Methods for the Detection and Quantification of Micro and Nanoplastics- A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Review Article

ABSTRACT

Over the past 35 years, synthetic or semi-synthetic polymers called "plastics" have been widely used across multiple fields due to their low cost, versatility, durability. Plastics have proved to be a boon to mankind. However, overuse of non- biodegradable plastics comes with its own downsides. Despite constant efforts to reuse and recycle plastics, these polymers substantially contribute towards the accumulation of debris hazardous to the environment. Plastic materials are slowly broken into fragments of micro- and nano plastics due to aging and weathering. Micro- and nano plastics were found capable of entering the food chain and hence are viewed as threats. This review paper revolves around methods used for the detection and quantification of micro- and nano plastics. Detection of micro- and nano plastics using methods like Raman spectroscopy, Infrared Spectroscopy, SERS, MALDI-TOF, and machine learning approaches are discussed here. The research efforts carried out in this article aims to further facilitate the R&D initiatives of Jozbiz Technologies.

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1. INTRODUCTION

Plastics are part of human life as it is mainly used in packaging. They are highly versatile, cheap, strong and durable. A day without plastics is unimaginable in the current era. Despite the various uses of plastic, it is still considered a threat to the environment due to it's non-biodegradability. Plastic pollution was first reported in 1974 and is a subject of major concern today. Initiatives to encourage sustainable use of plastics has increased.

The major classes of plastics in packaging material are Polyethene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET); and Polyvinyl chloride (PVC). These polymers are often broken down into tiny particles of plastics termed "microplastics" and are a form of man-made litter. They usually have diameters in the range of micrometers (0.06–0.5 mm in diameter). These particles contaminate seawater and affect aquatic organisms. Microplastics are found concentrated near the surface and samples can be visualized under a microscope using a lipophilic dye [1]. Marine aquaculture areas are highly polluted with plastics because of the plastic fishing aids used and their improper management [2]. This highly impacts the marine ecosystem. Hence, it is crucial to tackle the issues arising due to the extreme use and inappropriate disposal methods of plastic.

The integrity of plastics lies in its high molecular weight and any process that can drastically reduce it's average molecular weight will degrade the polymer. Plastics are not naturally biodegradable . The process of degradation can be brought about using different techniques which are as follows: biodegradation, photodegradation, thermo-oxidative degradation (slow oxidative breakdown at moderate temperatures), thermal degradation (action of high temperatures) and hydrolysis (reaction with water) [1].

Microplastics in oceans occur from the two main sources (a) direct introduction with runoff e.g., Microplastics used in cosmetics and (b) weathering breakdown of meso- and macroplastic debris.The latter is the major source of microplastics . Meso plastics are large plastic particles in the size range of 5-10mm. Macroplastics are the much larger plastics that are clearly visible [1]. The degradation rate of microplastics is very low and thus can cause negative impacts for years. Microplastics can be ingested by marine organisms which then is passed on to the higher organism in the food chain [2].

Many organisms ingest microplastics mistaking them for their prey. E.g., the dominant type of microplastics in zooplanktons were fibers. Similarly, the Planktivorous fish ingested blue polyethene fragments similar to their copepod prey [3]. Colour and size are hence important aspects of microplastic characterization.

The introduced microplastics in seawater attach to other microplastics to form biofilms. These biofilms constitute a new habitat named "plastisphere". Bacteria such as Vibrio and Pseudomonas are protected by these biofilms. Bacteria and microplastics cause major problems in fish production, human dietary health, ecological problems and these are carried away by wind causing it's spread across the oceans [2]. They are found relatively untouched by humans like in the Southern Ocean. The average abundance of microplastic in Antarctica ranged from 0.67 to 114 items m^{-2} [4].

Zhou et al. [5] showed that adding microplastics to compost affects the organic matter humification and fungal community succession during the composting process. It decreases humic acid content, maturity and polymerization of compost. These microplastics weakened the stability of the fungal community and decreased the symbiosis of fungi and increased the risk of presence of phytopathogenic fungi in compost [5].

Microplastics enter the human body along with food, water or day to day products we use. Microplastics are found in the gastrointestinal tract of marine organisms. The mean concentration of microplastic in gills ranged from 9.5 ± 2.87 to 52.6 ± 7.42 items/individual. And the average abundance of microplastic in guts varied from 8.8 ± 4.14 to 51.3 ± 7.24 items/individual [6] were the first to study the presence of microplastics in the human placenta through Raman Microspectroscopy.

12 microplastics (MP) fragments were isolated in 23 grams of four human placentas. The number of microplastics 5, 4 and 3 were found on the foetal side, maternal side and chorioamniotic membrane, this indicates microplastics are in every level of the placental tissues. Microparticles in the placenta can alter several

cellular regulating pathways and this may lead to adverse pregnancy results [6].

Even though plastic is causing trouble and is a concerning issue of the current century, it is also true that plastic is unavoidable. There are many ways to detect and quantify microplastics and nanoparticles in the environment.

