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Validation of Microplastics Accumulation on Edible Fruits and Vegetables

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ABSTRACT: Microplastics, which are omnipresent in the environment, particularly in agroecosystems, are the result of plastic particles that reach sizes between 5 mm and 1 m. This exacerbates the contamination caused by plastic. In this present study, frequent consumption of two variety fruits like *Vitis vinifera* and *Musa paradisiacal* as well as vegetables such as *Solanum melongena* and *Solanum tuberosum* from a local market in Trichy, Tamil Nadu, India, was collected and analysed for microplastics accumulation. Next, we extracted and determined the the size of the Microplastics (MPs), which ranged from 0.01 mm to 0.002 mm by using stereomicroscope and Scanning Electron Microscope. In FT-IR analysis was confirmed the different types of polymer functional groups like nylon, polypropylene, polystyrene and high-density polyethylene. Our research has highlighted the regularly consuming edible fruits and vegetables having MPs accumulation. In due course, the potential impacts of low range sized microplastics on food safety in edible fruits and vegetables and human health need to be urgently considered.

Keywords: Microplastics, Fruits, Vegetables, Agroecosystems, Public Health, Toxicology.

INTRODUCTION

Both society and the scientific community now face a significant challenge in the form of plastic pollution as a direct consequence of the rapid expansion in the use and production of plastic. According to Blasing and Amelung (2018), the vast majority of plastic waste is still dumped into the environment worldwide, despite some progress being made in plastic recycling and energy recovery. Microplastics are minute, have recently become the focus of environmental research due to their small size and potential harm to terrestrial ecosystems. Microplastic is a type of plastic that can be found in a variety of morphologies, including beads, fragments, fibers, and films, and has a diameter of less than 5 millimeters. Primary microplastics are those that are released directly into the environment where as secondary microplastics are produced by the fragmentation of larger-sized chunks. Microplastics have the potential to enter agro-ecosystems through a number of different pathways. The use of mulching film is one of these pathways (Qi *et al.*, 2018), the application of biosolids and compost, wastewater irrigation (Zhang and Liu 2018, Weithmann *et al.,* 2018), the addition of compost, and the use of biosolids (Heuchan *et al*., 2019), and last but not least, the application of fertilizer. The World Health Organization suggests consuming at least 400 g of fruit and vegetables on a daily basis, with the exception of potato, sweet potato, cassava, and other starchy roots and tubers. Inline, one-fifth of this amount may be consumed as MP. This would mean that approximately 80 g may be consumed on a daily basis, taking into consideration elements such as the potential of MP to bio-persist and translocate in plant.

In the end, agricultural systems benefit from a wide range of pollutants, including microplastics, whose effects are mostly unknown (Razzaghi *et al*., 2018).

A general lack of knowledge about the material fate and the harmful effects in the agricultural system leads to a break down in the food chain and an unidentified route of human exposure. Microplastics may be able to enter the cells of the seed, root, culm, leaves, and fruit, depending on their size and composition (Dietz and Hertz 2011). In most cases, the breakdown of MPs in soil is exceedingly sluggish, taking hundreds or even thousands of years to finish (Andrady, 2011). Particle plastics may be taken up by plant roots and then transported by transpiration flow from the roots to the stems and leaves of the plant. This will cause plastics particles to build up and be distributed differently throughout plant tissues (Li *et al.,* 2019). Microplastics have the potential to be ingested or inhaled into an animal body, most notably a humans, where they can subsequently build up in a variety of organs and show to a variety of unfavourable health problems, including cell destruction as well as inflaming and immunologic issues (Vethaak and Legler 2021). Microplastics contain a wide variety of hazardous chemicals, some of which include BPA, plasticizers, antiminitroxide, brominated flame retardants, polyfluorinated chemicals, and others. Each of these substances, along with microplastics, cause a notable warning to human health as well as the environment. The use of toxic plastics has been linked to a number of harmful effects on human health, such as eye irritation, vision loss, breathing difficulties, respiratory issues, liver dysfunction, cancers, skin conditions, lung problems, headaches, dizziness, birth defects, reproductive, cardiovascular, genotoxic, and gastrointestinal problems (Sarma *et al*., 2022).

