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A microscopic survey on microplastics in beverages: the case of beer, mineral water and tea†

Yinan Li,^{‡a} Lin Peng,^{‡a} Jianxin Fu,^a Xueli Dai^a and Guoqing Wang^{id} ^{*a,b}

It has been reported that microplastics exist ubiquitously in aquatic and terrestrial environments. Microplastic surveys on diverse daily foods with high consumption possibly containing microplastics have essential implications in clarifying the contamination routes, health risk assessment, and thereby preventing food pollution. Given the dependence of microplastic pollution on the regional environment, production and transportation, it further remains an open question on the number, size distribution and type of microplastics in foods from different countries worldwide. Here, we show that daily drinks produced worldwide, including beer, mineral water and tea, are all polluted with microplastics without exception. The number of microplastics investigated in this work lies in the range of 20–80 mL^{−1} for the beers, 10 mL^{−1} for the bottled mineral water, and 200–500 g^{−1} for the tea leaves. Quasi-spherical particles and irregular fragments dominate the shape of microplastics in beer and mineral water, whereas tea leaves carry numerous microplastic fibers. By identification through Raman spectroscopy, we observed the presence of polystyrene (PS) and polypropylene (PP) microplastics in beers, PP in bottled mineral water, and polyethylene (PE) and polyethylene terephthalate (PET) in tea leaves. Possible contamination sources include raw materials, atmosphere, and tools and containers that release microplastics. Given the facile adsorption of heavy metals and antibiotics to microplastics in beverages, public concern may arise regarding the accumulation of microplastics through the food chain and their synergetic harmful effect. Thus, our results should inspire further efforts that may contribute to the elimination and removal of microplastics from foods.

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Introduction

In the past few years, microplastic pollution and the resulting adverse consequences to the ecological system have been an issue of increasing concern.^{1,2} Degraded and fragmented from plastic products, personal care devices, industrial abrasives and others, microplastics are extensively observed in aquatic and terrestrial environments.³ Research endeavours have been made to analyze the distribution and abundance of microplastics in marine environments, lakes and rivers.⁴ It has also been reported that nowadays microplastics are widely present in air.⁵ Given the vulnerability of daily beverages to microplastic exposure during transportation, production and packaging,

microplastic pollution to our daily beverages seems inevitable. Although numerous studies were carried out on microplastic detection in foods,⁶ salts⁷ and drinks,^{8,9} it has remained an open question whether microplastic contamination of food is a common fact worldwide, and if so, what is the composition of these microplastics.¹⁰ The information is important, and may have implications in filling the knowledge gap of microplastic ingestion,⁴ food production and traceability¹¹ and health risk assessment.¹²

In this study, a microplastic survey was undertaken on the population and type of microplastics in a range of beverages, including beers, drinking water and tea. Prior to microscopic observation, suction filtration was performed to extract micro-particles from each beverage. Investigations on 15 types of beers produced worldwide and four types of Chinese tea leaves revealed the ubiquitous occurrence of microplastic pollution to all the daily beverages, despite characteristics of number and distribution for the shape of microplastics in each beverage. By coupling with the finger-printing feature of Raman spectroscopy,^{13–15} the presence of polystyrene (PS) and polypropylene (PP) microplastics in beers, PP in bottled mineral

^aCollege of Food Science & Engineering, Ocean University of China, 5 Yushan, Road, Qingdao 266003, China. E-mail: gqwang@ouc.edu.cn^bLaboratory for Marine Drugs and Bioproducts, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao 266237, China

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‡The authors contributed equally.

