniasamy et al., 2020). Of all the MPs, fibers made up the majority (97.5%), with fragments making up the remaining 2.5 percent.

The most prevalent type of MPs found in the milk samples, according to the results of Micro-Raman identification, was polyethersulfone (PES), a membrane material that is widely used in the dairy process.

Hernandez et al., (2019) discovered that plastic MPs and NPs were released into the beverage after immersing empty plastic tea bags in reverse osmosis water for five minutes at 95°C. A single cup of the beverage contained roughly 11.6 billion MPs and 3.1 billion NPs, all of which were derived from plastic teabags, according to the analysis. The particle sizes of the particles ranged from 520 nm to 270 µm.

Regarding single-use paper cups, it was discovered that LDPE-coated cups released up to 26 times more nanoparticles (NPs) than PLA-coated ones (maximum 1.9×10^7 nanoparticles per cup). Son et al., (2024).

A study reported that mechanical stress deteriorates the material properties and modifies the physical-chemical properties of the generated secondary nanoplastics in comparison to the original bulk source, making it more difficult to identify the compounds using spectroscopy. According to research, it is essential to comprehend material degradation processes in order to detect and measure nanoplastics in actual samples (Winkler, 2022).

The finding suggests that using a larger collector during the sampling process appears to increase the concentration of MPs in rainwater.

According to the second power of the inlet diameter, or the collector's inlet area, the number of MPs increased from 4 to 34 items/sample. This phenomenon was explained by the fact that rainwater collects at the same height regardless of the collectors' inlet diameter (Park., 2023). The concentration of fine dust in South Korea is highly variable, and atmospheric microparticulate matter would have a significant impact on the number of MPs in rainwater as reported in this study. (Hwang and Kim., 2020). Additionally, according to the study conducted the first rainfall produced less precipitation than the second, but there was a higher concentration of fine dust. As a result, the high air pollution level may have contributed to the high MP concentration seen in the previous rain event (Akbarizadeh., 2021).

As a result, 13 distinct varieties of MPs were detected; according to Do Taegu (Do, 2023) the most frequently found types were PP (46.76%), Acrylic (11.57%), and PES/PET (10.19%); PS (2.00%), SBR (1.93%), and PVC (0.34%) were less frequently found types.

The most successful fluid for breaking down organic matter and preserving swallowed plastics was potassium hydroxide (KOH) solution, according to a study done with six possible options. (Dehaut and others, 2016) Further investigation revealed that 10% KOH was useful for recovering PVC and LDPE film, PP and PET in fiber form, and rayon and acrylic fibers.

Moreover, it may be used to dissolve a wide range of biological tissues, even with particles as small as one millimeter. It's also the cheapest and quickest method for dissolving tissues. In a study, an easy-to-afford method for optimizing potassium hydroxide digestion—which permits the complete digestion of the viscera—was proposed. The gel in this investigation was treated with EtOH dilutions of KOH (1:1, 1:2, 1:4, and 1:10). The most flexible and efficient dilution was 1:10. Furthermore, the polymers showed no discernible changes upon the addition of EtOH (Dawson,et al ., 2020).

A study suggests that ultrafiltration or other separation techniques like flow field fractionation (FFF) and hydrodynamic chromatography (HDC) could be used to achieve size-based differentiation for nanoplastics (Wallace et al., 2016).

Because white and transparent microplastics (MPs) naturally emit fluorescence, fluorescence microscopy is also used to study microplastics. Fluorescence microscopy has been used in a number of studies conducted in the past few years on microplastics. Fluorescence microscopy, for instance, was used to investigate *T. japonicus* copepod's consumption of microplastics and nanoplastics. (Lee, et al., 2020) A consistent technique for staining MPs to view them under a fluorescence microscope is lacking in the literature.

While many dyes have been investigated for microplastic staining, Nile Red is the most well-researched and frequently utilized. This method enables a rapid quantification of microplastics, despite the fact that it does not disclose information about the chemical structure of the particles. Additionally, it is a reasonably quick, easy, and affordable method of determining the amount of microplastic in a sample. (Hengstmann, et al.,2017)

Another fluorescent dye that is frequently used to color textiles is rhodamine B (RhB). Fluorescently labeling nano polystyrene particles has been employed in research to evaluate the possible toxicity of nanoplastics on organisms in the environment. (Wong, et al., 2018) RhB was studied as a dye to determine whether it could stain plastics and to assess how well distilled water, ethanol, and acetone would dissolve the dye.

In addition, five distinct kinds of microplastics were labeled to explore its potential applications. It was also evaluated how stable the dye's fluorescence was under different circumstances. This investigation revealed that ethanol, as opposed to distilled water and ethanol, was the most effective solvent for RhB. Furthermore, it was shown that RhB fluoresces brightly even at low concentrations.

PVC particles turned a pale pink color, and amaranthine-colored PE, PP, and PU particles were detected. PS's outline had some staining, but it was difficult to see how the color had changed in other areas.

Under a fluorescence microscope, the particles displayed variable intensities of green fluorescence at excitation wavelengths between 450–490 and 515–565 nm. Furthermore, RhB was found to be fluorescently stable in a variety of experiment settings, including light, gut fluid, and even different solutions like KOH, nitric acid, and saturated sodium chloride. (Huiyan, et al.,2020).

In another study, the adsorption properties of RhB on different MPs were carefully examined in a range of temperature, humic acid, salinity, and pH settings. Because PVC was more polar than PS and PET, it was found that PVC could adsorb more RhB. Furthermore, when the sizes of MPs decreased, so did their absorption capacity. (Zhang, et al.,2022). For smaller microplastics, visual examination of the microplastics using fluorescent dyes may occasionally be imprecise. In addition, it is imperative to verify the plasticity of every visually identified item to prevent the overestimation or underestimation of the number of MPs in a given sample.

μ-Raman and μ-Fourier transform infrared (FTIR) spectroscopy can both verify this. (Gutow et.al.,2012). The degree and mode of human absorption of plastic particles are largely determined by the size of the particles. There are large gaps in our knowledge about the effects of nanoplastics on the environment and human exposure. This is a result of the dearth of research on the interactions and biological effects of nanoplastics in cells.

CHAPTER3:
METHODOLOGY

3.1 Sampling sites

3.1.1 Rain water sample

Rainwater sample was collected in a steel vessel from Rumdamol Davorlim Margao Goa (15.27556° N, 73.98319° E) during the months from August to September 2023.

3.1.2 Tap water sample

Tap water sample was collected from the laboratory at the School of Biological Sciences and Biotechnology, Goa University in a glass beaker.

3.1.3 Tetra pack and soft drink PET bottles

Tetra pack samples were collected from a local shop, in south Goa

3.1.3 Soft Drink PET bottles

Soft Drink PET bottels were collected from a local shop, in south Goa.

3.1.5 Paper cups

Paper cups were collected from local shop in North Goa

3.1.6 Milk cream

Milk cream was collected from a local shop in North Goa.

3.2 Sample collection and preparation

3.2.1 Rainwater sample

A steel container holding five thousand milliliters of rainwater was filled without any hindrance from Margao Goa during the month of August to September 2023.

After that, the water sample was put in a glass bottle and kept at room temperature while it was transported to the laboratory for examination and analysis

3.2.2 Tetra pack

Two samples of tetra packs were collected from a local store in Margao South Goa. Sample 1 from tetra pack (juice) which was newly manufactured and sample 2 (juice) from tetra pack which was near to expiry period. Both samples taken were at room temperatures.

3.2.3 Soft drink

Soft drink samples were collected from local shop in Margao South Goa. Sample 1 soft drink was from a chilled PET bottle, soft drink sample 2 was taken from a PET bottle which was exposed to sunlight for approximately 30 days and sample 3 was taken from a chilled glass soft drink bottle.

3.2.4 Paper cups

Paper cups were taken from a local shop in Taleigao North Goa. Tap water was collected in a glass beaker and boiled for 10 minutes and transferred into the cup as a test sample . Tap water at normal temperature was taken as control and kept at room temperature.

3.2.5 Milk cream

Milk was collected from a local shop in Bambolim, North Goa. The Milk cream was removed with a stainless steel spatula and was dried at room temperature. The sample was divided and transferred to two glass beakers. A 10% KOH (w/v) solution was added to the beaker containing milk cream and kept in the oven at 60℃ for 2 days till it digested completely.

3.3 Isolation of nanoplastics

3.3.1 Rainwater sample

The rainwater sample was taken in a beaker and the solution was filtered using a sieve having pore size of 0.45 mm.

Using a 20ml syringe, the filtered sample was then run through a 0.2µm diameter nucleopore membrane for nanoplastic characterization. The membrane was placed in a petri dish and was covered to dry overnight. After that, it was stained with Rhodamine B and allowed to dry for an additional night before being examined using an epifluorescence microscope with a 10X objective. Using a 20ml syringe, an additional 20 ml of fluid was filtered through a 0.2µm nucleopore membrane. Nile Red was used to stain the membranes overnight after they had been left to air dry. Using a 10X objective, the slide was examined under an epifluorescence microscope to check for nanoplastics.

3.3.2 Tetra pack

Using a 20 ml syringe, Juice from tetra pack sample 1, which had been newly manufactured and kept at room temperature, was run through a 0.2 µm nucleopore membrane. The membrane was placed in a petri dish to dry overnight. After that, it was stained with Rhodamine B and allowed to dry for an additional night before being examined using an epifluorescence microscope with a 10x objective. Using a 20ml syringe, an additional 20 ml of fluid was filtered through a 0.2µm nucleopore membrane. Nile Red was used to dye the membranes overnight after they had been let to air dry. Using a 10x objective, the slide was examined under an epifluorescence microscope to check for nanoplastics.

Using a 20 ml syringe, Juice from tetra pack sample 2, which had been near to expiry and kept at room temperature, was run through a 0.2 µm nucleopore membrane. The membrane was placed in a petri dish to dry overnight. After that, it was stained with Rhodamine B and allowed to dry for an additional night before being examined using an epifluorescence microscope with a 10X objective. Using a 20ml syringe, an additional 20 ml of fluid was filtered through a 0.2µm nucleopore membrane. Nile Red was used to dye the membranes overnight after they had been let to air dry. Using a10X objective, the slide was examined under an epifluorescence microscope to check for nanoplastics.

3.1.1 Soft drink

Following the collection of the soft drink samples, Sample 1 soft drink was extracted from the cold PET bottle and passed through a 20 ml syringe fitted with a 0.2 µm nucleopore membrane. The membrane was placed in a petri dish to dry overnight. After that, it was stained with Rhodamine B and allowed to dry for an additional night before being examined using an epifluorescence microscope with a 10X objective. Using a 20ml syringe, an additional 20 ml of fluid was filtered through a 0.2µm nucleopore membrane. Nile Red was used to dye the membranes overnight after they had been let to air dry. Using a 10X objective, the slide was examined under an epifluorescence microscope to check for nanoplastics. .Following the collection of the soft drink samples, Sample 2 soft drink was extracted from the PET bottle which was exposed to sunlight for 30 days and passed through a 20 ml syringe fitted with a 0.2 µm nucleopore membrane. The membrane was placed in a petri dish to dry overnight. After that, it was stained with Rhodamine B and allowed to dry for an additional night before being examined using an epifluorescence microscope with a 10X objective. Using a 20ml syringe, an additional 20 ml of fluid was filtered through a 0.2µm nucleopore membrane.

Nile Red was used to dye the membranes overnight after they had been let to air dry. Using a 10X objective, the slide was examined under an epifluorescence microscope to check for nanoplastics.

Following the collection of the soft drink samples, Sample 3 was extracted from the cold glass bottle and passed through a 20 ml syringe fitted with a 0.2 µm nucleopore membrane. The membrane was placed in a petri dish to dry overnight. After that, it was stained with Rhodamine B and allowed to dry for an additional night before being examined using an epifluorescence microscope with a 10x objective. Using a 20ml syringe, an additional 20 ml of fluid was filtered through a 0.2µm nucleopore membrane. Nile Red was used to dye the membranes overnight after they had been let to air dry. Using a 10X objective, the slide was examined under an epifluorescence microscope to check for nanoplastics.

