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Microplastic Contamination in the Human Gastrointestinal Tract and Daily Consumables Associated with an Indonesian Farming Community

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Abstract: Plastic is one of the most abundant pollutants in the environment. As a result of natural physical processes, large plastic waste is degraded into microsized particles (<5 mm) called microplastics. Because of their size, abundance, and durability, microplastics are widely distributed in the environment, contaminating food and water intended for human consumption. The extent of microplastic contamination in the human body is still unclear because there are few studies concerning microplastic contamination in human specimens and, in most studies, data were collected from city dwellers. Despite having the fourth largest population and being the fourth largest plastic waste producer in the world and second largest plastic polluter in the ocean, there are currently no data with respect to microplastic exposure for the Indonesian population. Several studies have reported on microplastic contamination in seafood and freshwater organisms from Indonesia, and it is likely that microplastics have contaminated the gastrointestinal tracts of Indonesians. Using Raman spectroscopy, we detected microplastic contamination in 7 out of 11 analyzed stool samples collected from a farming community in the highland village of Pacet, East Java, Indonesia. Polypropylene (PP) was the most abundant and prevalent type of microplastic observed, and it was found in four of the positive samples with an average concentration of 10.19 microgram per gram of feces (µg/g). Microplastics were also detected at high concentrations in tempeh (soybean cake, a staple protein source for Indonesians), table salts, and toothpaste, which were regularly consumed and used by the study participants. PP was particularly high in table salts (2.6 µg/g) and toothpaste (15.42 µg/g), suggesting that these products might contribute to the gastrointestinal contamination in the studied population. This pilot study indicated microplastic contamination in the rural Indonesian population and in their daily consumables, demonstrating the far-reaching extent of microplastic pollution beyond urban areas.

Keywords: microplastic; human stool; microplastic contamination; human exposure; waste management

1. Introduction

Plastic waste is one of the most abundant pollutants in marine and terrestrial ecosystems. It is estimated that 585 million tons of plastic waste is produced worldwide every year, of which only 9% is recycled, 12% is incinerated, and 79% goes to landfills or the natural environment [1,2]. Among the plastic producer countries, Indonesia is the fourth largest contributor, producing 20.5 million tons of plastic waste each year [2]. Indonesia is also the second largest contributor to marine plastic pollution, and it is estimated that 1.2 million tons of plastic enters the oceans around Indonesia each year [3]. Upon its release and accumulation in the environment, plastic waste is broken down into microplastic particles with sizes of less than 5 mm. Because of their small size, abundance, and durability, microplastics become widespread and ubiquitous in the environment [4]. In terrestrial environments, microplastics can be found abundantly in soil [5] and can be detected in various organisms, including earthworms [6], snails [7], human pets [8], and various livestock [9,10]. Microplastics are also entering the human food chain and can be found in drinking water and food consumed by humans [11–13]. In Indonesia, several studies have detected microplastics in commercial edible fish and bivalves [14–18].

In addition to food and water, microplastics are also detected in the air and in indoor and outdoor dust [19–21]. Therefore, microplastics could enter the human body not only via ingestion but also via inhalation. One study estimated that 74,000–121,000 microplastic particles could enter the human body each year via ingestion and inhalation [22]. Nevertheless, the extent of microplastic contamination in the human body is still unclear because studies using human specimen samples are still limited. Previous studies have reported microplastic contamination in human stool samples [23–25], colectomy samples [26], meconium [25,27], placenta [27,28], lungs [29], skin surface, head hair, and saliva [30]. Microplastics are detected in humans of all ages, from the placenta and meconium of newborn babies [25,27,28] to infant stool samples [25], young adult stool samples [24], and the lung tissue of elderly people [29]. The greatest concern with respect to microplastic exposure in humans is the potential toxicity and effects of microplastics on human health. Several studies using animal and human cells have reported potential adverse impacts of microplastic contamination on human health. Such contamination is reported to induce proinflammatory cytokines that cause immune responses [31,32], inhibit cell proliferation, cause morphological changes in lung cells [33], and cause various metabolic disorders associated with gut microbiota dysbiosis [34] and gut barrier dysfunction [35].