Fruits and vegetables are essential for the daily life of the world population, staple fruit and vegetable crops for approximately 30 % and 25 % of the worlds population, respectively. In Tamil Nadu, only the hilly districts of Dindigul, Nilgiris, Krishnagiri, and Erode have areas where the potato is farmed. The brinjal has grown areas in cuddalore, villupuram, vellore, thiruvannamalai and chengalpattu districts. In Tamil Nadu grape is cultivated in the districts of theni, coimbatore, dindugal, dharmapuri and krishnagiri. In Tamil Nadu, the theni district tops the list of regions where bananas are grown, followed by coimbatore, erode, and tuticorin districts. Peoples use these fruits and vegetables in their daily diet and hence we selected for our research. Studies on microplastic accumulation in common fruits and vegetables is relatively scanty.

We made the decision and used our method to determine whether commonly consumed fruits and vegetables contained microplastics smaller than 5 mm. This study aims to identify and characterize the microplastics using a different of analytical methods and investigate the presence of microplastics in commonly consumed fruits like grapes (*Vitis vinifera*) and banana (*Musa paradisiaca*) and vegetables like potato (*Solanum melongena*) and brinjal (*Solanum tuberosum*).

MATERIALS AND METHODS

Sample Collection and Preparation. Two varieties of samples, such as fruits (Two No. of Bananas and Grapes) as well as vegetables (Two No. of Brinjal and Potato) were obtained from the Tiruchirapalli, Tamil Nadu, India, local market. The samples were placed in a steel tray and kept closed. Sterilized steel knives were used to slice the collected samples. On clean, sterilized petri plates, the samples of sliced fruits and vegetables were kept. After that, the samples were dried for a day in a hot air oven at 60 degrees Celsius. A clean, sterilized mortar and pestle were used to grind the dried samples into powder. Grained samples were then extracted for microplastics and air-dried for four days (Conti *et al*., 2020).

Extraction of Microplastics. One gram of powdered material was treated overnight with 50 milliliters of 30% hydrogen peroxide to remove organic matter for the purpose of microplastics extraction. In a 100-ml glass beaker containing zinc chloride solution (1.5 g cm^3), microplastic particles were extracted through density separation following a second drying step. After being stirred, the sample was put through an ultrasonic bath for 15 minutes, and the beaker was covered overnight to allow other particles to settle out. The zinc chloride solution's surface was sampled with a syringe for potential microplastic particles, which were then filtered through gray, pre-washed cellulose nitrate filters with a grid of 3.1 mm and a pore size of 1.2 m. For further examination, the filters were dried (Loder and Gerdts 2015).

Stereomicroscopic Analysis. Stereomicroscope were used utilize to recognise the characterization of the microplastics morphology and determination of their size (Ainali *et al*., 2022). The recovered microplastic particles were then filtered through a filter paper and dried. The microplastics retained in the filter paper were separated and enumerated under a stereomicroscope (40×) (Masura *et al.,* 2015; James *et al*., 2020).

ATR-Fourier-Transform Infrared Spectroscopy Analysis (ATR FT-IR). FT-IR was used to detect the functional groups of the microplastics particles in given sample. The functional groups of extracted MPs in the fruits and vegetables were subjected to ATR FT-IR analysis (Perkin Elmer, Spectrum RX, USA). The spectrum of the recovered microplastics was recorded using spectra in the frequency range 4000-400 cm^{-1} (Jung *et al*., 2018).

Scanning Electron Microscope Analysis (SEM). A focused beam of electrons with a high energy is used in SEM to magnify a specific area of the sample. The recovered MPs particles were followed by the above determined method. Due to its micro- and nano-scale features at magnifications of up to 500,000 times, the SEM technique makes it possible to identify microplastics and the degradation process they undergo in a sample (SEM) (Carl Zeiss microscopy, ZEISS EVO). Microplastic particle sizes and elemental composition are determined using this method.

RESULTS AND DISCUSSION

The minutemicroplastics size was determine in the grapes and potato (0.002 mm), while the biggest ones was determine in the banana and brinjal samples (0.01 mm) were measured using a stereomicroscope (Fig.1). Similar results were recorded by Dyachenko *et al.* (2019) have reported the stereomicroscope to detect the existence of microplastics in secondary wastewater treatment plant effluent. Through stereomicroscopic research, Piehl *et al.* (2018) found that microplastics were present in soil. Chen *et al.* (2020) had stated that presence of MPs in domestic garbage soil through stereomicroscope. Beriot *et al.* (2021) have reported microplastics presence in plastic mulching in crop land with vegetables through stereomicroscope. Huerta *et al.* (2017) had stated that noticeable carry of microplastics to food web in plastic waste through stereomicroscope. Young *et al.* (2016) have reported MPs identified in environmental samples through stereomicroscope.