3.3.4 Paper cups

Tap water at normal temperature was taken as control and water after boiling transferred to the cups after 15 minutes was the test. The water was filtered by 0.2µm sized nucleopore membrane using 20ml syringe. The membrane was kept for drying overnight in a petri dish. It was then stained using Rhodamine B and kept overnight for drying later observed under epifluorescence microscope using 10X objective.

3.3.5 Milk cream

The solution was filtered once the sample had fully broken down. Using a 20ml syringe, the filtered material was then run through a nucleopore membrane with a 0.2µm pore size for the measurement of nanoplastics. The membrane was placed in a petri dish to dry overnight. After that, it was stained with Rhodamine B and examined with a 10X objective using an epifluorescence microscope.

Fig 3.1: Filtered rainwater solution passed through 0.22µm filter using a 20 mL syringe.

3.1 Visualization and analysis of obtained images

3.1.1 Epifluorescence microscopic analysis

The membranes stained with respective fluorescent dyes were observed under epifluorescence microscope in green light (495 - 570 nm) and examined for the color and intensity of the fluorescence the particles emitted.

3.1.2 Raman analysis

Depending on the kind of material, code names were assigned to nanoplastic samples. The prefix SD, which stands for Soft Drink, was used to assign code names to the nanoplastics found in the soft drink sample. The code name for the nanoplastics in the Rainwater sample was prefixed with RW, which stands for Rain Water. The prefix MC, which stands for Milk Cream, was used to assign a code name to the nanoplastics found in the milk cream sample. Wiley Online Raman Database's KnowItAll Information System 2021 was used to perform a similarity search for isolated particles.

CHAPTER 4 : ANALYSIS AND CONCLUSION

Nanoplastics due to their property of aggregation of tiny particles give the appearance of single entity. (Zhang, et al., 2022)

Standard PET and PE were stained with NR and observed in blue light in epifluorescence microscope as positive control.(Fig 4.0.1-4.0.4)

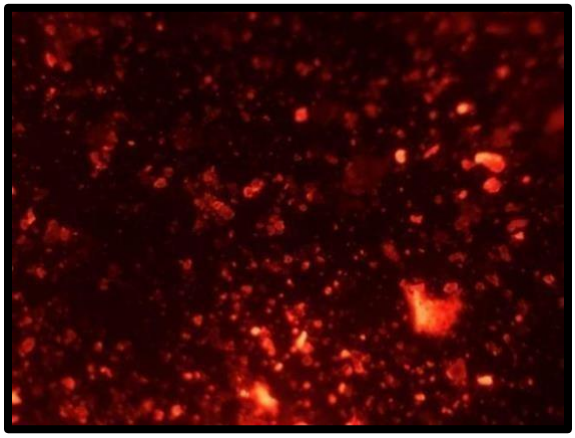

Fig 4.0.1

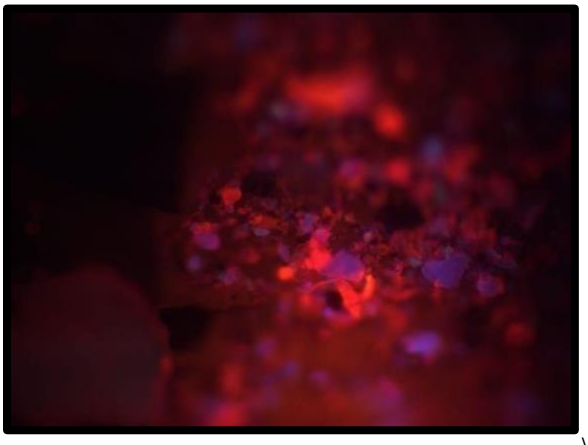

Fig 4.0.2

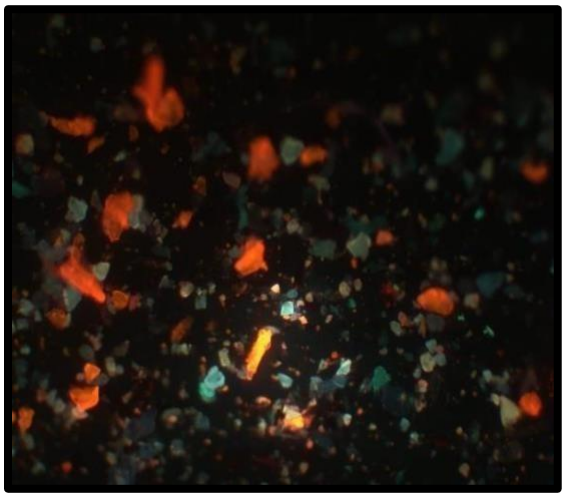

Fig 4.0.3

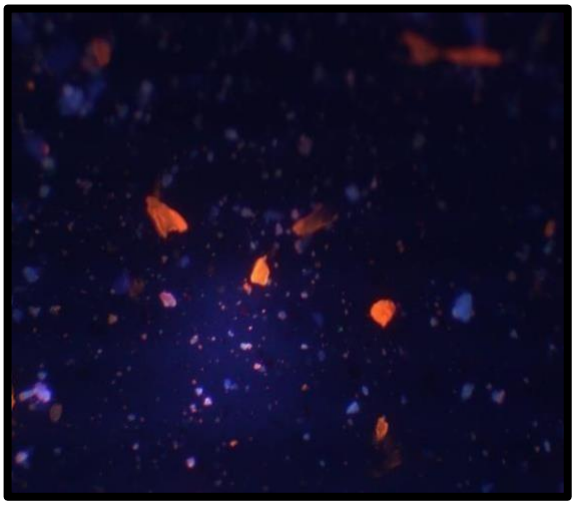

Fig 4.0.4

Figure 4.0.1- 4.0.4 S t a n d a r d Nanoplastics were observed under green light.

4.1. Rainwater sample

After the collection of rainwater it was filtered and assessed for NPs of sizes less than 200nm using Rhodamine B dye under epifluorescence microscopic and Raman spectroscopic techniques.

4.1.1 Nanoplastic analysis

20ml of filtrate passed was through 0.22µm and membrane was kept for drying overnight (WangS. H., 1941). The filter membrane were then stained using Rhodamine B overnight and observed under light microscope using 10x objective for particles having <200nm size. MilliQ water was taken as control(Fig 4.1.1.1)

Fig: 4.1.1.1 the stained filter used for filtering milli Q water under 10X magnification

Rainwater sample collected in non-plastic material passed through sieve followed by filtration through nucleopore membrane stained with rhodamine B was observed under 10X using green light showed presence of nanoplastic aggregates of lesser density (Fig 4.1.1.2 -4.1.1.8). The smaller particles (< 200 nm) emitted weak fluorescence. However, in Fig 4.1.1.4 the particle size appears to be larger and the fluorescence intensity of the particle is higher.

The reason for the same could be because of aggregation of nanoparticles that looks like one single particle as described by (Zhang, et al., 2022).

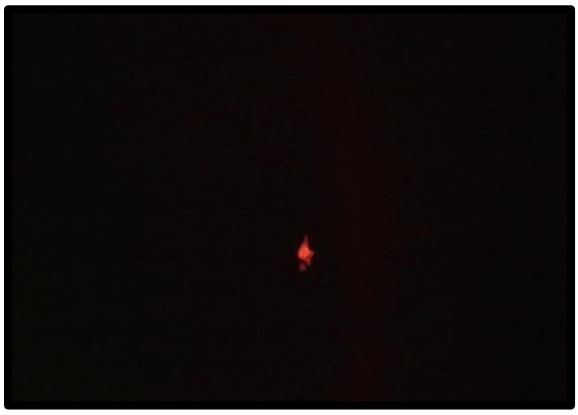

Fig 4.1.1.2

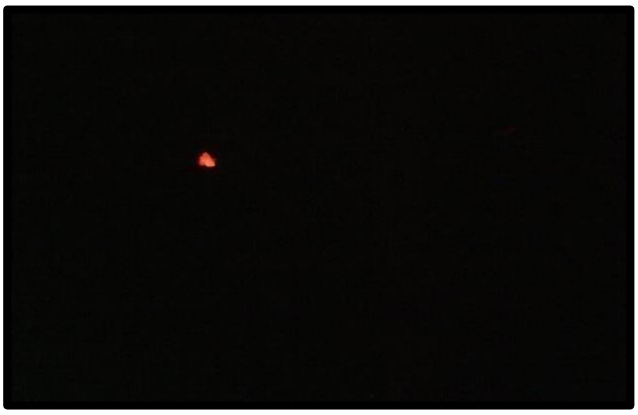

Fig 4.1.1.3

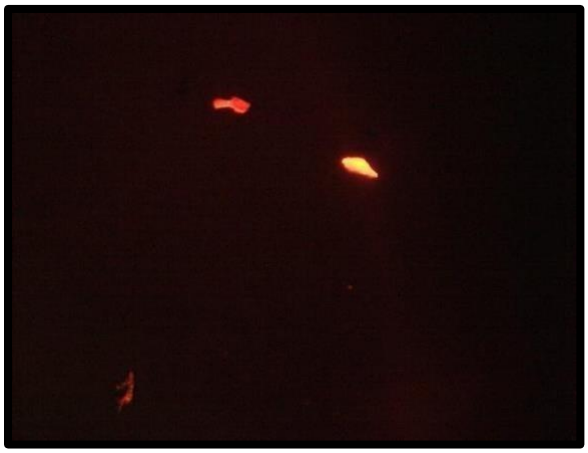

Fig 4.1.1.4

Fig 4.1.1.5

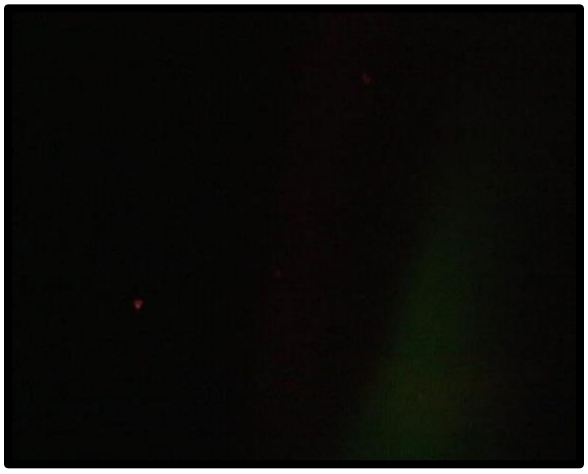

Fig 4.1.1.6

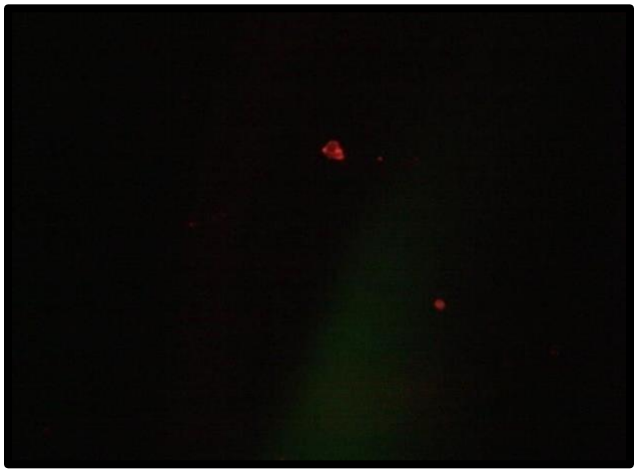

Fig 4.1.1.7

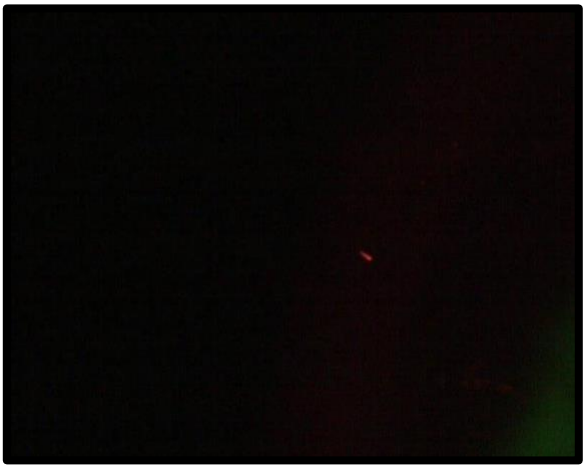

Fig 4.1.1.8

Figure 4.1.1.1 – 4.1.1.8 The stained filter used for filtering rain water under 10X magnification with Nanoplastics showing red fluorescence under green light.