Information regarding the extent of microplastic exposure in the human body is very important to formulate mitigation strategies and raise awareness. Currently, there are limited studies reporting microplastic exposure in human specimens, and in most studies, such samples were collected from city dwellers [23–30]. In addition, these published studies did not investigate microplastic contamination in daily consumables regularly used by the study participants, resulting in an information gap regarding the origin of the contamination.

Despite being the fourth most populous country and largest plastic waste contributor in the world [2], currently no data exist regarding microplastic contamination in the Indonesian population and in daily consumables in Indonesia. Here, we investigate the microplastic content in human stool samples and daily consumables collected from a farming community living in the highland village of Pacet, East Java, Indonesia. Even in highland rural villages, the use of plastic packaging for daily consumables is common. Therefore, microplastic contamination in consumables and the groundwater is possible. The results showed that microplastic contamination is observed in more than 50% of the stool samples, of which polypropylene (PP) is the most common contaminant. In addition, microplastics are found at high concentrations in the staple food, table salts, and toothpaste frequently used by the study participants. Finally, the results indicate the far-reaching contamination of microplastics in the human body, food, and hygiene products, and because of the potential adverse effect of microplastics on human health, the current study suggests systematic measures to reduce microplastic contamination in the human environment.

2. Materials and Methods

Here, we investigate microplastic contamination in stool samples representing the human gastrointestinal tract. The samples were collected from a farming community living in an Indonesian rural area because studies of rural populations are underrepresented and no previous data existed concerning microplastic exposure in the Indonesian population. Because plastic is generally used for food packaging and waste is poorly managed in Indonesia, we hypothesized that, despite their rural setting, the studied population had been exposed to microplastics and carried microplastic contamination in their gastrointestinal tracts. The majority of the microplastic contamination likely results from consumed food, water, and oral hygiene products. To trace the potential origin of the contamination, interviews were conducted to collect data regarding the participants' dietary habits. On the basis of this information, we collected samples of drinking water, food, and hygiene products that were frequently consumed by the participants, and the samples were subjected to a microplastic analysis using Raman spectroscopy.

2.1. Study Participants and Stool Sample Collection

The objective of the study was to investigate the extent of microplastic contamination in a rural Indonesian population. Accordingly, stool samples were collected from 11 healthy participants (5 males and 6 females) from a farming community living in a rural highland village in Indonesia. The participants came from three different hamlets in the village of Pacet, Mojokerto, East Java, Indonesia, and the GPS coordinates for the hamlets are provided in Table 1.

Table 1. Geographical locations of the sample collection areas.

Hamlet	GPS Coordinate
Warubinatur	07°36.367053′ S, 112°33.134887′ E
Sumbergayam	07°36.309465′ S, 112°32.970971′ E
Randegan	07°37.271696′ S, 112°31.726817′ E

The participants were chosen based on the following criteria: generally healthy, within the age range of 20–50 years, from a farming family, and having not consumed any antibiotics for 2 months prior to the sample collection. We also recorded the details of the participants' diets and general health conditions for 7 days prior to sample collection. The collected data included the frequencies that the participants consumed seafood and products with plastic packaging.

To ensure that there was no microplastic contamination during stool sample collection and processing, we provided the participants with glass containers with lids and spoons made of steel. We also used glass and steel utensils during sample preparation and microplastic analysis.

2.2. Collection of Drinking Water, Staple Foods, Table Salts, and Toothpaste Samples

To trace possible sources of any microplastic contamination, the recorded dietary data from the participants were used to collect drinking water, staple foods, table salts, and toothpaste commonly used and consumed by the participants. Drinking water was collected from three natural springs that were communally used by all of the participants. Meanwhile, staple foods, table salts, and toothpaste were collected from local markets where the participants usually acquired these products. Three replicates were collected and measured for each item.