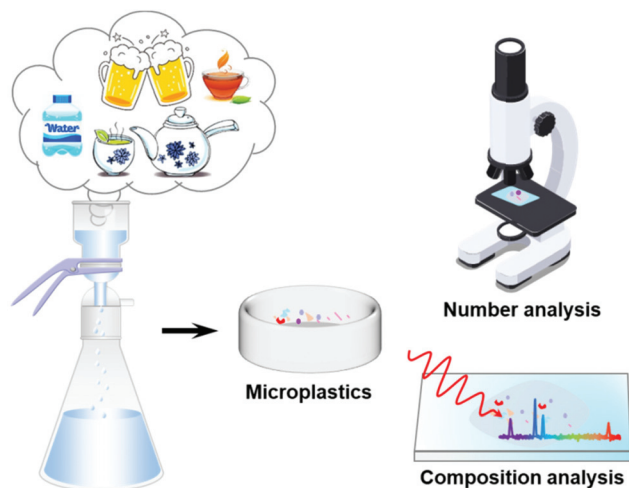


Fig. 1 Schematic of the microscopic survey on the microplastics extracted from various beverages, including beers, teas and mineral water.

water, and polyethylene (PE) and polyethylene terephthalate (PET) in tea leaves were identified (Fig. 1). The possible contamination routes for the beverages may be through raw materials, air¹⁶ and tools and containers.^{17,18} Given the widespread pollution of microplastics and the adsorption of toxic substances,¹⁹ public concern may arise regarding the bioaccumulation of microplastics through the food chain and the enhanced harmful effect. This work provides essential information for environmental protection, food processing and packaging, and health effect assessment.

Experimental

Chemicals and apparatus

Four types of teas were obtained from a local supermarket, including green tea, black tea, dark tea and white tea. The black tea was stored in iron cans, while the green tea, white tea and dark tea were packaged in paper bags. Fifteen types of beers of different brands worldwide were purchased online. The detailed information of brand and type are listed in Table S1.† All beers were packaged in reusable glass bottles. Anhydrous ethanol (Sinopharm, China) and water purified by Sartorius (Germany) were used during sample processing. Filtration was conducted using a vacuum filtration device equipped with an SH/T0093 vacuum pump (Shanghai Zhixin, China). A nitrocellulose film of 0.22 μm (diameter 25 mm) was used for filtration to retain the particles. An SB-5200D ultrasound machine (Ningbo Scientz Biotechnology, China) and a constant-temperature evaporator were used to treat the samples. A NIKON/Ni-E electrodynamic fluorescence microscope (NIKON, Japan) was used for the observation of microplastics. An inVia Raman microscope (Renishaw, UK) was used for Raman spectral analysis.

Collection of microplastics from beverages

For each beer and mineral water, 330 mL of sample beer was subjected to suction filtration, a method that has been reported elsewhere.²⁰ Specifically, the supernatant was subjected to vacuum filtration through a 0.22 μm -nitrocellulose membrane-based filter. The vacuum pump was operated at a vacuum pressure of about 1.0 bar. When the filter was blocked during filtration, a new filter was used to replace it to continue the suction filtration for the rest of the suspension. To calculate the average number of microplastics in each sample, three sets of filtration experiments were performed parallelly.

For tea leaves, 20 g of each tea leaf was added to 1000 mL H_2O , and subjected to a water bath at 90 $^\circ\text{C}$ for 60 min. Furthermore, the system was left without disturbance at room temperature for 3 days. Then, microplastics were extracted from 500 mL of the suspension from the upper part *via* suction filtration.

All filters for each sample were carefully transferred to a new beaker using clean metal tweezers, where usually, 1–2 filters were used for each beer and mineral water, whereas 3–4 filters were needed for the vacuum filtration of each tea sample. Each filter was then immersed in 1–2 mL anhydrous ethanol and subjected to sonication for 10 min. Each sample was concentrated to 1–2 mL upon ethanol evaporation using a water bath and then stored in a glass vial at room temperature for future analyses. As a control sample, pure water was treated under identical procedures for comparison with the beverage samples.

Microscopic observation and analysis

The extracted microparticles in ethanol were drop-wise added onto a glass surface. After ethanol evaporation, the microparticles were observed and counted under a fluorescence microscope under different magnifications using a bright field. For Raman spectral analysis, the microparticles were surveyed under a Raman microscope and irradiated with a laser at a wavelength of 785 nm using the Raman spectrometer. Raman spectra were collected using the *Xpspeak* software and measured with a resolution of 0.96 cm^{-1} in a wavenumber range of 500 to 1550 cm^{-1} . It should be noted that the instrument was calibrated prior to spectral collection for samples.