The reason could be because industrial processes either directly or indirectly introduce nanoplastics into the atmosphere through other contaminants. When precipitation occurs, air currents have the ability to carry these nanoplastics, which then end up deposited on surfaces, including aquatic bodies. The finding of nanoplastic deposition in the rainwater samples suggests that air travel may be a major source of plastic waste into isolated areas. as stated by Deonie Allen,et al., (2019)

4.1.2 Raman analysis of rainwater

The similarity of Raman spectra of the isolated MPs of each category was compared to standard polymers using Wiley Science Solution's KnowItAll Raman Spectral Database collection. The similarity varied from 58-62%. Three different spectra were observed as three different sections of the filter were tested for the analysis.

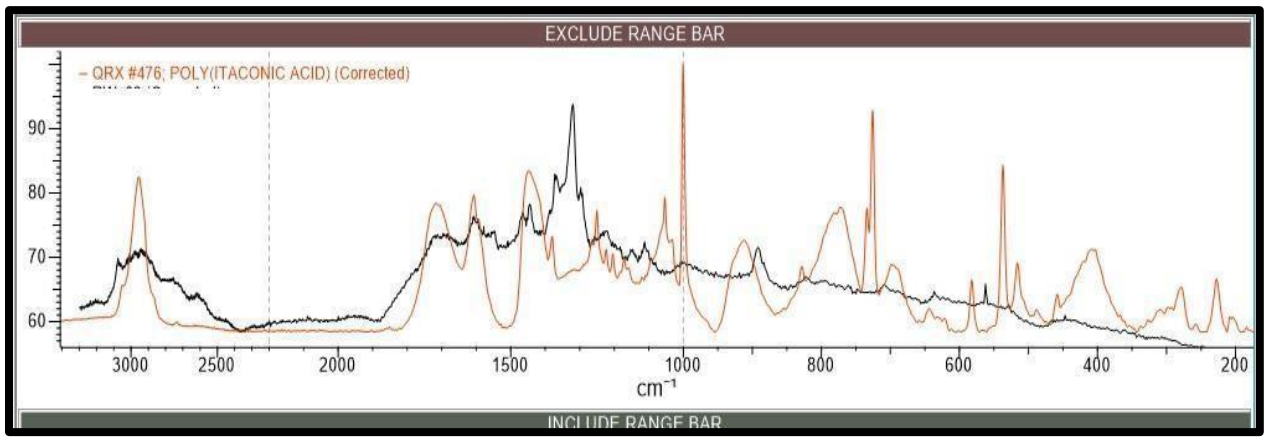

Fig 4..1.2.1 Raman spectra of RW01A

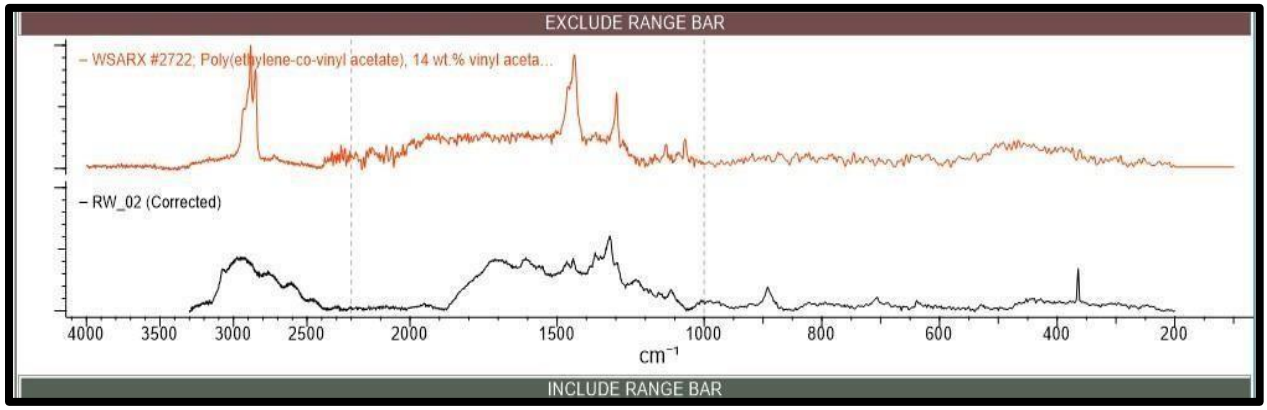

Fig 4.1.2..2 Raman spectra of RW01B

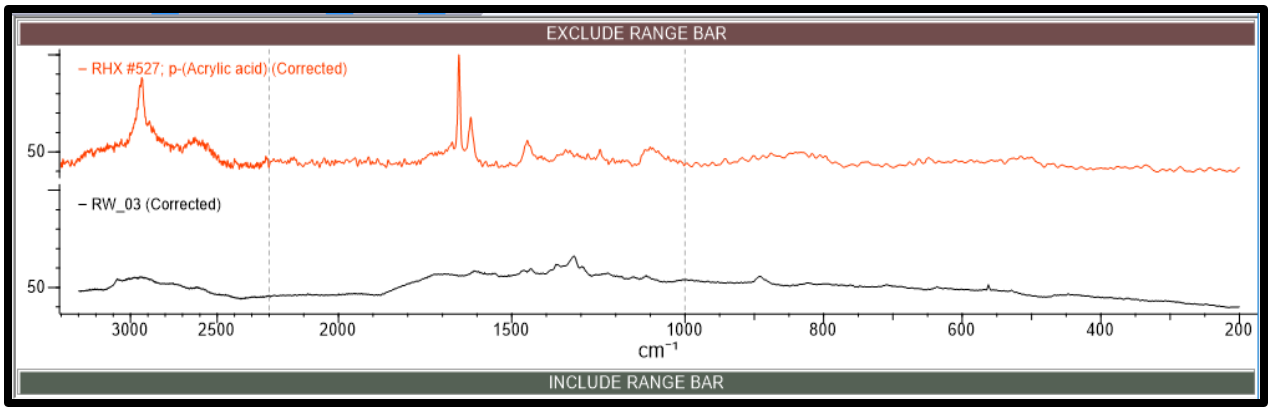

Fig 4.1.2.3Raman spectra of RW01C

Table 1 : Raman analysis for rain water

Sr. no.	NP as given for Raman	Similar to
1	RW01A	PIA
2	RW01B	PEVA
3	RW01C	PAA

Raman spectrum for RW01A showed 62.90% similarity to Poly Ictonic acid of and RW01B showed 56.07% similarity with Polyethylene co- vinyl acetate. Three different spectra are observed as three different sections of the filter were testes for the analysis. RW01C was found 58.32% similar to PAA. RW01B was PEVA, it's possible that PEVA particles or residues from products made with PEVA could enter the environment through various pathways. Runoff from urban areas, landfills, and trash areas can contaminate rivers, streams, and other water bodies with plastic debris, especially objects containing PEVA. Through the water cycle, these plastics may subsequently be carried by water currents and wind up in rainfall (Cai et. al., 2020)

4.2 Tap water

Nanoplastic analysis of tap water was done Using 20 ml syringe, tap water was filtered using a 0.2 µm nucleopore membrane and stained using Rhodamine B in control which was milli Q water showed no aggregates of nanoplastics (Fig 4.2.1)

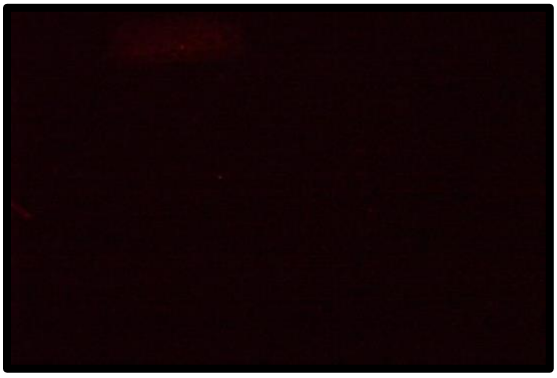

Fig 4.2.1 stained filter of milli Q water

Sample tap water fig 4.2.1 – 4.2 6 has shown the presence of nanoplastics which fluoresce red under green light. Fig 4.2.3 has shown nanoplastics in bright colour due to greater intensity of light.

Fig 4.2.2

Fig 4.2.3

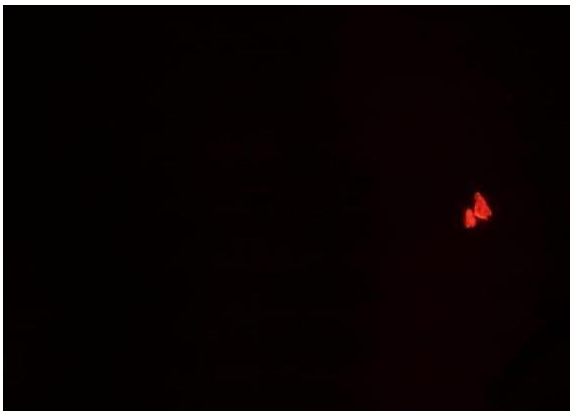

Fig 4.2.4

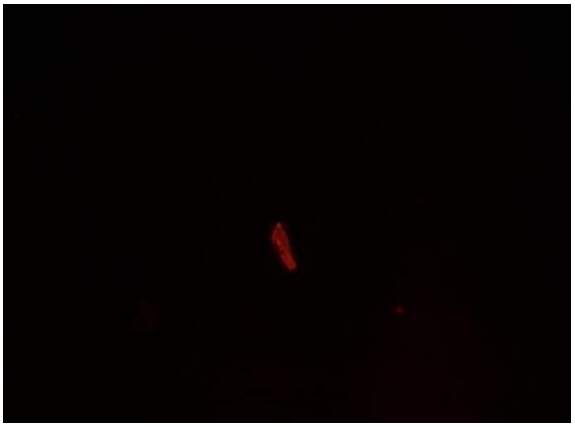

Fig 4.2.5

Fig 4.2.6

Figure 4.2.1- 4.2.6 Nanoplastics in the tap water showing red fluorescence under green light .

This result is in accordance to Chowdhury et al., (2020) the study that detected microplastics, along with nanoplastic particles, in tap water samples from Canadian cities. The possible reasons for the same could be aging of the pipeline, source of tap water.

4.3 Tetra pack

For nanoplastic analysis in juice from newly manufactured tetra pack was tested and showed no presence of nanoplastics (Fig 4.3.1 – 4.3.2)

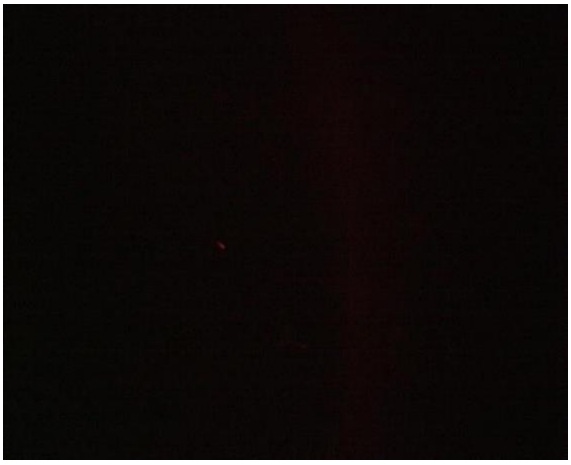

Fig 4.3.1

Fig 4.3.2

Figure 4.3.1-4.3.2 Microscopic images of tetra pack juice under green light.

The test sample was taken from tetra pack which had neared expiry at room temperature detected nanoplastics aggregates using green light under 10X stained by Rhodamine B after filtering through 0.22 µm membrane. The aggregates observed emitted weak fluorescence ranging from red to green. (Fig 4.3.3 – 4.3.9). The results are similar to the study reported Brands packaged in Tetra pack had the highest concentration of microplastics. (Altunışık, 2023). Test sample was chosen as tetra pack that had neared expiry due to its exposure to various temperature fluctuations and examined for naoplastics had presence of nanoplastics aggregates.