SEM images of MPs were shown in Fig. 3 (a-d). In our results were revealed that MPs particles were captured with different magnification range of 402 KX, 2.00 KX and 5.00 KX were observed. Lian *et al.* (2020) have been detected the presence of microplastics presence in fruits and vegetables. Li *et al.* (2021) had stated the presence of microplastics presence of cucumber plants through SEM.

Li *et al.* (2020), microplastics of sizes 0.2 µm and 2 µm were present in *Triticum aestivum.* Wang *et al.* (2020) have been reported the presence of microplastics in *Zea mays* L. at size of 100-154 µm. Urbina *et al.* (2020) have been reported the accumulation of microplastics in *Zea mays* L. at size of 3 µm. Wu *et al.* (2020) have reported the presence of microplastics in *Z. mays* at size of 100-154 µm. Boots *et al.* (2019) had been describe the accumulation of microplastics in *Lolium perenne* L. at 65.6 and 102.6 µm. Dong *et al*. (2020) have been reported the accumulation of microplastics in *Oryza sativa* at 10 µm. Zhou *et al.* (2021) had stated the accumulation of microplastics in *T. aestivum* at size of 50-1000 µm. Li *et al*. (2021) had stated the microplastics were found in *Hordeum vulgare* L. at size of 5.64 µm and 96.75 µm. Verla *et al.* (2020) have been reported the accumulation of microplastics at size ≤5mm in *Citrus aurantium*. SEM has traditionally been used to image the microplasticsin inside plant cells (Conti *et al*., 2020; Jiang *et al*., 2019; Li *et al*., 2020; Li *et al*., 2020). Li *et al*. (2019); Zhou *et al*. (2018) demonstarted the ability of edible land plants to absorb microplastics from soil is a worrying topic because to potential health risks.

In FT-IR analysis were then used to identify the various polymer functional groups that was present in the fruits and vegetables using the microplastics that had been recovered (Fig. 3). The spectral range of a banana sample was measured at 3444.68 cm^{-1}. The peak range at 1633.55 cm^{-1} relates to C=O stretching. The peak range at 693 cm^{-1} represents aromatic CH. The peak range at 1271.00 cm^{-1} measures C-N stretching. The spectral range of grapes was measured at 3436.06 cm^{-1}, and 1633.82 cm^{-1} pertains to C=O stretching. 1270.76 cm^{-1} measures NH bending. In potato, the spectral range was measured at 3435.84 cm^{-1}. The frequency 1634.11 cm^{-1} represents C=O stretching, and the frequency 684 cm^{-1} shows C=O bending. The C=O stretching line at 3435.82 cm^{-1} in the brinjal sample's spectral range, along with the 1120.93 cm^{-1} and 696 cm^{-1} peak ranges, are representative of the acrylonitrile butadiene styrene (ABS) group (Table 1 and Fig. 2).

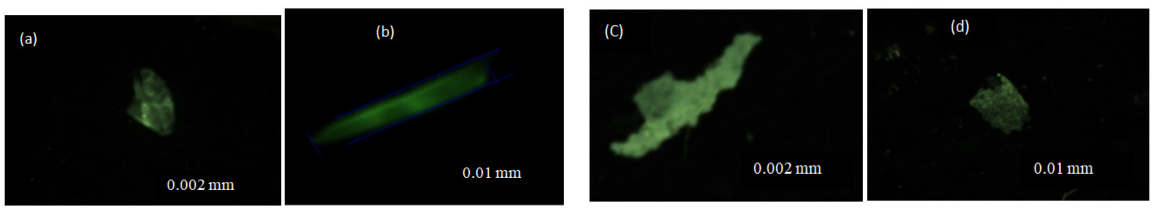
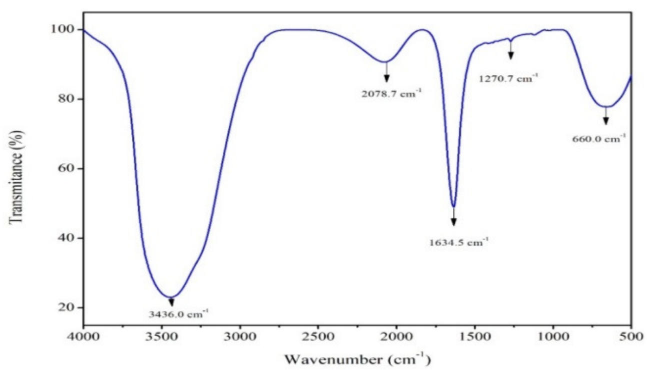
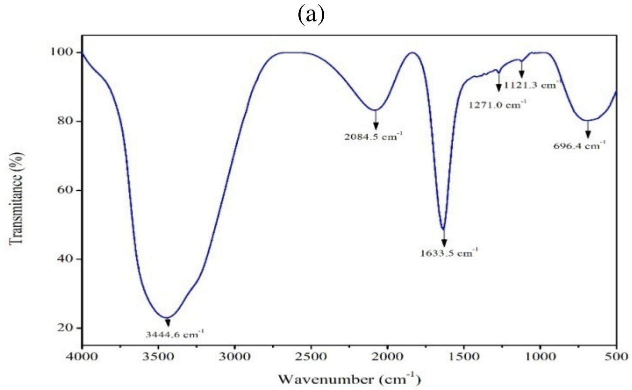