Quality assurance of samples

To avoid contamination of samples by microplastic from external sources, including appliances and/or airborne/water-borne particles, it was necessary to maintain strict cleaning conditions during the experiments. Samples were processed inside a fume hood. The inner and outer surfaces of the tools and containers were washed with deionized water three times to avoid any plastic contamination. Cotton laboratory coats were worn without latex gloves to prevent plastic particles on clothes and gloves. To ensure that hands were free of contamination, hands were washed with deionized water three times. Every filter membrane used for pure water filtration was also viewed using the bright field of the fluorescence microscope,

and images were captured as the control results. Moreover, pure water was analyzed as a control sample in parallel for comparison purposes and to estimate the background contamination that may occur during beverage sample processing.

Results and discussion

Considerations on the selection of beverages for the microplastic survey

Beer, tea and mineral water are among the most popular drinks worldwide, and the production of these beverages inevitably makes use of water resources from the environment. It has yet been documented that there is an occurrence of microplastics in almost all aquatic matrices. Accordingly, the daily beverages with large consumption may be inevitably contaminated by microplastics, which is a problem of increasing public concern. Furthermore, there is a possibility that existing microplastic particles inside may exert potential risks on human health after entering our bodies. Indeed, previous studies have revealed that microplastics can cause a range of harmful effects on organisms.^{21,22} Nevertheless, it remains a question on the number, size distribution and type of microplastics in the beverages, depending on the regional environment, production process, and transportation of the beverages. By microscopic survey on the abundance and the composition

of various extraneous particles, this study aims to increase the knowledge on microplastic particles in different daily beverages.

To this end, beers from various brands from 15 countries were chosen for microscopic investigation, including a beer of a local brand (Qingdao Beer) (Fig. S1†). A bottled mineral water was also selected for analysing plastic contamination. Furthermore, typical types of teas, including green, white, black and dark teas, were prepared using the corresponding tea leaves, which are popular daily drinks not only in East Asia but also in Europe (*e.g.*, black tea). By looking into exogenous microparticles in these samples, we hope to provide a microplastic pollution profile for the representative beverages worldwide.

Observation of microplastic particles from the beverages

To look into the particles extracted from the beverages, microscopy was applied with bright field observation capable of identifying objects of a few micrometres in size. As shown in Fig. 2, the particles displayed various shapes that ranged from fiber to debris and colourful fragment. In order to testify whether the microparticles originated from the beverages adapted, control experiments on the microplastic extraction from pure water were conducted under identical procedures. Few particles with negligible diameters compared to those shown in Fig. 2 were observed (Fig. S2†). Since all polymeric