Fig 4.3.3

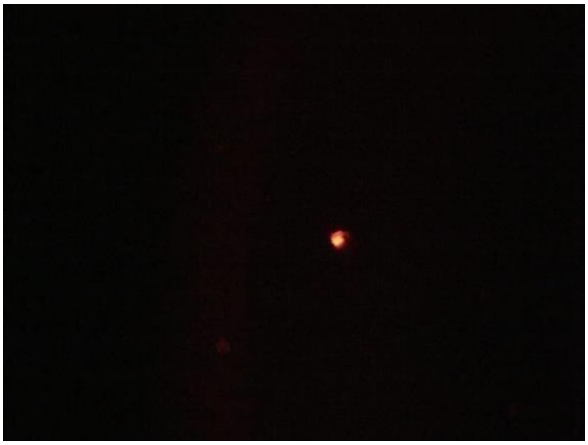

Fig 4.3.4

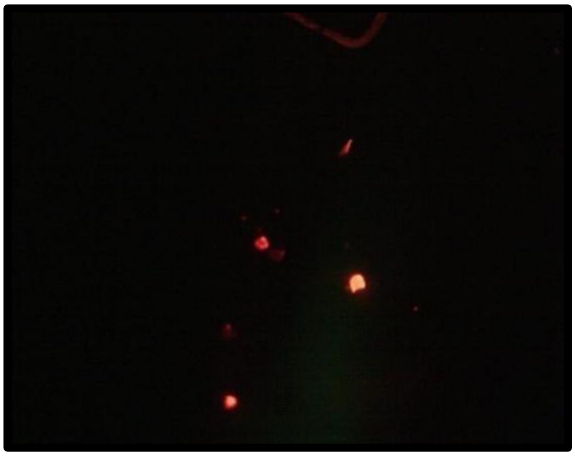

Fig 4.3.5

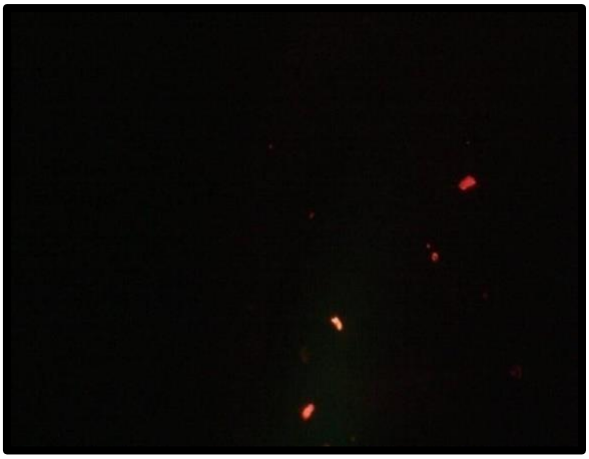

Fig 4.3.6

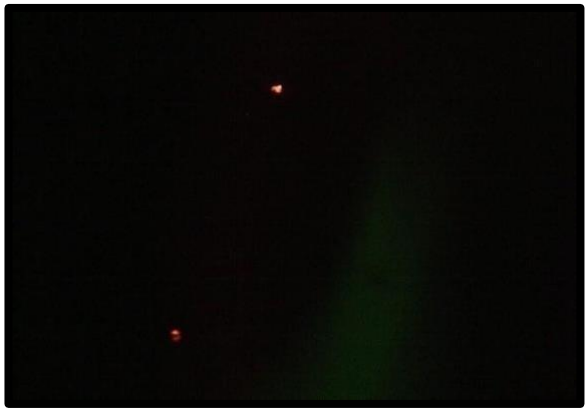

Fig 4.3.7

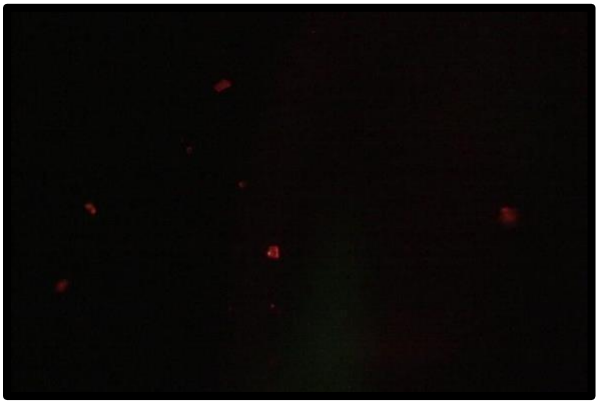

Fig 4.3.8

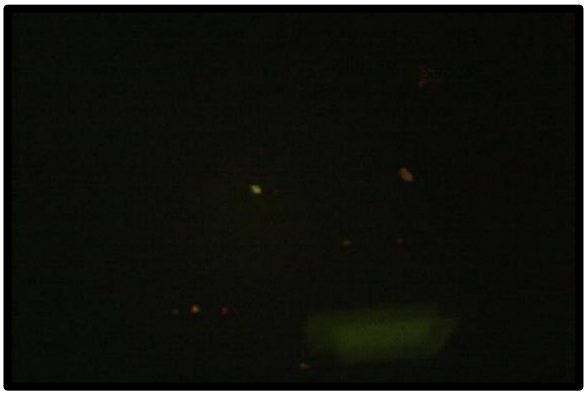

Fig 4.3.9

Figure 4.3.3 – 4.3.9: Nanoplastics detected in tetra pack near to expiry showing red fluorescence under green light

4.4 Soft drink

Soft Drink was exposed to various conditions and filtered through the nucleopore filter and dried overnight and stained with respective stains and visualized. Fig 4.4.1.1 Nanoplastic aggregates from sample was taken from chilled pet bottle and was filtered subsequently stained using rhodamine B, visualized under green light showed aggregates of nanoplastics (Fig 4.4.1.1 -4.4.1.6)

a) Sample 1 soft drink from PET bottle chilled were analysed for nanaoplastics .

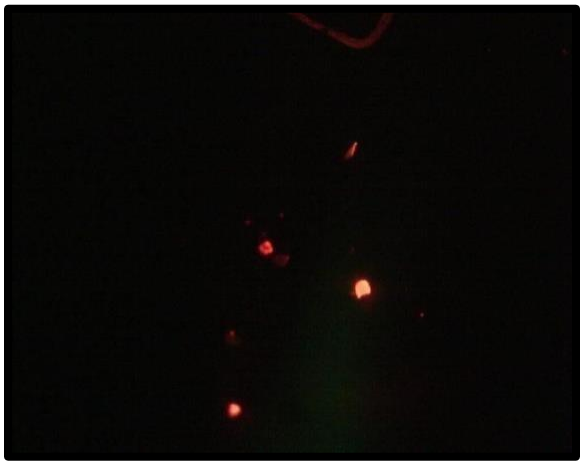

Fig 4.4.1.1

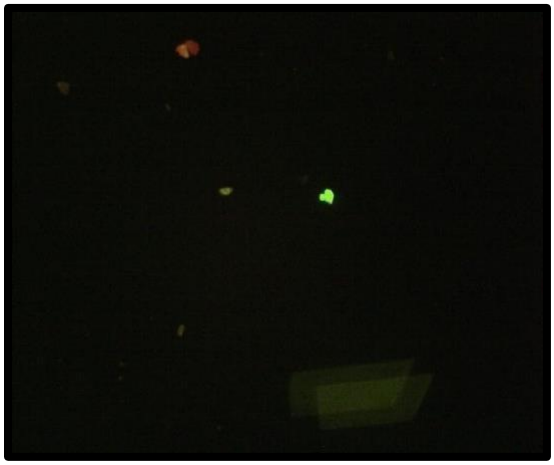

Fig 4.4.1.2

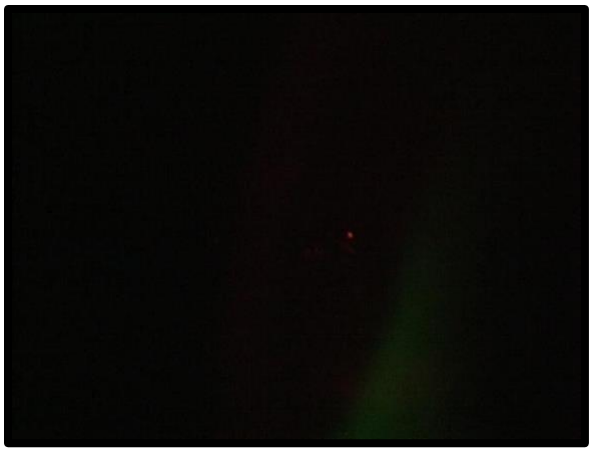

Fig 4.4.1.3

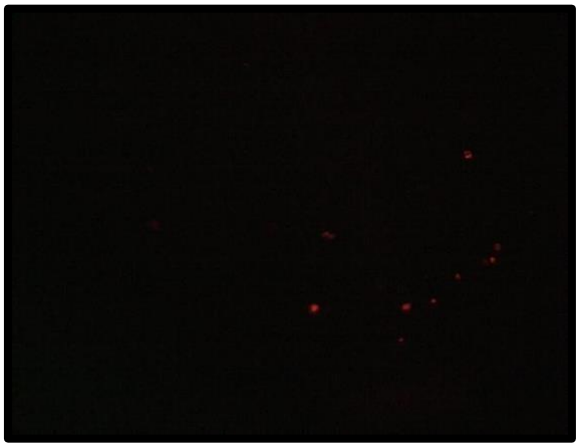

Fig 4.4.1.4

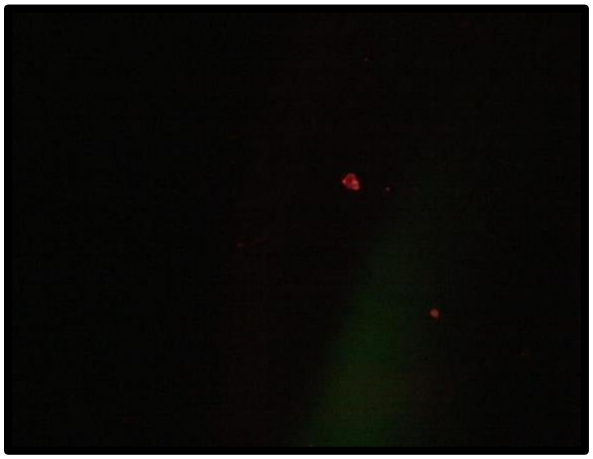

Fig 4.4.1.5

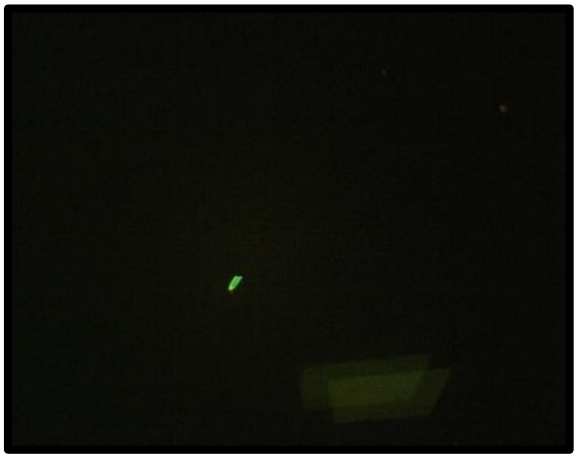

Fig 4.4.1.6

Figure 4.4.1.1- 4.4.1.6 Nanoplastics in soft drink PET bottle showing red and green fluorescence under green light.

4.4.2 Raman Analysis

Raman spectra were compared to standard plastic polymer spectrum for each of the NP classes (Fig 4.4.2.1 – 4,4,2,3) using Wiley Science Solution's KnowItAll Raman Spectral Database Collection. Three different spectra were observed due to the testing of three different sections of the filter membrane.