2. HISTORY OF METHODS

2.1 Detection of Microplastics in *Scrobicularia plana* (clams) Using IR Spectroscopy

Ribeiro et al. [7] says that Clams are suitable bio monitors in the environmental risk assessment of PS (polystyrene) microplastics as they are prominent targets of PS microplastics ecotoxicity.

In order to check the presence of PS microplastic in the tissues of peppery furrow shell and assess further, [7] used Infrared (IR) spectroscopy in the mode of diffuse reflectance. IR spectroscopy basically detects the absorption of light (in the IR region of electromagnetic spectrum) by a compound or molecule containing a bond within that exhibits dipole moment. It is used for structural determination of a compund or a molecule.

Initially, two stock solutions of PS microplastics of 100 mg/L, one using ultra-pure water and another using natural sea water were prepared, which were maintained under constant aeration. Clams were collected and stored for 7 days under 12-hour light of photoperiod. Three aquaria containing 20L natural sea water, each with 1 mg/L of microplastics and sixty clams were used. Aeration was done through glass Pasteur pipettes. Water was changed for every 24 hours with subsequent addition of PS microplastics, before which stock solution of 100 mg/L was sonicated for 30 minutes in purified water. Abiotic parameters such as temperature, salinity, pH and percentage of oxygen saturation were checked and maintained using multiparametric probe TRIPOD. Clams were collected over 0, 3, 7 and 14 days respectively and were depurated for 7 days. Immediately after which clams hemolymph were collected by intermittent suction using a sterile hypodermic syringe which was gently penetrated into the posterior adductor muscle. The gills and digestive gland were stored at -80°C after dissection.

A qualitative analysis was done using optical microscopy analysis of hemolymph of control (0

days exposure) and exposed clams on 14th day in order to check the presence of microplastics in the tissue of clams. The gills and digestive gland tissues were lyophilized at -40 °C and Infrared spectroscopy was used for their analysis. The lyophilized samples were diluted in the ratio of 1:4 with potassium bromide and ground finely in an agitator. They were stuffed into a 11mm diameter sample cup and pressed in order to produce a smooth surface.

And over carrying out IR spectroscopy and optical microscopy for qualitative analysis, the hydrodynamic diameter of PS microplastics was found to be 18.4 ± 1.33µm from DLS measurement. And PS microplastics were found to have highest negative surface charge in seawater ($-12.4 ± 2.36$ mV) than in Milli-Q water ($-52.6 ± 2.34$ mV) from the zeta potential measurements which indirectly shows the tendency of aggregation of PS microplastics in seawater. Observing hemolymph under optical microscope confirmed the presence of microplastics as small aggregates. Accumulation of PS microplastics from the seawater was found in clam's gills (noted after 14 days) and digestive glands. Microplastics were found in digestive glands by the ingestion through the inhalant siphon which was subsequently transported to the mouth and to the digestive gland. Presence of aggregates of microplastics determines that they travelled through the circulatory system too.

So [7] concluded that clams consumed PS microplastics dominantly by gills (accumulation) and also in digestive gland (storing). Gills were found to be more effective in response than the digestive glands by inducing oxidative stress for PS exposure.

Through this method IR spectroscopy was found to be more efficient in studying the effects of microplastics in clams and also in measuring the microplastics in sea water.

2.2 Microplastics Detection in Streaming Tap Water using Raman Spectroscopy

Microplastic analysis usually consists of sampling, sample processing, identification and quantification of the microplastic contents present in the sample. For microplastics present in smaller volumes, sampling is often done using sieves and filters. Later, the filtrate is treated in order to get rid of organic compounds and the polymer content will be analyzed.

Ann-Kathrin [8] have mentioned that Fourier-Transform Infrared (FTIR) spectroscopy and Raman micro-spectroscopy are extensively used in this method as they are non-invasive and can be directly applied on the filter which holds extracted particles. And among FTIR and Raman spectroscopy methods. FTIR is a sensitive, fast and a reliable technique used for the structural determination and quantification of various compounds which includes biomolecules. Raman spectroscopy is widely used as it can be applied directly for aquatic samples, it basically detects the shift in wavelength of the inelastically scattered radiation which provides chemical and structural information [9-11].

Kniggendorf et al. [8] had come up with a setup of direct detection of microplastics of size 0.1 mm in streaming tap-water (at the rate of 1 L/h) using Raman spectroscopy, which can be directly attached for a tap and it didn't include primary steps sampling and sample processing. And that setup was tested for microplastics of five common polymers Polyamide (PA), Polyethylene (PE), Polymethyl-methacrylate (PMMA), Polypropylene (PP) and Polystyrene (PS) against autofluorescence and few other contaminants with same size as background.

The five different microplastic particles used in this method and their physical properties were as per the Table 1.