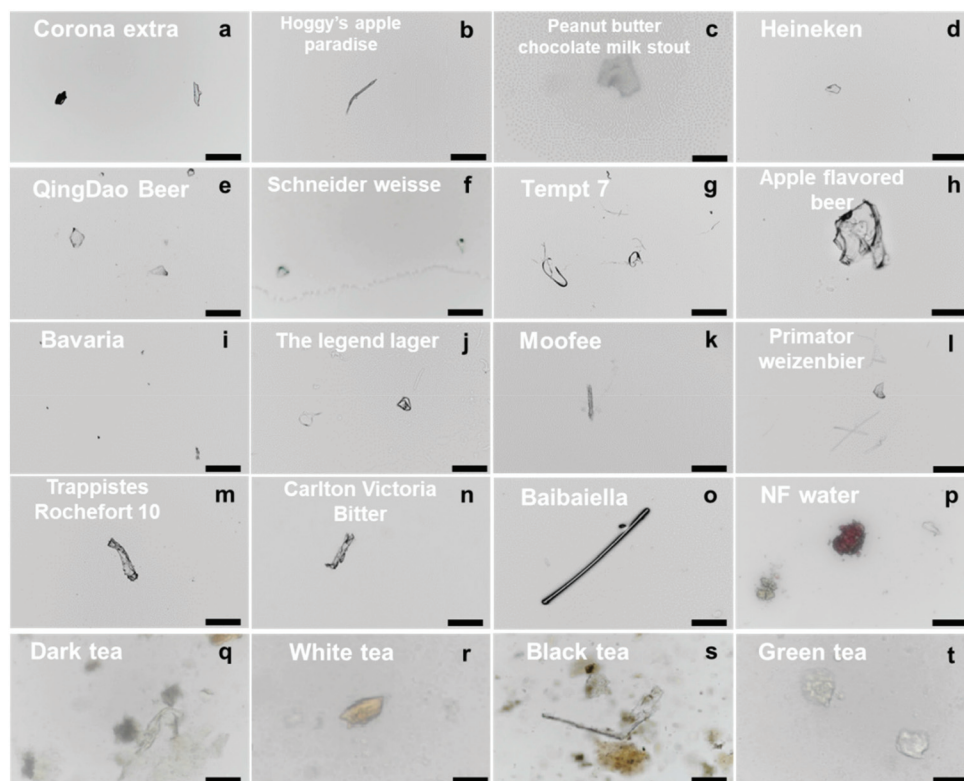


Fig. 2 Typical bright field images of particles found in the various beverages, including beers (a–o), bottled mineral water (p) and teas produced with tea-leaves (q–t). Scale bar: 100 μm . The type of drink is depicted in the corresponding image.

particles of ≤ 5 mm in size were collectively referred to as microplastics,²³ these results could directly validate the presence of microplastics. By visual identification based on their colour and structural characteristics,²⁴ microplastics were observed in all the beers from different countries, which could be divided into fibers and fragments. In the optical images (Fig. 2), for instance, microplastic fibers longer than 100 μm were found in beers *Hoggy's apple paradise* (Estonia), *Tempt 7* (Denmark) and *Baibaiella* (Brazil). Relatively small fragments could be observed from *Corana extra* (Mexico), *Peanut butter chocolate milk stout* (United States), *Qingdao beer* (China) and *Apple flavoured beer* (Israel). Some longer fragments with diameters of about 100 μm were perceived in *Moofee* (Belgium), *Trappistes Rochefort 10* (Belgium) and *Carlton Victoria Bitter* (Australia), which is smaller than the fibers in aspect ratio aforementioned. We also noted the presence of some small microplastics in *Bavaria* (Holland).

According to our statistical analysis, overall, it is suggested that fibers and fragments are present in each type of beer despite different numbers and proportions. By counting the number of microplastic in each sample, depending on the volume, it was estimated that the number of microplastic fragments ranged from 929 to 9154 per 100 mL beer. As a minor component, fibrous microplastic occupies a fraction of 24.6% on average. In total, there are 1212 microplastics per 100 mL *Tempt 7* (Denmark), while the number is as large as 9659 in *Barbarella* (Brazil) per 100 mL. The average number of microplastics per 100 mL of beer lies in the range of 1212–9659 in the beers, which would signal several microplastic pollutions to beverages. In contrast, fragmentary microplastics dominantly existed in the mineral water tested. Furthermore, the number of the microplastic fragment is as few as 929 per 100 mL of the mineral water. Compared to the number and distribution of microplastics found in Qingdao beer, the results may imply the occurrence of microplastic contamination to beers in the course of production, processing and transportation in China.