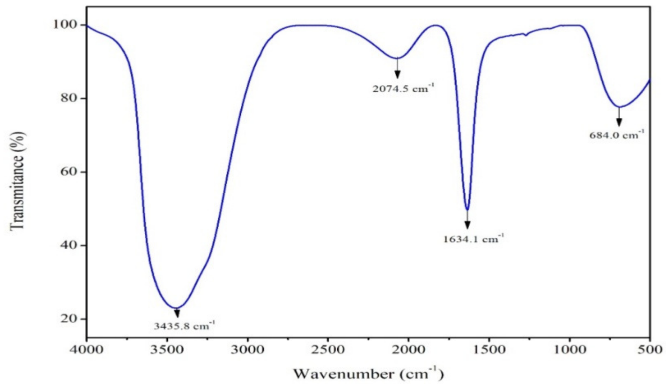
Fig. 1. Stereomicroscopical image was shows of microplastics presence in fruits and vegetables: (a) Grapes scale bar 0.002mm; (b) Banana scale bar 0.01mm; (c) Potato scale bar 0.002 mm; and (d) Brinjal scale bar 0.01mm.



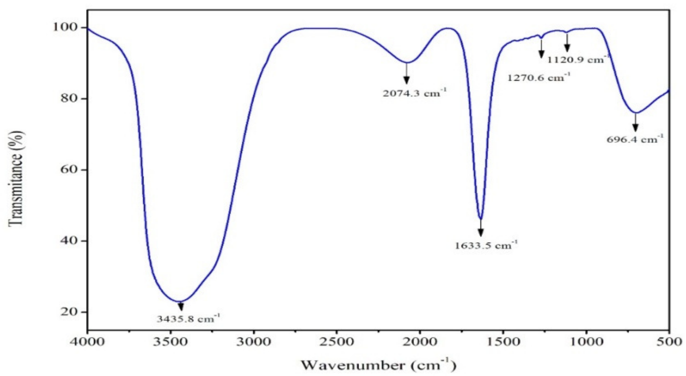
(a)



(b)



(c)



(d)

Fig. 2. FT-IR images represents the accumulation of microplastic chemical groups in fruits and vegetables: (a) Grapes; (b) Banana; (c) Potato; and (d) Brinjal.