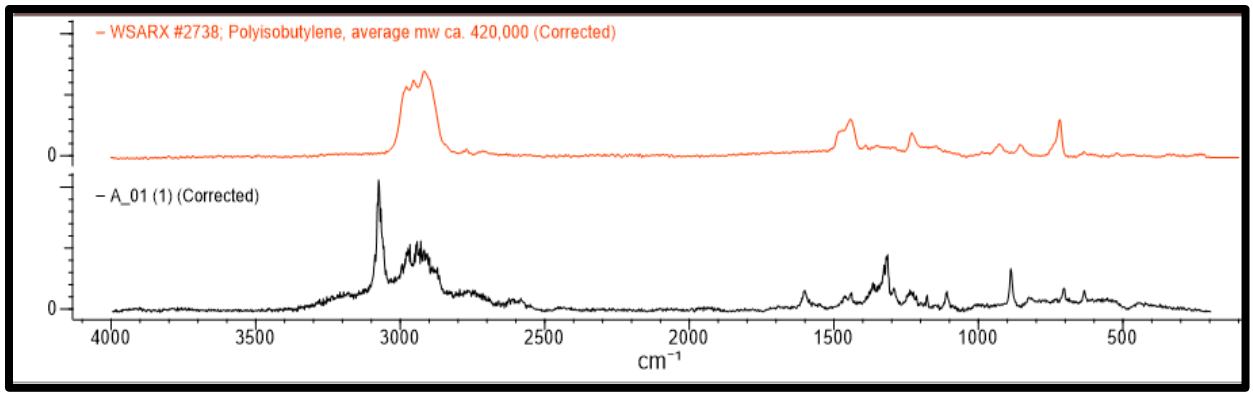

Fig 4.4.2.1Raman Spectra of Soft Drink 01A

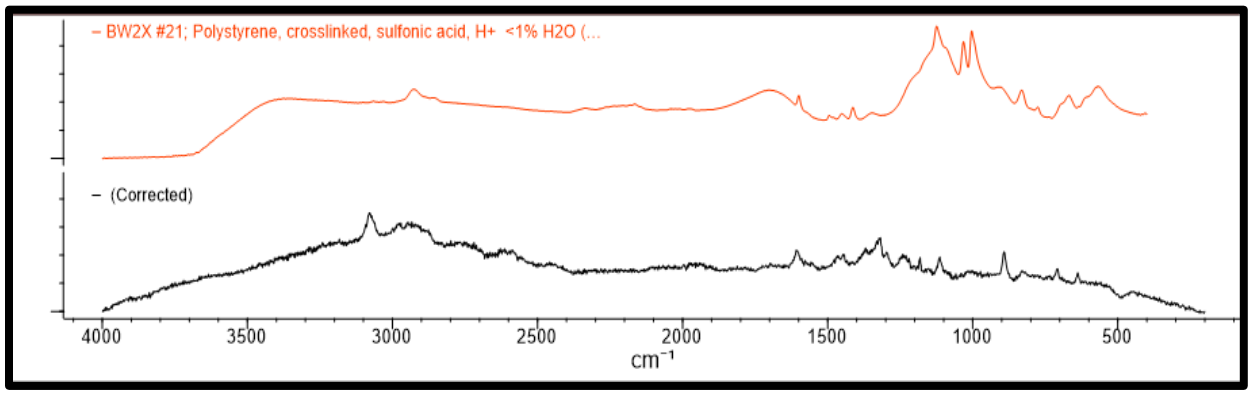

Fig 4.4.2.2 Raman Spectra of Soft Drink 01 B

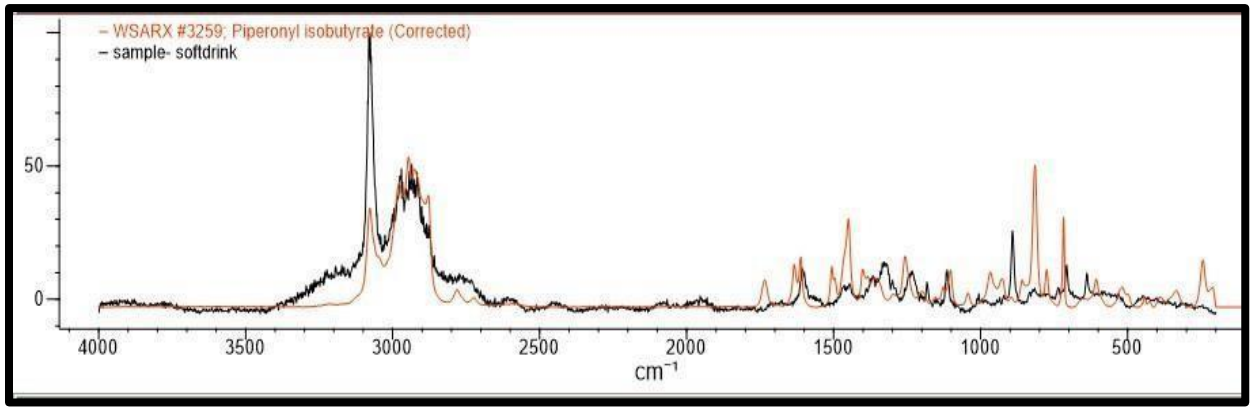

Fig 4..4.2.3.3Raman Spectra of soft drink 01 C

Table 2: Raman analysis for soft drink

Sr. no.	NP as given for Raman	Similar to
1	SD01A	Polystyrene
2	SD01B	Polyisobutylene
3	SD01C	Piperonyl isobutrate

Raman spectrum for SD01A showed similarity to polyisobutylene. SD01B is similar to polyisobutylene having 55.97% similarity and SD01C shows similarity to piperonyl isobutrate. (Fig 4.4.2.1 – 4.2.3.3) This result is of soft drink sample chilled in a PET bottle. Iced carbonated beverage bottles release MPs/NPs at levels between 68 and 4.66×10^8 particles/L (Chen, 2023) which signifies that nanoplastics are released from soft drink PET bottle.

b) Soft drink from PET bottle exposed to sunlight Using Rhodamine B dye showed the presence of nanoplastic aggregates when observed under 10X. (Fig 4.4.3.1 -4.4.3.8)

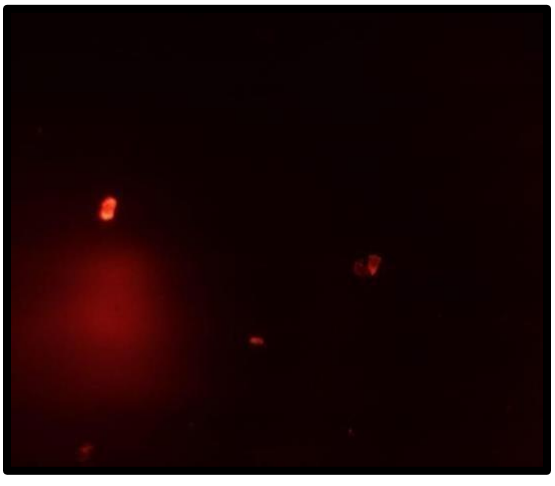

Fig 4.4.3.1

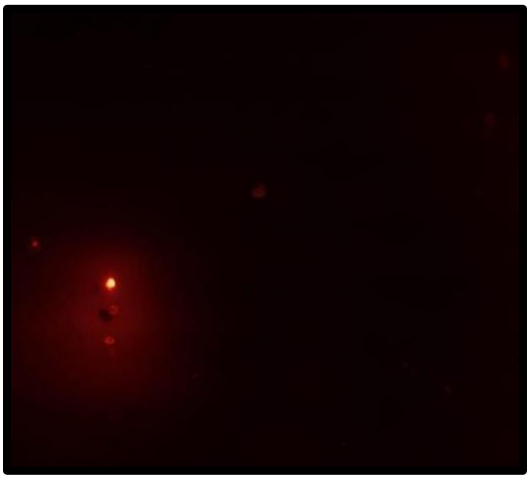

Fig 4.4.3.2

Fig 4.4.3.3

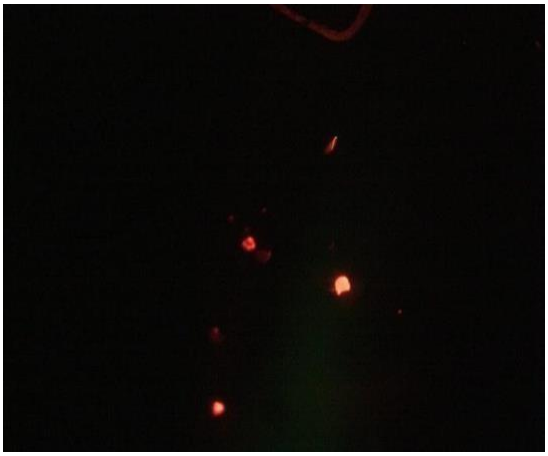

Fig 4.4.3.4

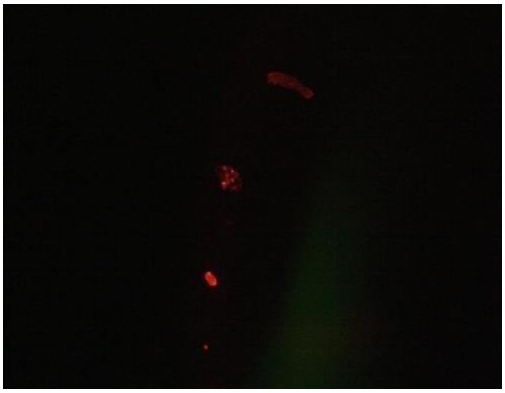

Fig 4.4.3.5

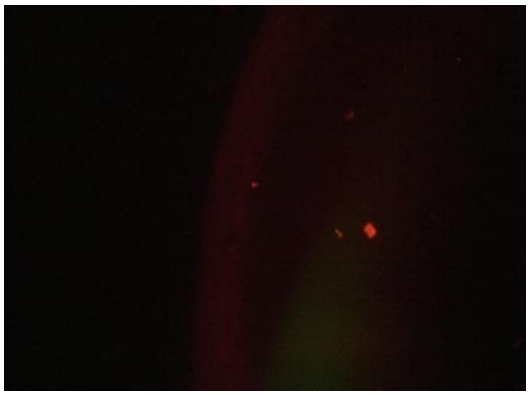

Fig 4.4.3.6

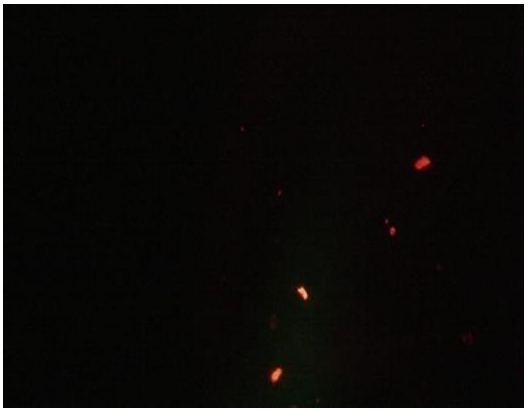

Fig 4.4.3.7

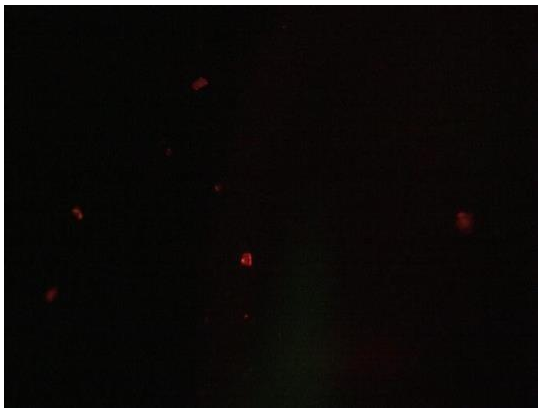

Fig 4.4.3.8

Fig 4.4.3.1- 4.4.3.8: Nanoplastics present in soft drink sample showing red colour under green light using Rhodamine B dye.

c) Soft drink from PET bottle exposed to sunlight and stained Using Nile red dye when observed under 10 X contained nanoplastics as seen in fig 4.4.4.1 – 4.4.4.2

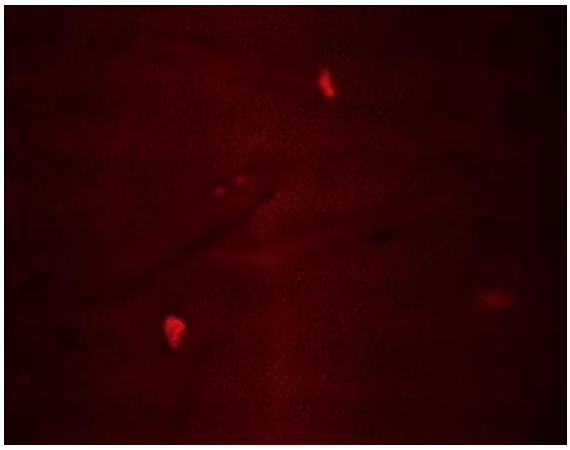

Fig 4.4.4.1

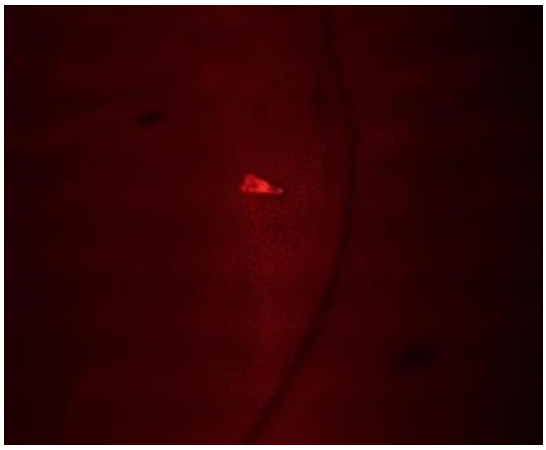

Fig 4.4.4.2

Fig 4.4.4.1-4.4.4.2 Nanoplastics in soft drink from Pet bottle exposed to sunlight showing red fluorescence under green light when stained using Nile red dye.