Considering the leading role of tea in beverage products for the world population, understanding microplastic pollution and distribution in tea leaves is also important. Both Fig. 2 and Fig. S3† indicate the presence of fragments and fibers in the teas prepared with the tea leaves. The yellow colour of the microplastics observed from white and black tea leaves may be attributed to the staining effect of the corresponding tea. For the tea in 1000 mL prepared with 20 g tea leaves, fibrous microplastics were detected to be the major exogenous solid component for black, white and dark teas (Fig. 3). Around 5000 fibers were estimated to be present in each of black, green and white tea leave per 20 g. Similarly, the number of 4433 for fibers was estimated to exist per 20 g of green tea leaves, whereas 6570 fragments were found, which are the main form of microplastics in green tea. Compared with beer and drinking water, tea leaves may be easily swept along microfibers during their production due to easy adhesion. This rationalizes the higher portion of fibers that have a longer length than fragments. Unlike liquid foods, also, tea leaves

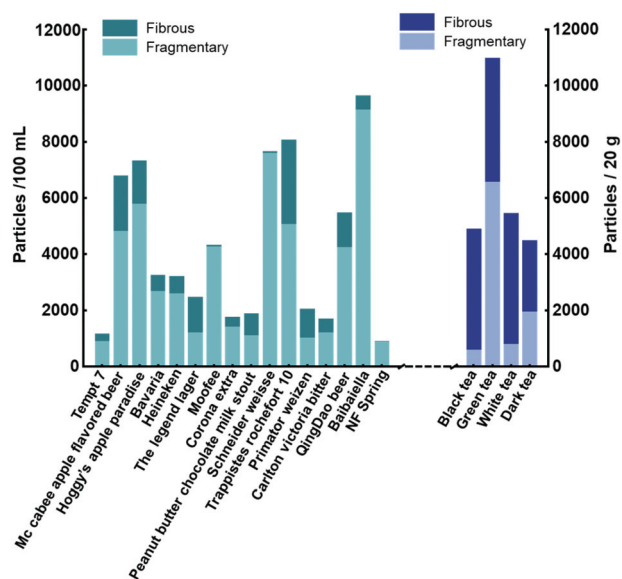


Fig. 3 Histograms showing the number of microplastics in various beverages. Totally 15 types of beers, 4 kinds of tea leaves and 1 mineral water were investigated.

may be contaminated by airborne microplastics and those released from the package. The above results also have implications on how microplastics are affecting instant granules. It is worth noting that the filtration process based on the use of a 0.22 μm -nitrocellulose membrane may not be applied to the extraction of some nanosized plastics. Also, the presence of a tiny plastic particle in the beverages cannot be ruled out. Although small nanoplastics can be detected by an electron microscope,⁹ they may be omitted by the bright-field microscopic observation.

Identification of microplastic composition from the beverages

Owing to the highly resolved spectral bands and operation over a wide range of excitation wavelengths, Raman spectroscopy was utilized to identify the chemical composition of the microplastics extracted from the beverages. As standard samples, commercially available microplastics were first analyzed by Raman spectroscopy for comparison purposes, including polyamide (PA), polycarbonate (PC), PE, PET, polymethylmethacrylate (PMMA), PP, PS, and polyvinyl chloride (PVC) (Fig. S4†).

To identify the chemical structures of the microplastics extracted from the diverse beers, the suspended microplastics were transferred to a glass substrate under the Raman spectrometer. The irradiation of a laser at a wavelength of 785 nm generated Raman signals for the microplastics. As shown in Fig. 4a, the Raman spectra are listed and compared with those reported in previous studies.²⁵ For the microparticles from Primator Weizen beer, the Raman spectra mainly vibrate at 622 cm^{-1} (aromatic C=C in plane ring deformation), 791 cm^{-1} (aromatic C=C out of plane deformation), 997 cm^{-1}