The test solutions were prepared by dissolving 1g of the above mentioned microplastics in 1L purified water respectively and were stored in a glass flask with glass stopper at room temperature. The sample solutions were prepared by dissolving 0.5g of microplastic and respective contaminants in 1L tap water (contaminant was either 50 ml/L surfactant or 10g/L humic acid) and was stored in a glass flask with glass stopper at room temperature. The particles were kept in the water column until the experiment was done by forcing them into the column through continuous shaking of closed flasks using a magnetic stirrer.

For the water circuit setup, the flow cell was composed of a rectangular tube of borosilicate glass with dimensions of 4mm length, 4mm breadth and 50mm width with 0.5mm thick wall. The tubes' internal diameter was 5mm. The final setup had PTFE tubing which showed the least number of adhering particles for all microplastics and the tube was attached directly to the flow cell and was sealed using Parafilm. 1L of each test solution was pumped through the components at 1L/h flow rate with the help of a programmable peristaltic pump equipped with six-roller head and PTFE coated pump tubes in order to test component compatibility with microplastics before draining and drying them. And microplastics in the test suspensions which were in contact with the surface of area 5 cm^2 were analyzed using a microscope. 70% Ethanol was used to rinse the surface of the setup.

The optical setup (Fig. 1) was set on a base plate of area 300×450 mm^2 and was shielded on all four sides with aluminium plates of 5mm thickness. The aluminium wall was drilled for opening for laser fibre, water tubes and a USB endoscope camera (to monitor the setup during operation).

For excitation, a fiber-connected continuous-wave Nd:YAG laser was used at a wavelength of 532 nm and the beam which was exiting the laser fiber was made parallel to a beam width of 1mm using the lens L1 and was directed by reflection at an angle of 8^0 on the edge filter F1 for 532 nm along the flow cell. The resultant backscattered light which passed through F1 was collected by the lenses L2 and L3 through F2 which is a second edge filter to the Raman spectrometer which had 50 µm slit and a 1200 lines/mm grating blazed for 700 nm, spectral resolution of 0.61 nm, recording spectra in the range 200 and 4000 rel. cm^{-1}, and an average quantum efficiency of 64% between 625.1 and 641.1 nm and the quantum efficiency of the inbuilt camera was 27%. The signal-to-noise ratio (SNR) of the spectrometer was 300:1. A USB endoscope camera was used to observe the beam path for the confirmation of spectra corresponding to only individual particles which were passing through the laser beam. The flashes of elastically scattered light were used to determine the number of particles within the path of the beam for each spectrum during the integration time [12-14]. The complete spectral range of the spectrometer was continuously recorded. The spectral range for polymer identification was limited to 2800-3100 rel./cm to allow for the automated recognition within the recording time of the next spectrum. The background was removed by averaging 500 Raman spectra of flowing tap water without added contaminants and was subtracted from the Raman spectra which was recorded. After which the obtained Raman spectra were smoothed using a filter.

Table 1. Physical properties of five different types of microplastic particles [8]

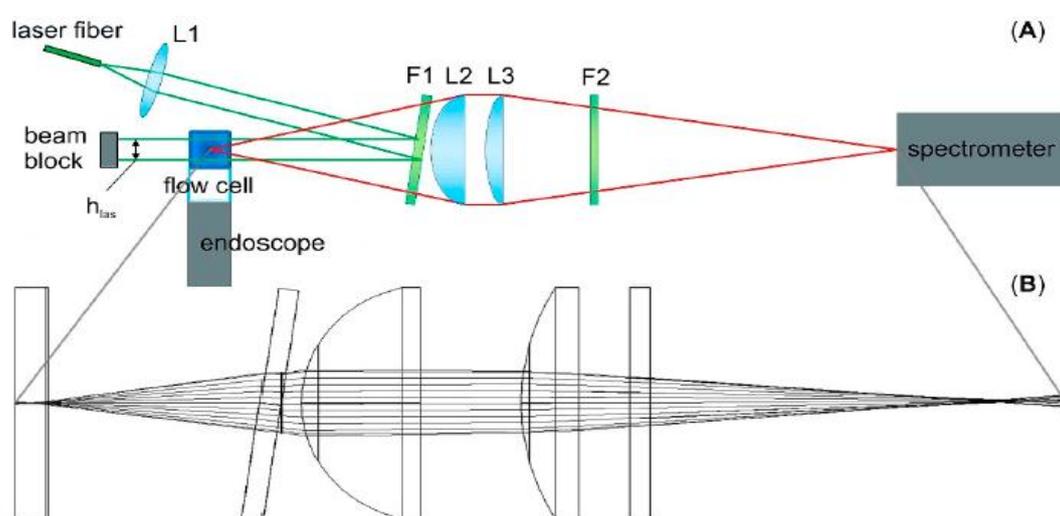| Polymer	Particle Size (µm)	Particle shape	Density (g/cm^3)	Raman bands (rel. cm^{-1})
Polyamide (PA)	1-315	fragments	1.14	2875,2903,2928
Polyethylene (PE)	1-315	fragments	0.92	2850,2884
Polymethyl-methacrylate (PMMA)	15-150	microbead	1.18	2848,2955,3002
Polypropylene (PP)	150	microbead	0.91	2842,2886,2961
Polystyrene (PS)	106-125	microbead	1.05	2855,2907,3058