According to (Astner et al., 2019) high temperatures may be a cause for plastic polymers to break down and degrade, releasing microplastics (MPs) into common food and drink items

d) Glass bottle chilled

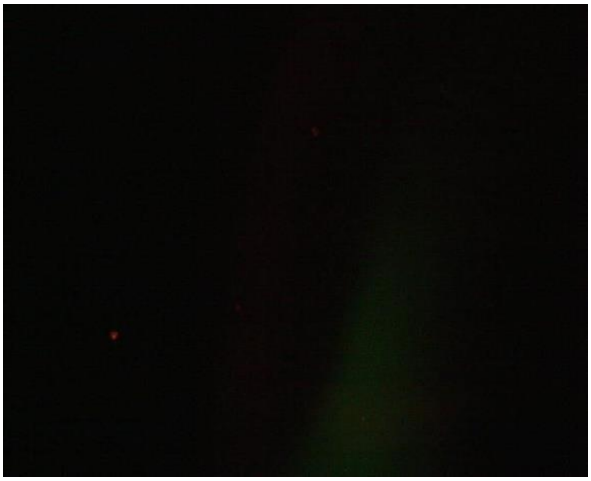

Fig 4.4.4.3 Soft drink from chilled glass bottle sample under fluorescence microscope no nanoplastics aggregates present.

Fig 4.4.3.7 nanoplastic aggregates in sample exposed to sunlight and stained using rhodamine B using green light. Fig 4.4.4.1 nanoplastic aggregates under 10X using Nile red stain under green light. Fig 4.4.4.3 sample from chilled glass soft drink bottle showed no presence of nanoplastics aggregates. According to a study it was reported that multiple freeze-thaw cycles (from -18°C to 25°C) increased the release of MPs and NPs (Yalin, 2023) could be a possible reason for the presence of nanoplastics in chilled PET bottles.

4.4 Paper cups

For the analysis of nanoplastics, water at room temperature was taken as control and directly transferred in the cup and was kept for 15 minutes. It was then filtered using 0.22 um nucleopore membrane followed by staining. The results showed no presence of nanoplastics aggregates (Fig 4.5.1)

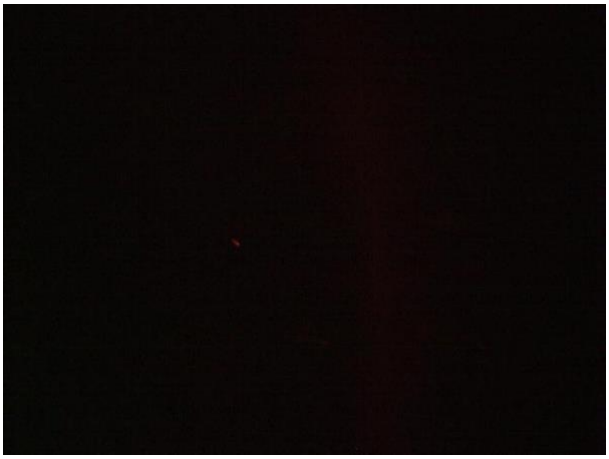

Fig 4.5.1: Room temperature filtered water from paper cup

For the test sample the water was boiled properly and transferred to the paper cups . then passed through the 0.22um filter membrane and was stained with Rhodamine B stain until dried. And observed under epifluorescence microscope. Fig 4.5.2 – 4.5.7 show the presence of medium intensity nanoplastic aggregates when observed under 10X

Filtered Boiled water from the paper cup after 15 minutes was taken as the test sample.

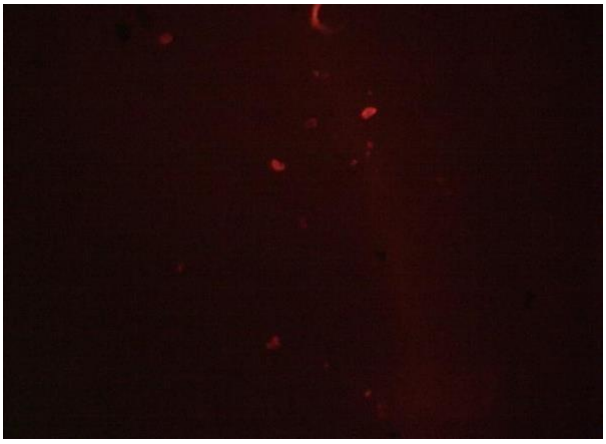

Fig 4.5.2

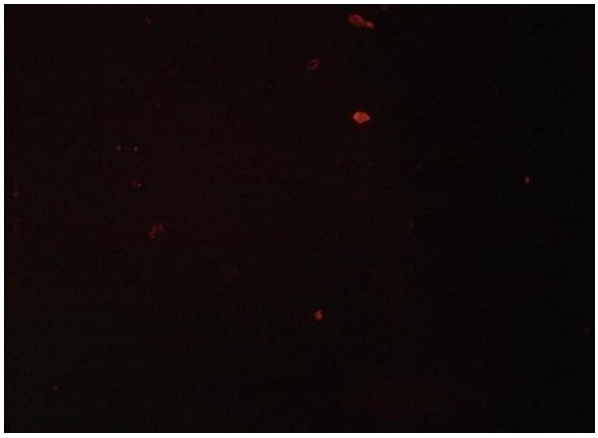

Fig 4.5.3

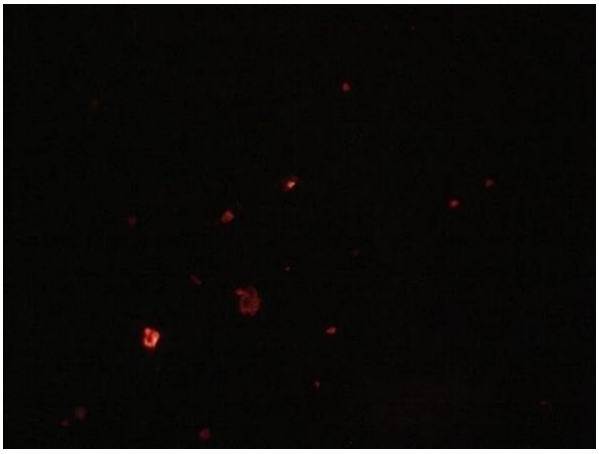

Fig 4.5.4

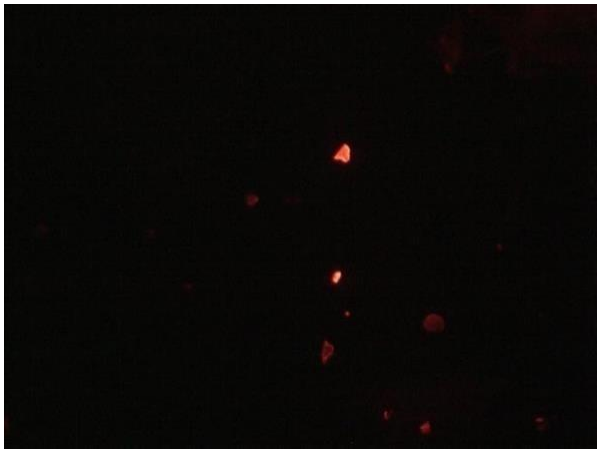

Fig 4.5.5

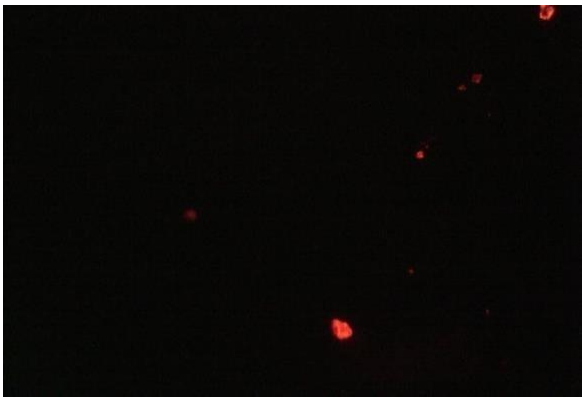

Fig 4.5.6

Fig 4.5.7

Figure 4.5.2 – 4.5.7 Nanoplastics in hot water showing red fluorescence under green light.

Hernandez et al. (2019) found that plastic microplastics (MPs) and nanoparticles (NPs) were released into the beverage when empty plastic tea bags were immersed in reverse osmosis water for five minutes at 95°C

4.6 Milk cream

Milk cream was digested using KOH in and kept for 2 days in the hot air oven at 60 °C

(Fig 4.6.0.1-4.6.0.4)

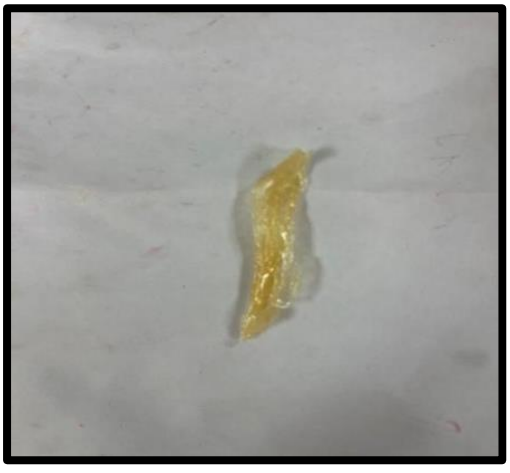

Fig :4.6.0.1 milk cream before digestion

Fig: 4.6.0.2 Milk cream digestion day 0

Fig 4.6.0.3: Milk cream digestion day 1

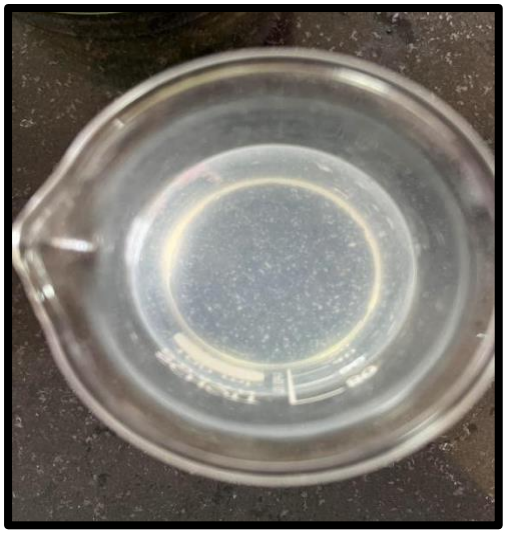

Fig 4.6.0.4 Milk cream digestion day 2

4.6.1. Nanoplastic Analysis

After the digestion of milk cream sample with 10% KOH, it was assessed for NPs using microscopic and Raman spectroscopic techniques for the detection of NPs of size less than 200nm.