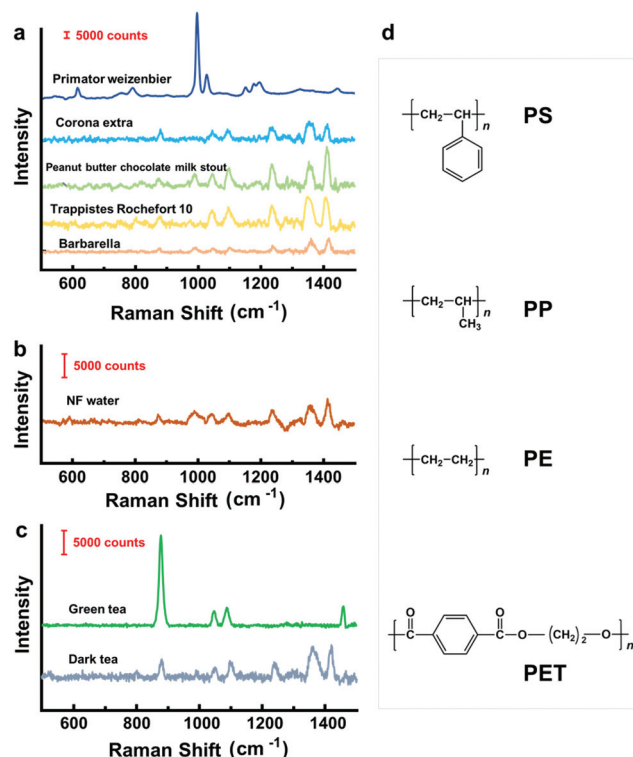


Fig. 4 Identification of chemical compositions of microplastics. (a–c) Raman spectroscopic characterizations of microplastics extracted from beers (a), mineral water (b) and teas (c). Histograms showing the number of microplastics in various beverages. (d) Chemical structures of the various plastics for reference.

(aromatic ring breathing mode), 1027 cm⁻¹, 1150 cm⁻¹ (aromatic C–H in plane deformation), 1178 cm⁻¹ (aromatic ring stretching), 1445 cm⁻¹ (CH₂ deformation). The above wave-number information is consistent with the characteristic bands for PS, as confirmed by both the previous report and the Raman peaks for the standard PS sample (Fig. S4†). We also observed that the Raman spectra of the microparticles extracted from Corona Extra-beer, peanut butter chocolate milk stout-beer, Trappistes Rochefort 10-beer and Barbarella-beer display similar profile with well-overlapped peaks. Vibrations occur at the 881 cm⁻¹ (C–C stretching), 990 cm⁻¹ (CH₃ rocking), 1046 cm⁻¹ and 1099 cm⁻¹ (C–C stretching), 1352 cm⁻¹ (CH₃ deformation), and 1410 cm⁻¹ (CH₂ wagging). The above peaks were found to be closely matched with the Raman bands of PP reported elsewhere.²⁶ For microparticles from the other 10 types of beers, a similar Raman spectrum was obtained for each (Fig. S5†). Only a single Raman band centered at 881 nm was observed. Currently, we have not found a match of microplastic Raman spectrum with it. It should be pointed out that the Raman spectra shown in Fig. 4 are not corresponding to the images shown in Fig. 2. In other words, the compositions of microplastics found in the beverages may not be limited to those inferred here.

It is worth noting that all these beers were packaged in glass bottles as received. Since the packages do not release plastic and polymeric particles, the microplastics were possibly introduced from raw materials (*e.g.*, water source, grains), work clothes and the production environment. For instance, microplastics may exist in water from polluted rivers and lakes, and be released from water pipes. Barley and hops, which are necessary, in beer production are also vulnerable to plastic pollution. Indeed, a large number of microplastic fragments and fibers have been found in wheat and rye grains.²⁷

For the microparticles obtained from NF mineral water, as shown in Fig. 4b, the spectrum indicates a major vibration at 809 cm⁻¹ (C–H rocking vibration), 971 cm⁻¹, 990 cm⁻¹, and 1048 cm⁻¹ (C–C stretching), 1322 cm⁻¹, 1360 cm⁻¹ and 1459 cm⁻¹ (CH₂ bending). The above peaks agree well with that for PP reported elsewhere.^{25,28} Considering the bottling process, the release of plastic particles from the packaging may be caused by water injection into the bottle. Moreover, the stress exerted physically on the bottle cap when decapping could also be a causative factor. The numbers and compositions identified in the various beverages in the present work were also summarized in Table S1.†