Fig. 1. (A) Schematic of the optical setup with orientation of the flow cell perpendicular to the image plane. (B) Beam path from the centre of the cell to the entrance slit of the spectrometer [8]

The optical setup was evaluated using uncontaminated tap water suspended with microplastic particles which was pumped through the flow cell at 1 L/h flow rate and 1.54 W/mm^2 Laser power. In order to avoid repeated detection of microplastics inside the flow cell, 1 mm was the beam width kept. And from the Raman spectra of those five particles, it was understood that the SNR of minimum 5 was helpful in detecting the particles and the Raman lines at the highest wavenumbers of 3058 rel.cm^{-1} for PS, 3002 rel. cm^{-1} for PMMA, and 2928 rel. cm^{-1} for PA are mounted on the broad Raman band between 3200 and 3600 rel.cm^{-1} which belongs to the OH stretching of water. However, smoothing the spectra by removing water associated background improved the SNR to 10, which helps in identifying the microplastic particles even in the contaminated water.

Keeping the SNR to 5 also helped in separating Raman lines of microplastics from that of

biological components (such as microalgae and bacteria) clearly and microbial residues of 100µm or more size were also detected and were seen as non-polymers.

Using Raman spectroscopy, the five different microplastic particles in the tap water were detected individually. Also in this method, no animals were harmed. This way, the Raman spectroscopy method seemed to be more reliable.

2.3 MALDI-TOF and Thermal Degradation

Analytical techniques like mass spectrometry are widely employed to determine the mass-to-charge ratio of ions from a sample. This in turn helps measure the molecular weight of the components of the sample [15-17].

Matrix-assisted laser desorption/ionization (MALDI) coupled with the mass analyzer- time of flight (TOF) is one such mainstream mass

spectrometric technique. MALDI is a soft ionization method where the sample/ analyte mixed with an excessive amount of matrix is irradiated by the laser beam briefly. The matrix molecules absorb laser energy and carry the vaporized sample molecules. During this process of ablation, the analyte molecules are either protonated or deprotonated with the matrix molecules. A fixed potential energy is applied to these charged particles due to which they enter a field free region of known length of the mass analyzer and drift until they reach the detector. The lighter ions drift faster than the heavier ones and the time taken to reach the detector helps measure the m/z ratio. MALDI-TOF is commonly used to identify and quantify biomacromolecules.

Lin et al. [18] delineated a new technique based on MALDI-TOF and thermal degradation/ fragmentation for the identification and quantification of Micro- and nanoplastics (MNPs).

Polystyrene (PS) is one amongst the commonly used plastics and its non-biodegradability is proved to be hazardous. [18] applied the new technique to identify and quantify standard PS MNPs, PS MNPs in fish and river water samples. Further, to prove the universality of the method, it was validated with other types of MNPs like Polyethylene glycol terephthalate (PET).

Six PS standards of different molecular weight were purchased. Their sizes were determined to be less than 3mm. PS standards were dispersed in ethanol and sonicated for 10 min to obtain TEM images. The PS standards were dispersed in ultrapure water under sonication for 10 min for DLS measurements. The TEM results were consistent with the DLS results. This helped in the characterization of PS MNPs.

To demonstrate the technique's applicability on real samples, 5 fish samples and river water samples were collected beside standard PS MNPs. Two fish samples were killed and mixed with PS MNPs. Both the samples were studied by the comparison of results. The other three fish samples were fed with fish food contaminated with PS MNPs three times a day for three days. The river water samples were spiked with a mixture of standard PS MNPs.

The fish were eventually killed and immersed in 1% KOH solution at 50℃ for 36 hr. This solution was filtered using a filter membrane. Fish bones were separated from the solids collected on the membrane. The solids were mixed with saturated NaCl and left undisturbed for 10 min. The top layer was filtered again and dried in an oven and solids were collected for further analysis. The sample preparation procedure remained the same for water samples.

All the 3 types of samples (PS standards, pre-treated fish and river water samples) were subjected to thermal degradation at 380℃ for 10 min. MALDI- TOF was later carried out. Tetrahydrofuran (THF) was used to dissolve the samples. Dithranol (DI) and Silvertrifluroacetate (AgTFA) were prepared in THF and were used as matrix and ionization reagent in the ratio of 1:10:1 for MALDI. Matrix is crucial to absorb the laser beam and to transfer the protons. According to A. [19] a sufficient number of matrix molecules are required to study a certain number of molecules. Thus, the matrix concentration was optimized. Polymers are difficult to be ionized and hence an optimal concentration of a monovalent cationic reagent was used. A laser with the frequency of 100 Hz was used and spectra was recorded summing 500 laser shots. As the negative ion mode failed to produce significant signals, the experiments were carried out in the positive ion mode. Multiple fingerprint peaks were obtained for the PS MNPs. A series of peaks for styrene with mass of 104 was regularly observed in the low mass range and the high mass range of low molecular weight standards. The repeated peaks were absent in the high mass range for plastics of higher molecular weight. This indicates that the monomer repeated units were difficult to produce for polymers with high molecular weight. This successfully proved that the fingerprint peaks of MALDI in the high mass range can be used to establish the differences in the molecular weights of MNPs.