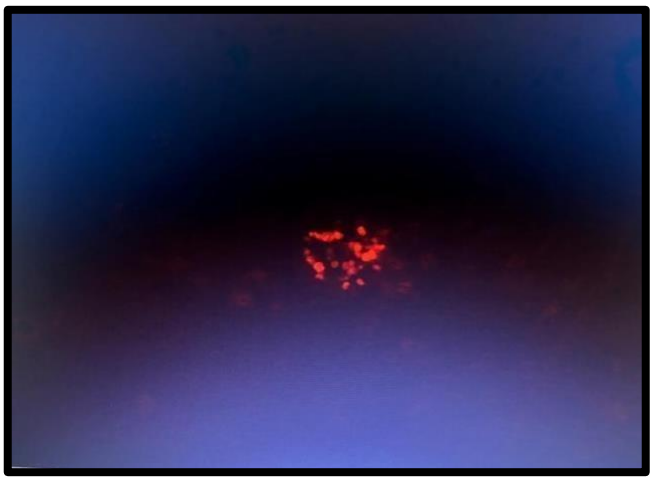

Fig 4.6.1.1

Fig 4.6.1.2

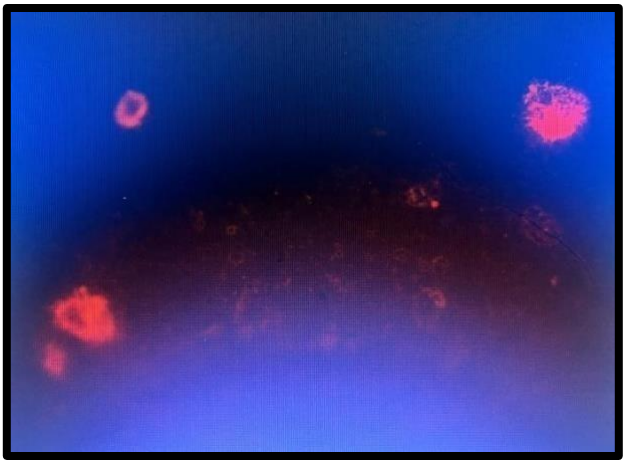

Fig 4.6..1.3

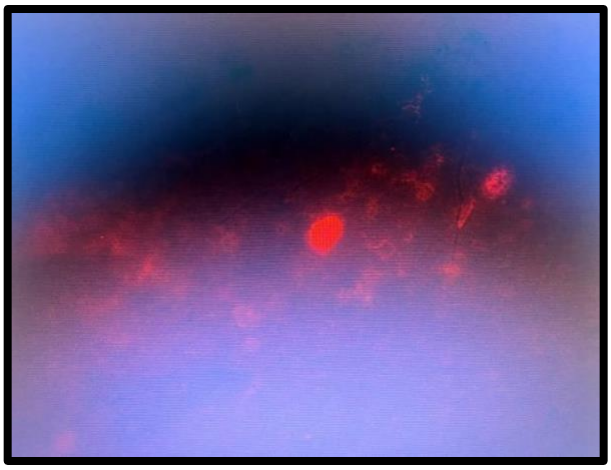

Fig 4.6.1.4

Fig 4.6.1.5

Figure 4.6.1.1- 4.6.1.5 Nanoplastics in milk cream showing red fluorescence when observed under green light.

This sample when observed under the epifluorescence microscope showed the presence of nanoplastic aggregates under 10X (Fig 4.6.1.1 – 4.6.1.5). The emitted fluorescence is weak due to low intensity of light. Microplastic contamination in milk may occur during inhalation and feeding of the cows which can end up in the milk and also might originate from each step of the production processes and packaging (Diaz-Basantes et al., 2022).

4.6.2. Raman analysis for milk cream

Raman spectra were compared to standard plastic polymer spectrum for each of the NP using Wiley Science Solution's KnowItAll Raman Spectral Database Collection. Three different spectra were observed as three sections on the same filter were analyzed .

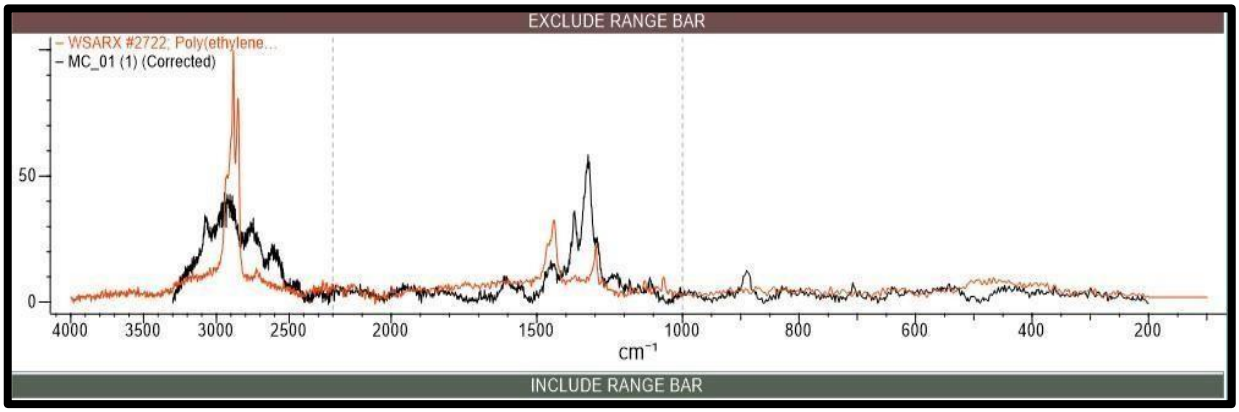

Fig 4.6.2.1 Raman spectra of milk cream MC01A

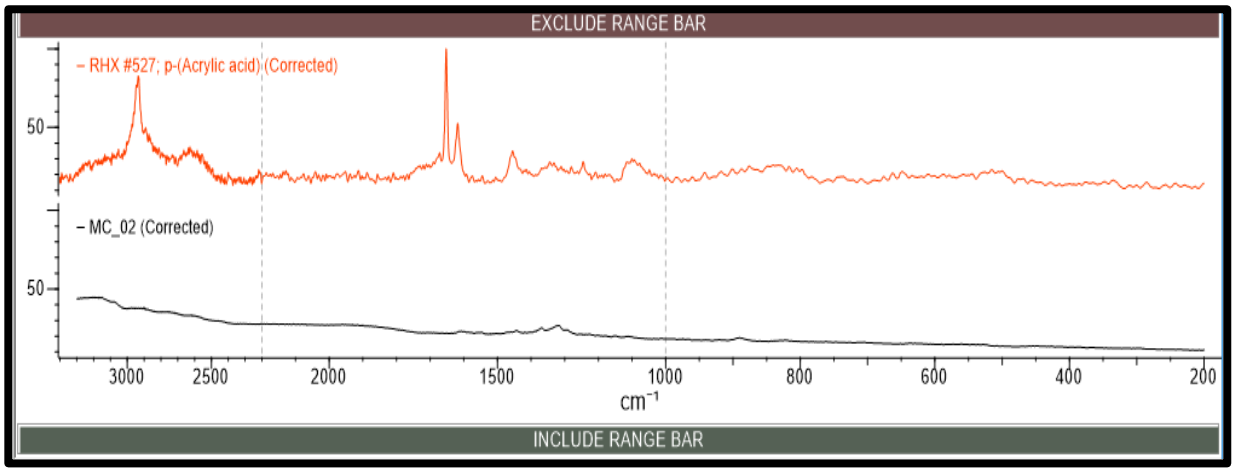

Fig 4.6.2.2 Raman spectra of milk cream MC01B

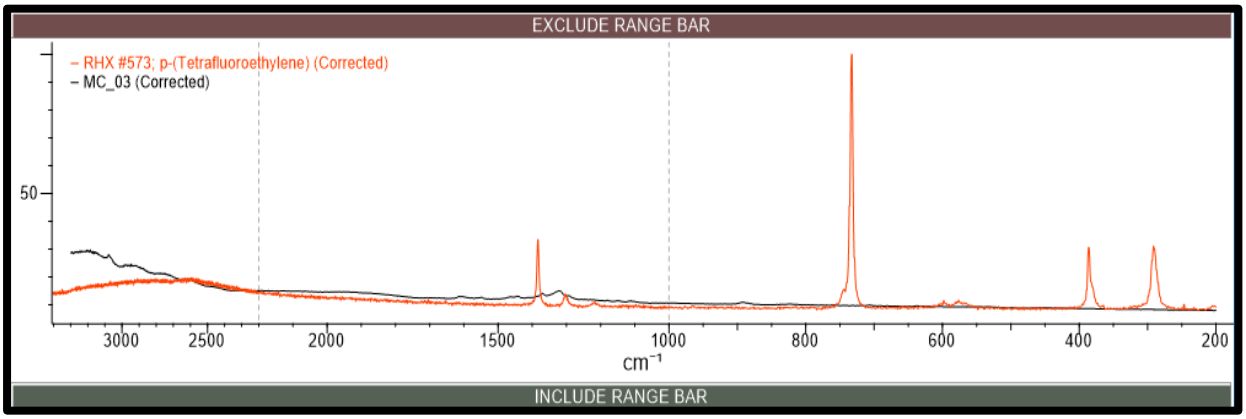

Fig 4.6.2.3 Raman spectra of milk cream MC01C

Table 3: Raman analysis for milk cream

Sr. no.	NP as given for Raman	Similar to
1	MC01A	PEVA
2	MC01B	PAA
3	MC01C	PTFE

Raman spectrum for MC01A showed 53.93 % similarity to PEVA and MC01B showed 61.47 % similarity to PAA. MC01C showed 60.77% similarity with PTFE. (Fig: 4.2.6.1 – 4.2.6.3) The different spectrum observed are because three sections from the filter were taken for Raman analysis. The results obtained are in accordance to the literature PEVA is the most dominant plastic polymer found in milk (Diaz-Basantes et al.,2022).

REFERENCES

1. Alprol, A. E., Gaballah, M. S., & Hassaan, M. A. (2021). Micro and Nanoplastics analysis: Focus on their classification, sources, and impacts in marine environment. *Regional Studies in Marine Science*, *42*, 101625. https://doi.org/10.1016/j.rsma.2021.101625

2. Altunışık, A. (2023). Prevalence of microplastics in commercially sold soft drinks and human risk assessment. *Journal of Environmental Management*, *336*, 117720. https://doi.org/10.1016/j.jenvman.2023.117720

3. Amobonye, A., Bhagwat, P., Sindhu, R., Singh, S., & Pillai, S. (2021). Environmental Impacts of Microplastics and Nanoplastics: A Current Overview. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.768297

4. Cai, H., Xu, E. G., Du, F., Li, R., Liu, J., & Shi, H. (2021). Analysis of environmental nanoplastics: Progress and challenges. *Chemical Engineering Journal*, *410*, 128208. https://doi.org/10.1016/j.cej.2020.128208

5. Campanale, C., Massarelli, C., Savino, I., Locaputo, V., & Uricchio, V. F. (2020). A detailed review study on potential effects of microplastics and additives of Concern on human health. *International Journal of Environmental Research and Public Health/International Journal of Environmental Research and Public Health*, *17*(4), 1212. https://doi.org/10.3390/ijerph17041212

6. Cella, C., La Spina, R., Méhn, D., Fumagalli, F., Ceccone, G., Valsesia, A., & Gilliland, D. (2022). Detecting Micro- and Nanoplastics Released from Food Packaging: Challenges and Analytical Strategies. *Polymers*, *14*(6), 1238. https://doi.org/10.3390/

7. Chen, Y., Xu, H., Luo, Y., Ding, Y., Huang, J., Wu, H., Han, J., Du, L., Kang, A., Jia, M., Xiong, W., & Yang, Z. (2023). Plastic bottles for chilled carbonated beverages as a source of microplastics and nanoplastics. *Water Research*, *242*, 120243. https://doi.org/10.1016/j.watres.2023.120243