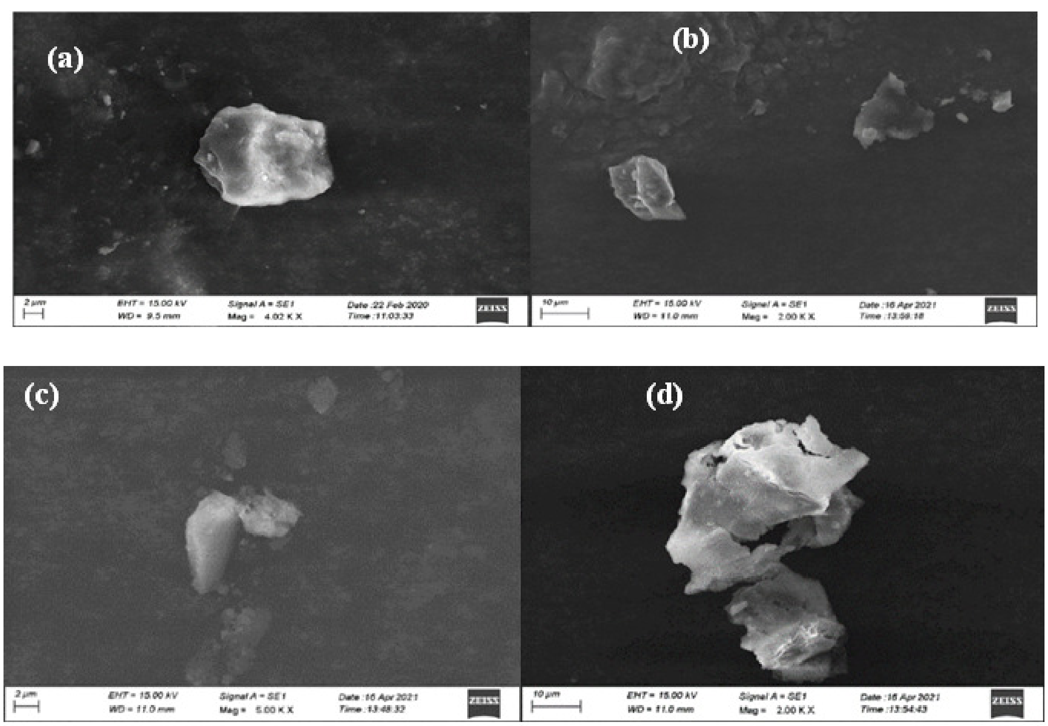


Fig. 3. SEM Images Shows the Microplastics Presence in Fruits and Vegetables with Different Magnification: (a) Grapes (b) Banana (c) Potato and (d) Brinjal.

Table 1: FT-IR Characteristics Peak Assignments for Various Types of Microplastics.

Sr. No.	Sample Name	Bond	Spectral range of the bands (Cm^{-1})	Microplastic Group
1.	Grapes	C=O stretching NH bending	3436.06 $cm^{-1,}$ 1633.82 cm^{-1} 1270.76 cm^{-1}	Nylon
2.	Banana	C=O stretching	3444.68 cm^{-1} 1633.55 cm^{-1} 1271.cm^{-1} 1121 cm^{-1} 693 cm^{-1}	Nylon High density polyethylene terephthalate (HDPET) Polyethylene oxide Polystyrene
3.	Potato	C=O stretching C=O bending	3435.84 cm^{-1} 1634.11 cm^{-1} 684 cm^{-1}	Nylon
4.	Brinjal	C=O stretching	3435.82 cm^{-1} 1120.93 cm^{-1} 696.45 cm^{-1}	Nylon Polyethylene oxide Acrylonitrile butadienestyrene (ABS)

According to Mecozzi *et al.* (2016), a nylon group peak was found, and it displays C=O stretching in the 3350 cm^{-1} to 3600 cm^{-1} region of the FT-IR spectrum study. Same results were found in our samples of banana, grapes, potatoes, and brinjals at spectral ranges 3444.68 cm^{-1}, 3436.06 cm^{-1}, 3435.84 cm^{-1}, and 3435.82 cm^{-1}. The C=O stretching shows that nylon polymer at 3435.82 cm^{-1}, and the spectral peak at 1250-1350 cm^{-1} shows that there is a C-N stretching resulted HDPE polymer group presented. Similar peaks were discovered in our banana sample, which demonstrates that the peak range 1271.00 cm^{-1} contains C-N stretching and that it refers to the HDPET polymer group. The peak range for the C-N stretch was found to be 1050 cm^{-1}-1150 cm^{-1}. This range pertains to the polyethylene oxide group. The similar findings was discovered in a sample of bananas at a range of 1121.33 cm^{-1}, which exhibits C-N stretch and pertains to the polyethylene oxide group. There was observed to be a C-N stretch in the peak range of 1050 cm^{-1} -1150 cm^{-1}, and this stretch pertains to the polyethylene oxide group. The same results were found in brinjal in the peak range of 1120.93 cm^{-1}, and they refer to the polyethylene oxide group.

Jung *et al.* (2018), there were 1634 cm^{-1} peaks, which are part of the nylon group. Furthermore, even though a same finding were present in the banana sample at peak range 693 cm^{-1}, our results were similar. The peak range in the banana sample was 1633.55 cm^{-1}, which shows C=O stretching and indicates the nylon polymer group, and the peak range in the polystyrene group sample was 694 cm^{-1}, which represents aromatic CH out of plane bending. The peak ranges of 1633.55 cm^{-1}, 1270.76 cm^{-1}, 1270.83 cm^{-1}, 1633.82 cm^{-1}, and 1634.11 cm^{-1} in grapes, potatoes, and brinjal demonstrate C=O stretching, which stands for the nylon group. The acrylonitrile butadiene styrene (ABS) group was produced as a result of aromatic CH out-of-plane bending, which was represented by peak range 698 cm^{-1}. Similar findings were observed for the acrylonitrile butadiene styrene (ABS) group in the peak range of 696.45 cm^{-1} in the brinjal sample.