Despite the use of both high- and low- mass regions for MNP identification, the large variations in the peak intensity of samples with different molecular weights interfered in the accurate quantification of real samples. As a consequence of the scanty concentration of MNPs, the sensitivity of the technique needed further enhancement to improve the MS responses of the fingerprint peaks.

Lin et al. [18] suggests thermal pretreatment to induce pyrolytic fragmentation of PS to produce smaller molecules of styrene and its trimers. This treatment enhanced the peak intensity by 8.8-folds in the low mass regions and brought about

several changes in the peaks at the high- mass regions. The repeated peaks for styrene I the high m/z range for low molecular weight plastics declined. Contrarily, the styrene peaks in the high- mass regions for heavier plastics were enhanced. The above results hence proved that the technique of thermal degradation could be successfully wielded to differentiate and identify PS and quantify PS MNPs regardless of different molecular weights. The universality of this method was displayed by following the same protocol on PET MNPs. MALDI- TOF was hence established as a method suitable for the detection and quantification of MNPs.

2.4 Surface-Enhanced Raman Spectroscopic Method

Traditional Raman Spectroscopic method cannot be used to detect microscopic particles less than 1μm. [20] suggests the use of a facile method of "Surface-Enhanced Raman Spectroscopy" to overcome the above-mentioned drawback.

SERS (Surface-enhanced Raman spectroscopy) amplifies the Raman response of an analyte when it is interacting with the surface plasmon of metals. This technique is sensitive enough to facilitate the detection of a single molecule. The mechanism of SERS has not exactly been deduced. Currently, there are two theories that describe the enhancement effect of SERS. They are the electromagnetic theory and the chemical enhancement theory. The former is based on the excitation of surface plasmons that are localized, whereas the latter is based on charge transfer complex formation of the adsorbed molecule.

Xu et al. [20] used Klarite- a commercial SERS substrate for studying nanoparticles of polystyrene (PS) and polyethylene methacrylate (PMMA). PS and PMMA spheres were compared on both Klarite and silicon wafers. Klarite has inverted pyramid shaped pits that are ordered. Nine pyramidal pits were generated in the design modeler and these had equal dimensions. PS and PMMA particles were diluted with deionized water to a ratio of $1:4 \times 10^4$ with the volume of 4 ml in order to access individual particles. Microplastic sample solution containing PS, PMMA was added dropwise onto Klarite and dried at room temperature. An external cavity diode laser operating at 25mW was used to excite the sample. A diffraction grating having 1200 lines per mm was utilized. Linearly polarized light was focused onto the hotspots of Klarite. Raman Microspectroscopy was conducted and the spectra were collected.

The corresponding optical images were taken from bright-field microscopy. It was found that the Raman signal from the silicon wafer over-powered the weak signals from PS spheres. This was determined to be due to the larger laser spot size of the spectrometer compared to the PS particle size. In contrast to the silicon wafers, the ordered structure of Klarite provided a means to index the location of every pyramidal pit under SEM and optical microscopy, thereby yielding better results. PS particles smaller than 5μm could be clearly identified using Klarite substrate. Klarite was found to have the potential of enhancing Raman signals.

The location of PS spheres on Klarite was determined to be influential on the signal strength. The relationship between the both was established by placing the PS spheres of the same size in and out of the pyramidal pits and carrying out the experiment independently. The Raman peaks were found to have a lower intensity when the spheres were placed outside the pits. The spheres too large to fit into the pits were found to have no significant benefits from the electric field enhancement.

To prove the supremacy of the Klarite substrate, Raman spectra of PS on different substrates such as glass and Al foil were studied. In both cases, barely any characteristic peaks of PS were observed for spheres smaller than 1 μm.

The Enhancement Factors (EF) of SERS was quantified using the following formula: -

$$EF = (I_{SERS} / N_{SERS}) \div (I_{NRS} / N_{NRS})$$

Where I_{SERS} and I_{NRS} are defined as peak intensities detected by the SERS substrate and non-SERS substrate respectively; N_{SERS} and N_{NRS} refer to the number of molecules that contribute to the intensity of SERS and non-SERS Raman peak intensities respectively.

EF was low for particles present at the top of the Klarite pits compared to the particles located within it. The variations in the EF values indicated the dependence of peak intensity on particle size and particle location. The results obtained in this experimentation successfully highlighted the capacity of klarite to enhance Raman Scattering with tiny amounts of smaller particles that would otherwise be undetected. PS

spheres were successfully identified, located and measured using SERS.