8. Da Costa Filho, P. A., Andrey, D., Eriksen, B., Peixoto, R. P., Carrères, B. M., Ambühl, M. E., Descarrega, J. B., Dubascoux, S., Zbinden, P., Panchaud, A., & Poitevin, E. (2021). Detection and characterization of small-sized microplastics (≥ 5 µm) in milk products. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-021-03458-7

9. Du, H., Zhang, Y., Jiang, H., & Wang, H. (2022). Adsorption of rhodamine B on polyvinyl chloride, polystyrene, and polyethylene terephthalate microplastics in aqueous environment. *Environmental Technology and Innovation 27*

10. Gao, Z., Wontor, K., & Cizdziel, J. V. (2022). Labeling Microplastics with Fluorescent Dyes for Detection, Recovery, and Degradation Experiments. *Molecules/Molecules Online/Molecules Annual*, *27*(21), 7415. https://doi.org/10.3390/molecules27217415

11. Gaylarde, C. C., Neto, J. a. B., & Da Fonseca, E. M. (2021). Nanoplastics in aquatic systems - are they more hazardous than microplastics? *Environmental Pollution*, *272*, 115950. https://doi.org/10.1016/j.envpol.2020.115950

12. Gigault, J., Ter Halle, A., Baudrimont, M., Pascal, P., Gauffre, F., Phi, T., Hadri, H. E., Grassl, B., & Reynaud, S. (2018). Current opinion: What is a nanoplastic? *Environmental Pollution*, *235*, 1030–1034. https://doi.org/10.1016/j.envpol.2018.01.024

13. Hernandez, L., Yousefi, N., & Tufenkji, N. (2017). Are there nanoplastics in your personal care products? . *Environ Sci Technol Lett 4(7):*, 280–285. doi:https://doi.org/10.1021/ acs.estle tt.7b00187

14. Huang, Y., Wong, K. K., Wei, L., Zhao, H., Wang, T., Stanescu, S., Boult, S., Van Dongen, B. E., Mativenga, P., & Li, L. (2022). Characteristics of nano-plastics in bottled drinking

water. *Journal of Hazardous Materials, 424*, 127404. https://doi.org/10.1016/j.jhazmat.2021.127404

15. Huiyan, T., Jiang, Q., Zhong, X., & Hu, X. (2020). Rhodamine B dye staining for visualizing microplastics in laboratory-based studies. *Environmental Science and Pollution Research*, 4209-4215

16. Ivleva, N. P., Wiesheu, A. C., & Nießner, R. (2016). Microplastic in aquatic ecosystems. *Angewandte Chemie*, *56*(7), 1720–1739. https://doi.org/10.1002/anie.201606957

17. Jin, M., Wang, X., Ren, T., Wang, J., & Shan, J. (2021). Microplastics contamination in food and beverages: Direct exposure to humans. *Journal of Food Science*, *86*(7), 2816–2837. https://doi.org/10.1111/1750-3841.15802

18. Karakolis, E., Nguyen, B., You, J., Rochman, C., & Sinton, D. (2019). Fluorescent dyes for visualizing micro plastic particles and fibers in laboratory-based studies. *Environmental science and technology*, 334–340.

19. Kaseke, T., Lujic, T., & Veličković, T. Ć. (2023). Nano- and Microplastics Migration from Plastic Food Packaging into Dairy Products: Impact on Nutrient Digestion, Absorption, and Metabolism. *Foods*, *12*(16), 3043. https://doi.org/10.3390/foods12163043

20. Kiran, B. R., Kopperi, H., & Mohan, S. V. (2022). Micro/nano-plastics occurrence, identification, risk analysis and mitigation: challenges and perspectives. *Reviews in Environmental Science and Bio/Technology*, *21*(1), 169–203. https://doi.org/10.1007/s11157-021-09609-6

21. Lai, H., Liu, X., & Qu, M. (2022). Nanoplastics and human Health: hazard identification and biointerface. *Nanomaterials*, *12*(8), 1298. https://doi.org/10.3390/nano12081298

22. Mariano, S., Tacconi, S., Fidaleo, M., Rossi, M., & Dini, L. (2021). Micro and Nanoplastics identification: classic methods and innovative detection techniques. *Frontiers in Toxicology*, *3*. https://doi.org/10.3389/ftox.2021.636640

23. Mitrano, D. M., Wick, P., & Nowack, B. (2021). Placing nanoplastics in the context of global plastic pollution. *Nature Nanotechnology*, *16*(5), 491–500. https://doi.org/10.1038/s41565-021-00888-2

24. Mortensen, N. P., Fennell, T. R., & Johnson, L. D. (2021). Unintended human ingestion of nanoplastics and small microplastics through drinking water, beverages, and food sources. *NanoImpact*, *21*, 100302.https://doi.org/10.1016/j.impact.2021.100302

25. Mourik, L., Veen, I., Velzen, M., Groenewoud, Q., & Leslie, H. (2022). Plastic Particles in Livestock Feed, Milk, Meat and Blood: A Pilot Study. *Environment & Health.*

26. Nguyen, B., Claveau-Mallet, D., Hernandez, L. M., Xu, E. G., Farner, J. M., & Tufenkji, N. (2019). Separation and analysis of microplastics and nanoplastics in complex environmental samples. *Accounts of Chemical Research*, *52*(4), 858–866. https://doi.org/10.1021/acs.accounts.8b00602

27. Novotná, K., Čermáková, L., Pivokonská, L., Cajthaml, T., & Pivokonský, M. (2019). Microplastics in drinking water treatment – Current knowledge and research needs. *Science of the Total Environment*, *667*, 730–740. https://doi.org/10.1016/j.scitotenv.2019.02.431

28. Oßmann, B. E., Sarau, G., Holtmannspötter, H., Pischetsrieder, M., Christiansen, S., & Dicke, W. (2018). Small-sized microplastics and pigmented particles in bottled mineral water. *Water Research*, *141*, 307–316. https://doi.org/10.1016/j.watres.2018.05.027

29. Rodríguez-Seijo, A., & Pereira, R. (2017). Morphological and physical characterization of microplastics. In *Comprehensive analytical chemistry* (pp. 49–66). https://doi.org/10.1016/bs.coac.2016.10.007

30. Schymanski, Goldbeck, C., Humpf, H., & Fürst, P. (2018). Analysis of microplastics in water by micro-Raman spectroscopy: release of plastic particles from different packaging into mineral water. *Water Res 129:*, 154–162. doi:https://doi.org/10.1016/j.watres.

31. Sharma, V. K., Ma, X., Lichtfouse, É., & Robert, D. (2022). Nanoplastics are potentially more dangerous than microplastics. *Environmental Chemistry Letters*, *21*(4), 1933–1936. https://doi.org/10.1007/s10311-022-01539-1

32. Shen, M., Zhang, Y., Zhu, Y., Song, B., Zeng, G., Hu, D., Wen, X., & Ren, X. (2019). Recent advances in toxicological research of nanoplastics in the environment: A review. *Environmental Pollution*, *252*, 511–521. https://doi.org/10.1016/j.envpol.2019.05.102

33. Shorny, A., Steiner, F., Hörner, H., & Skoff, S. M. (2023). Imaging and identification of single nanoplastic particles and agglomerates. *Scientific Reports*, *13*(1). https://doi.org/10.1038/s41598-023-37290-y

34. Shruti, V., Pérez-Guevara, F., Elizalde, I., & Kutralam-Muniasamy, G. (2021). Toward a unified framework for investigating micro(nano)plastics in packaged beverages intended for human consumption. *Environmental Pollution*, *268*, 115811. https://doi.org/10.1016/j.envpol.2020.115811

35. Son, J., Nam, Y. S., & Kim, C. (2024). Nanoplastics from disposable paper cups and microwavable food containers. *Journal of Hazardous Materials*, *464*, 133014. https://doi.org/10.1016/j.jhazmat.2023.133014

36. Sohail, M., Urooj, Z., Noreen, S., Baig, M. M. F. A., Zhang, X., & Li, B. (2023). Micro- and nanoplastics: Contamination routes of food products and critical interpretation of detection strategies. *Science of the Total Environment*, *891*, 164596. https://doi.org/10.1016/j.scitotenv.2023.164596

37. Tamminga, M., Hengstmann, E., & Fischer, E. (2017). Nile Red Staining as a Subsidiary Method for Microplastic Quantification: A Comparison of Three Solvents and Factors Influencing Application Reliability. *SDRP Journal of Earth Sciences & Environmental Studies*. doi:10.15436/JESES.2.2.1

38. Tong, H., Jiang, Q., Zhong, X., & Hu, X. (2020). Rhodamine B dye staining for visualizing microplastics in laboratory-based studies. *Environmental Science and Pollution Research*, *28*(4), 4209–4215. https://doi.org/10.1007/s11356-020-10801-4

39. Toussaint, B., Barbara, R., Angers-Loustau, A., Gilliland, D., Kestens, V., Petrillo, M., Rio-Echevarria, I. M., & Van Den Eede, G. (2019). Review of micro- and nanoplastic contamination in the food chain. *Food Additives & Contaminants: Part A*, *36*(5), 639–673. https://doi.org/10.1080/19440049.2019.1583381

40. Vitali, C., Peters, R., Janssen, H., & Nielen, M. W. F. (2023). Microplastics and nanoplastics in food, water, and beverages; part I. occurrence. *TrAC Trends in Analytical Chemistry*, *159*, 116670. https://doi.org/10.1016/j.trac.2022.116670

41. Wallace, H., Alexandar, J., Bignami, M., & Cortrill, B. (2016). Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA 14(6)*. doi:10.2903/j.efsa.2016.4501

42. Winkler, A., Fumagalli, F., Cella, C., Gilliland, D., Tremolada, P., & Valsesia, A. (2022). Detection and formation mechanisms of secondary nanoplastic released from drinking water bottles. *Water Research*, *222*, 118848. https://doi.org/10.1016/j.watres.2022.118848

43. Xu, J., Thomas, K., Luo, Z., & Gowen, A. (2019). FTIR and raman imaging for microplastics analysis: state of the art, challenges and prospects. *Trends Anal. Chem. 119, 115629.* doi:https://doi.org/10.1016/j.trac.2019.115629

44. Xing, G., Gao, J., Wang, H., & Liu, Y. (2023). New Fluorophore and its applications in visualizing polystyrene nanoplastics in bean sprouts and HELA cells. *Molecules/Molecules Online/Molecules Annual*, *28*(20), 7102. https://doi.org/10.3390/molecules28207102

45. Yee, M. S., Hii, L., Looi, C., Lim, W., Wong, S. F., Kok, Y., Tan, B., Wong, C. S., & Leong, C. (2021). Impact of microplastics and nanoplastics on human health. *Nanomaterials, 11*(2), 496. https://doi.org/10.3390/nano11020496

46. Zhang, E., Kim, M., Rueda, L., Rochman, C., VanWormer, E., Moore, J., & Shapiro, K. (2022). Association of zoonotic protozoan parasites with microplastics in seawater and implications for human and wildlife health. *Scientific Reports*. doi:https://doi.org/10.1038/s41598-022-10485-5

APPENDIX

Reagents preparation

10% KOH

10% KOH was prepared in a screw cap bottle by weighing 10g of KOH pellets and dissolving them in 100mL of distilled water. The bottle was marked as corrosive and stored in a cool, dry place.

Nile Red

Nile Red (NR) solution was prepared at 1 mg/mL in acetone in a clean 5ml vial. The vial was covered with aluminium foil and stored at room temperature away from light until use.

Rhodamine B

Rhodamine B (RhB) solution was prepared at 0.2mg/mL in ethanol in a clean 5ml vial. The vial was covered with aluminium foil and stored at 20˚C until use.

Similarity search of plastic polymers done using Wiley Science Solutions's KnowItAll Raman Spectral Database Collection

New search option was selected in KnowItAll information system 2021 and spectrum in .spc format was opened. A list of all the compounds having similar functional groups was obtained and was checked for the polymers of interest. The software gave percent similarity based on the functional groups present in the standard and the sample. The graph of the standard polymer was compared to that of the sample. Snipping tool was used to take a snap of the spectra obtained.