Drugs like erythromycin, clindamycin, clarithromycin, ciprofloxacin, doxycycline, tetracycline, miconazole, and many more present in biosolids, according to Chenxi *et al.* (2008); Garca-Santiago *et al.* (2016). These chemicals will stay in soil and may therefore be soak up by plants, according to Carvalho *et al.* (2014); Vodyanitskii and Yakovlev (2016). Biological accumulation of these medications in their stems, leaves, and fruits, which stunts plant growth and causes phytotoxicity, according to studies (Eggen *et al*., 2011; Goldstein *et al.,* 2014). (Carvalho *et al*., 2014). According to Rezania *et al.* (2018), intake of those plants by people and vertebrate that shall impacts the food web and is very toxic for human health.

Li *et al.* (2020) conducted a study that provides the fact that plants have absorbed MPs and other findings point to the possibility of flexible cell wall pores that make MP particle penetration easier. Due to the size and composition of microplastics, it is possible for them to easily pass through the seeds, roots, stems, leaves, fruits, and plant cells of plants. This uptake of microplastics has been observed to occur under controlled conditions. As observed by Ullah *et al.* (2021) MPs have the ability to penetrate plant cell walls as well as membrane barriers, which can lead to the obstruction of cell pores and the death of cells.

This was described by Li *et al*. (2019) that microplastic can be transmitted to the roots of fresh vegetables, where they can accumulate, and that these MPs can be passed from the roots to the shoot tissues. According to the findings of Ng *et al.* (2018), the uptake, transport, and presence of microplastics varies from one plant species to another based on the functional and structural properties of the plant. Li *et al.* (2019), MPs have the potential to be transferred to and stored in the roots of fresh vegetables, as well as transfer from root to shoot tissues. Furthermore, they have the capacity to penetrate from the roots to the shoot tissues. A number of aspects of a plants anatomy, including its roots and xylem, as well as its rate of development, transpiration, water and lipid fractions, tonoplast and plasma membrane potential, and the pH of its vacuoles and cytoplasm, all have a role in the plants ability to take in microplastics.

According to Ullah *et al.* (2021) the initial sources of microplastics buildup in plant and soil contains insecticides, herbicides, and fertilizers. Microplastics can be found in mineral fertilizer. According to Allen *et al.* (2019), treated wastewater and biosolids are applied as fertilizer for irrigation reasons. Because of this, microplastics are able to enter plants through this route. Composts, digestives, and percolate-leachates from digestion, all of which are utilised as liquid fertilizer, have been described by Weithmann *et al.* (2018) as being a way for the entrance of microplastics into the environment. Enyoh *et al.* (2019), humans have the potential to eat 80 g of microplastics daily through the consumption of plants as a source of food. MPs accumulation in plants affects various problems like metabolomic systems and enzymatic activity impairment, increased H_2O_2 and O_2 roots concentration and phytohormone changes (Li *et al.,* 2021). Zhou *et al.* (2021); Wu *et al.* (2020) have reported microplastics can affect the plants in shoot and root reduction jasmonic acid and lignin inhibition. When microplastics make their way into plants and begin to collect, this opens the door for the possibility that they will enter the food chain, where they may undergo biomagnification and lead to a various of major health concerns in humans. In their study on the effect that MPs have on agricultural soil, Zhang *et al.* (2019) found that moremicroplastics concentrations led to rise soil mobility, which in turn posed potential risks to both crop plants and humans.

CONCLUSION

The preliminary study was investigated the accumulation of MPs in edible fruits like grapes and banana and vegetables like potato and brinjal. This was identified and characterized through various analytical methods like Stereomicroscope, FTIR and SEM. Our findings demonstrates MPs particles 0.002mm upto 0.01mm have identified in edible fruits and vegetables that microplastics have been found in quantities that are frequently consumed by people, raising concerns that they may be transferred to humans through this ingestion. These findings imply the possibility of MPs gaining entry into the food chain and the potential health hazards. It is imperative that governmental organisations and health authorities move rapidly in order to enact and put into practise environmental regulations that will monitor the production, utilisation, and disposal of plastics.

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Conflict of Interest. None.

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