Investigations with PAMMA were conducted to understand the versatility of Klarite for SERS detection and identification of microsplastics. EF for PAMMA was found to be clearly lesser PS. This was suggested to be the result of low normal Raman cross section and low sensitivity to Klarite substrate.

To understand the applicability of SERS using Klarite on real samples, the ambient aerosol microplastics were extracted, treated and deposited on Klarite substrate and the spectra were collected. PS and PET were both detected. This detection proved Klarite to be a promising substrate in detection of various nanoplastics in the environment.

2.5 Microplastics counting, Quantification, Classification through Machine Learning Approach

A method supported by the utilization of open-source computer vision (OpenCV) algorithms to extrapolate this information from the analysis of an acquired image was developed to classify and count the particles [21].

The overall workflow or the procedure is shown below within the sort of flow chart (Fig. 2).

The steps are as follows:

1. Sampling: Freshwater as a sample was collected in a glass jar.
2. Extraction: The sample was then transferred to labeled glass jars at 4° C then wet sieving was administered to retrieve the material from the sample. Sample material larger than 5mm was discarded Wet peroxide oxidation (30% H_2O_2 and 0.05 M Fe (II)) was done. Density separation with NaCl was performed leaving the extract behind. This was done to digest labile organic fraction. At the highest level, an extra chemical identification of a subset of particles was also performed by Pyrolysis-Gas chromatography or Mass-Spectrometry to verify the particle's synthetic origin.
3. Image acquisition: Once the samples are processed and extracted, simple to use digital 12-megapixel smartphone camera had to be used to take pictures. Microplastics need to be distributed by increasing the contrast between particles and the background.
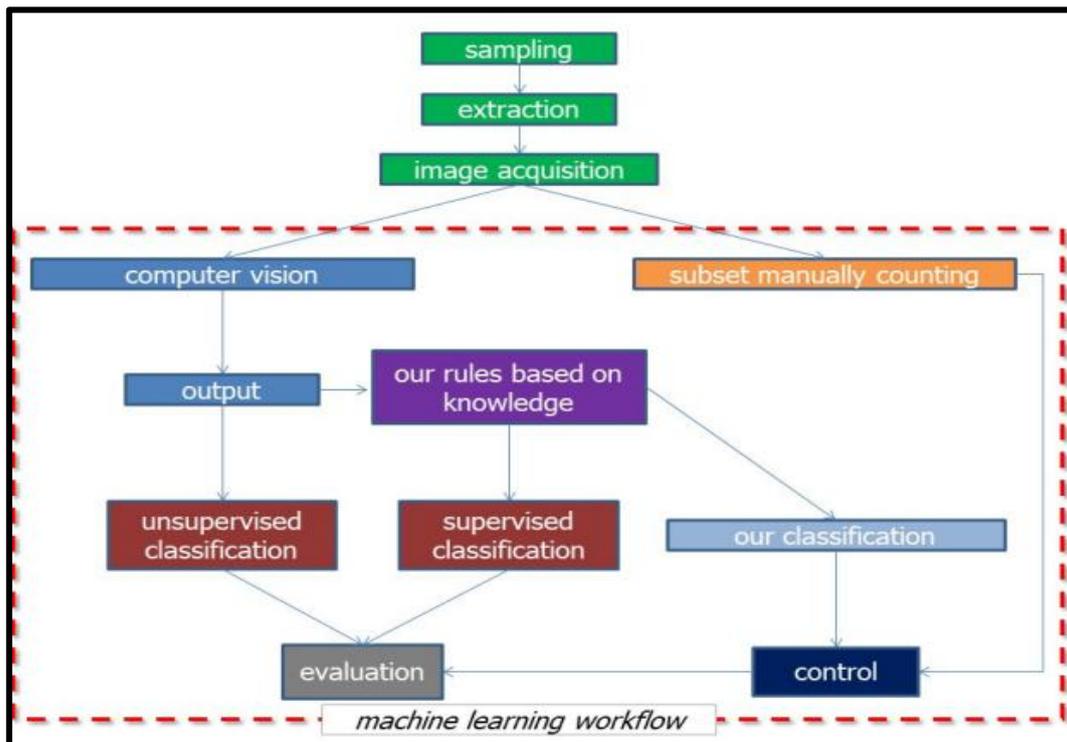
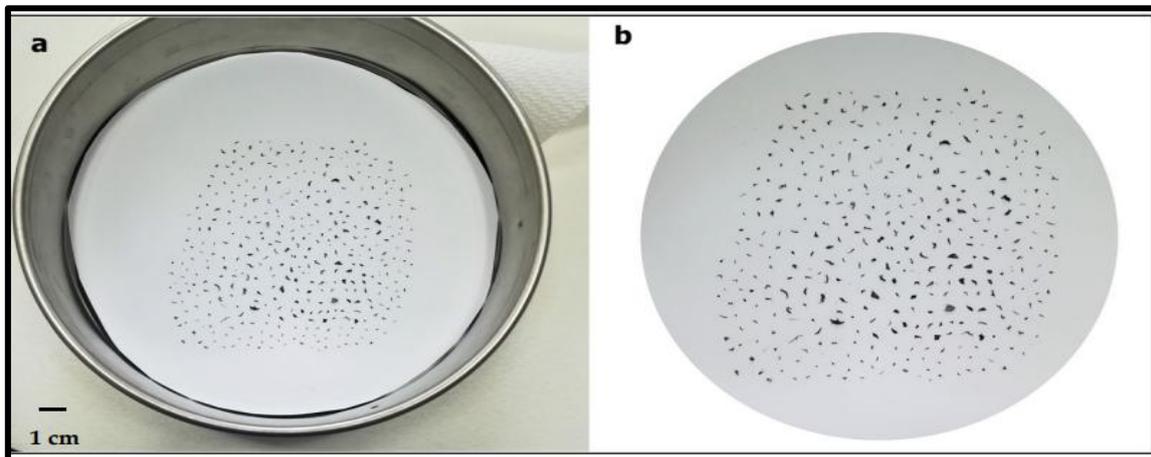