The spectra in Fig. 4c show the Raman bands of the microplastics found in dark tea and green tea. We observed the Raman peaks for the green tea samples at 858 cm⁻¹ (aromatic C=C out of plane deformation), 1091 cm⁻¹ (stretching vibration of the carbon atoms between the ring and the carboxyl groups), 1283 cm⁻¹ (CH₂ twisting, aromatic in plane CH deformation), and 1461 cm⁻¹ (CH₂ bending), which are in line with the bands of PET.^{25,28,29} On the other hand, the extracted microparticles from the dark tea exhibit characteristic bands at 1418 cm⁻¹, 1369 cm⁻¹ and 1295 cm⁻¹ (CH₂ bending), 1129 cm⁻¹ and 1051 cm⁻¹ (C–C stretching), which are in good agreement with that of PE.^{25,28} Accordingly, we inferred the chemical compositions of the detected microplastics in dark tea and green tea to be PE and PET, respectively (Fig. 4d, Fig. S4†).

The Raman spectra of the microplastics found in white tea mainly vibrate at 858 cm⁻¹ and 1091 cm⁻¹ (Fig. S6†). The Raman spectra of the microplastics found in black tea mainly vibrate at 858 cm⁻¹, 1091 cm⁻¹ and 1459 cm⁻¹. Indeed, the above peaks likely resemble that of PET, which represents the most commonly applied polymer for plastic packaging purposes (*e.g.*, plastic bags, caps, bottles, film, containers, and pipes). However, the concordance with the Raman bands of PET is lower than 70%. The slightly different Raman characteristics of the microplastics from that of PET may be due to the presence of other chemical species in the suspension.⁸

Contamination of tea leaves may start from the growth process itself. Due to the ubiquitous nature of microplastic in environment,³⁰ it is hard to guarantee that the plants are free of microplastic contamination. Therefore, airborne microplastic is a possible source of contamination. Furthermore, although a clean environment is maintained in tea production, the tools and containers may be another source of microplastics during the stir-fry or steaming process. For instance, recent tea manufacturers have shifted to the adoption of

plastic tea bags, which may release small plastic particles when brewing tea.⁹ Also, airborne microplastics may be introduced during sun drying for tea production.

Conclusions

The production of beverages necessitates the use of water from aquatic matrices, whereby there is the occurrence of microplastics in most cases. Therefore, understanding the possible distribution of microplastics in our daily beverages provides essential information for environmental protection, food processing and packaging, and health effect assessment. In this extensive survey, we discovered that microplastic particles widely exist in our daily drinks worldwide, such as beer, mineral water and tea. The shape of microplastics ranges from irregular quasi-spherical particles to fragments and fibers. The number of microplastics distributed is in the range of 20–80 mL⁻¹ for beers, 10 mL⁻¹ for bottled mineral water, and 200–500 g⁻¹ for teas that were investigated in this work. Regarding the compositions of the microplastic particles, PS and PP were detected in beers by Raman spectroscopy, whereas PP in bottled mineral water and PE and PET in teas are of abundant types. Possible contamination sources for the beverages are the raw materials, such as barley and hops for beers and water for beers and mineral water. For teas, microplastic pollution may have started from their growth. Another possible route is the production process that necessitates the use of tools and containers that release microplastics. This study has important implications for the following reasons: first, the occurrence of microplastic pollution in other commercial foods such as biscuits and instant food still requires additional efforts. Given the powerful fingerprinting feature, Raman spectroscopy is capable of identifying microplastics in more complex food systems.^{31–33} Coupled with the microplastic distribution data reported in this work, measures need to be taken to assess health risks and to establish a tolerable daily intake of microplastics in foods and beverages. Second, given the widespread pollution of microplastics and the facile adsorption of toxic substances, including heavy metals and antibiotics, a public concern may arise with regard to the bioaccumulation of microplastics through the food chain and their synergetic harmful effect as well. Finally, we hope this study will inspire further endeavours that may contribute to the elimination and removal of microplastics from our food.

Author contributions

G. W.: Conceived and designed the research. Y. L. and L. P.: Conducted the experiments. G. W., Y. L. and L. P.: Wrote the manuscripts. All authors: discussed the results.

Conflicts of interest

There are no conflicts of interest to declare.

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