Fig. 2. The overall workflow [22]

Fig. 3. Image preprocessing step: a. picture taken within the sieve, b. cropped image [22]

4. Machine learning flow: This method is predicated on open CV python application programming interfaces. It is based on two processes:

   a. Feature engineering- Supports a code implementation to find out the best way to represent data that will be used in machine learning techniques.
   b. Feature extraction- It is implemented with Linear Blend Threshold, Binarization, Bounding Box generation, extraction of particle features, and Classification supported size and morphology.

Edge value was identified before processing the information. An algorithm that uses hand-codes of "if-then-else" is used to process the information. The ratio between the two main spatial dimensions was considered to classify MP. After extracting the particle, the subsequent step was to get the output for each particle in the image into one among the morphology and size. The image differentiates particles according to their morphology (fragments, pellets, lines, and fibers).

Below shows the pseudo code for the classification of the Microplastics:

```
START
 For each particle within the image do this:
 #here we consider a primary geometric relationship of the shapes of Micro plastics
 If the ratio between the 2 dimensions of the bounding box is bigger than 3.5:
If mean pixel intensity is bigger than 130.0 (on a scale of 0 to 255):
 Assign classification 'fiber'
Else assign classification 'line'
 #here we considered a second geometric relationship of the shapes of Micro plastics
Else if the ratio between numbers of not null pixels and thus the result of the two
 Spatial dimensions of the particle are less than 0.4:
If the mean pixel intensity is bigger than 130.0:
Assign classification 'fiber'
Else assign classification 'line'
Else if the ratio between numbers of not null pixels and thus the result of the two
Spatial dimensions of the particle are greater than or equal to 0.4:
If the ratio before calculated is bigger than 0.7 and the, therefore, the ratio between the 2 spatial
dimensions of the particle is bigger than 0.9 but smaller than 1.1:
Assign classification 'pellet'
Else assign classification 'fragment' to all other cases
End
```

Code 1: Pseudocode for microplastic classification.

Two classifications are chosen for the classification of the MP:

1.   Supervised classification:

In this K-Nearest Neighbor (K-NN) algorithm was used to predict new data supported by its neighbor which may be a non-parametric method that wants to classify data with discrete labels and regression models. Set the value of K to 3. This provided an honest compromise between an inaccurate classification. The information was bifurcated as follows:

☐ Training phase- It is aimed to coach a machine learning model on the set of knowledge called "training dataset".
☐ Test phase- It is a phase during which the finalized machine learning model on a new set is evaluated.

The distance chosen was supported by standard Euclidean distance implemented with Scikit-learn from which we will measure similarity [23]. The algorithm was implemented with Python and a machine learning application in which OpenCV is combined with Python, Scikit-learn, and Matplotlib. To assess the results and the goodness of supervised classification a report/ summary of classification was created. The metrics are defined in terms of true, false positives, and negatives respectively. For every class accuracy, precision, recall, F1 score, and support are calculated.

2.   Unsupervised classification

In this k-means clustering was used to search for a predetermined number of k clusters within an unlabeled dataset. It is supported by two simple assumptions [24]:

☐ The center of every cluster is simply the arithmetic mean of all the points belonging to the cluster;
☐ Each point within the cluster is closer to its center than to other cluster centers.

The goal was to spot the intrinsic properties of knowledge points that make them belong to an equivalent subgroup. The number of clusters was set to 4. Furthermore, to assess the proposed early ML algorithm's validity, the particles were manually counted for five selected images corresponding to five different samples. Positive results were obtained The classification was done in the different classes. Additionally, simple software with a graphical interface (GUI) was created to facilitate the manual counting by a click. Discovering hidden features to know and understand the unfolding within the image is one of the goals of the unsupervised classification.
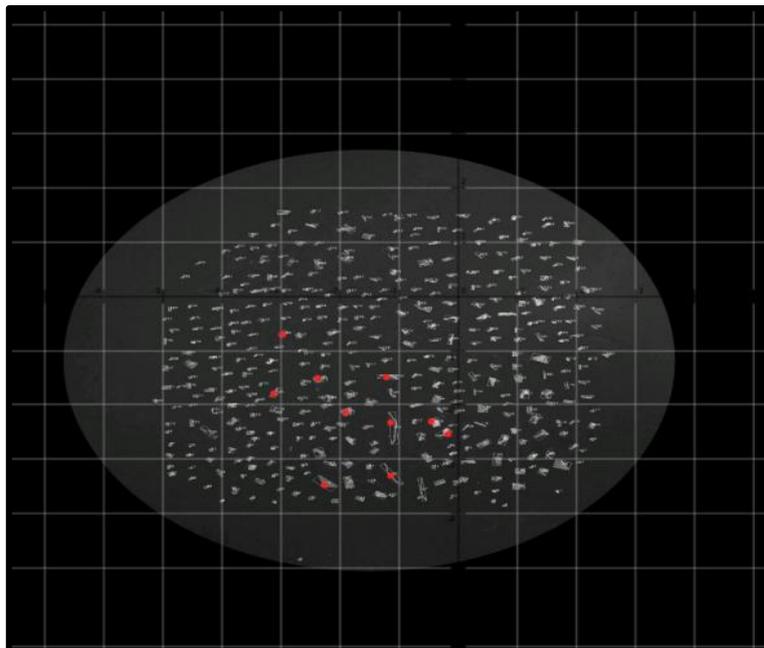

Fig. 4. Details of GUI used for manual counting [22]

3. CONCLUSION

MNPs are the major concern of the current time. Tons of plastics reach the oceans in many ways. Plastics are mainly used in packaging like single used plastics. Most of the daily use products have microplastics in them e.g., cosmetics. It has been found in the body of the marine organism, birds and recently is also been detected in all levels of the placenta. There are many ways to detect and quantify micro and nano plastic using methods discussed in the paper.

Infrared spectroscopy is a widely used analytical tool used to characterize and quantify analyte molecules. It is a non-destructive technique and does not require special sample preparation. However, water content in the sample can interfere with the spectra and detection of MNPs would require appropriate instrumentation since the method is not highly sensitive. The FTIR spectroscopy discussed here is advantageous over the traditional IR spectroscopy. It provides high power output and scans through the frequencies of the source rapidly. The disadvantage of FTIR is that the results obtained are not highly reliable as certain materials can completely absorb infrared radiations.

Raman spectroscopy is a technique that provides information about the chemical structure of the analyte molecule by probing the analyte sample. Light from a high intensity laser source is scattered by the molecules in the analyte sample. A small amount of light is scattered at different wavelengths depending on the chemical structure of the sample. This is called "Raman Scatter".

The Raman spectra produced for a molecule acts as a unique chemical fingerprint for that particular molecule thus enabling the identification of the molecule or differentiation from other molecules. The light scattering technique also provides information about phase, polymorphism, intrinsic stress/strain and contamination of the sample. This method can hence be used for characterization, identification and quantification of MNPs without destroying the sample. Sample throughput is high. The non-destructive technique is both qualitative and quantitative. Analysis requires only a small quantity of microplastic samples. Raman spectra can be collected from a small volume. This method requires no sample preparation and raman spectra can be obtained rapidly.

Unlike IR spectroscopy, the spectra from Raman scatter aren't bothered by water, and the method offers better selectivity. This is a huge advantage over IR spectroscopy since it enables quantification of MNPs from liquid samples. Raman Spectroscopy is superior to FTIR- the gold standard of molecular spectroscopy. Despite all the advantages Raman spectroscopy cannot be considered as the best method for quantification of MNPs due to the weak Raman signals. The signals from impurities can mask or weaken the raman signals. The accurate detection would require sensitive and optimized instrumentation.

The disadvantage of Raman spectroscopy can be dealt with by the use of SERS. Raman scattering can be highly enhanced upto the level of 10^{14} by SERS. The raman-active molecules are adsorbed onto metal surfaces which help in the enhancement of Raman signals. Another major advantage of SERS over other methods is the ability of the technique to detect single molecules. The paper discussed in the review established klarite as a suitable metal surface to enhance raman signals. This helps reduce the cost associated with the common use of gold and silver metals.

The rapid turnaround time and high accuracy sets MALDI-TOF apart from the other techniques discussed. MALDI-TOF coupled with thermal degradation of MNPs enhanced peak intensity and brought about accurate identification of MNPs.

We highly believe that the next generation techniques must focus on methods with high sensitivity and encourage quantification at a lower cost. Marine ecosystems today are plagued with micro- and nanoplastics. In order to save marine lives, it is extremely important to deal with the current issue regarding proper use and management of plastic.

The detection of micro- and nano plastics using methods like Raman spectroscopy, Infrared Spectroscopy, SERS, MALDI-TOF, and machine learning approach can pave the way for better handling of plastic wastes in the environment.

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COMPETING INTERESTS

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