2.3. Sample Preparation

Sample preparation was performed to digest the non-microplastic content in the samples, facilitating proper identification and quantification of the microplastics in the samples. Microplastics were extracted from the stool, food, salts, and toothpaste according to Yan et al. (2021) [36], with some modifications. In brief, the collected stool, food, salt, and toothpaste samples and other samples were incubated at 70 °C for at least 1 week until the weights of the samples were constant. The dried stool samples (1 g for each sample) were mixed with 50 mL of Fenton's reagent and incubated at room temperature for 5 h. The mixture was then subjected to filtration using a cellulose nitrate–cellulose acetate filter with a pore size of 0.8 µm. The filters were then digested using 65% HNO_3 at 50 °C for 30 min, followed by incubation at 70 °C for 10 min. The solution obtained following digestion was then diluted with distilled water at a ratio of 1:2, and the microplastic content in the samples was measured using Raman spectroscopy. Conversely, the collected drinking water samples were directly subjected to Raman spectroscopy measurements without any preparation.

2.4. Microplastic Analysis

Microplastic identification and quantification from the digested samples were performed using Raman spectroscopy (off-resonance) with an excitation wavelength of 785 nm. A StellarNet Raman HR-TEC-785 was used with a 785-nm (100-mW) diode laser as the optical excitation. For the reference spectra, several concentrations of microplastics were prepared by dispersing different types of microplastics separately, e.g., polyethylene terephthalate (PET), polystyrene (PS), PP, polyethylene (PE), high-density polyethylene (HDPE), and low-density polyethylene (LDPE), in 0.1 M HNO_3. The spectrum profiles produced by each type of microplastic were then used as calibration curves to quantify the microplastic content in the samples.

To identify the microplastic content in the samples, Raman spectra within the range of 500–2750 cm^{-1} were used with the spectral identifier of each reference polymer. The Raman spectra obtained from the samples were then fitted to the reference spectra and calculated using the particle swarm optimization algorithm [37,38], with the following assumption:

$$I(\omega) = \sum_{i=1}^{n} \alpha_i I_i(\omega) \quad (1)$$

where $I(\omega)$ indicates the Raman spectrum of the sample, $I_i(\omega)$ indicates the Raman spectrum of the microplastic reference, α indicates the fraction of the microplastic mass, and i indicates the microplastic type index, e.g., PET, PS, PP, PE, LDPE, or HDPE

The values of α were then used to determine the intensity contribution of Raman spectral identifier from each type of identified microplastic in the samples and the intensity contribution values were used to quantify the microplastic content in the samples using the calibration curve.

2.5. Statistical Analysis

To determine possible connections between microplastic contamination and the participants' dietary habits, the correlations between the seafood and plastic-packaged food consumption frequencies and the total microplastic content in the stool samples were analyzed using Pearson's correlation, with the significance defined as $p < 0.05$.

3. Results and Discussion

A large number of studies have shown that microplastics are ubiquitous in nature, are widely distributed in both aquatic [39] and terrestrial environments [40], and are found incorporated in the food chain [41]. Microplastics are also detected in human consumables, such as drinking water [11], rice [42], livestock [9,10], seafood [12], and salt [13]. In addition, microplastics are found in various hygiene products [43,44] and cosmetics [45].

Nevertheless, studies of microplastics in human specimens are still limited, and the origin of such contamination is still unclear. Here, we report, for the first time, microplastic exposure in the Indonesian population and its association with daily consumables and oral hygiene products.

3.1. The Abundance and Characteristic of Microplastics in Human Stools

Microplastics were detected in 7 of the 11 collected stool samples. Of the seven samples containing microplastics, the microplastic concentration found in the stools ranged from 6.94 micrograms of microplastic per gram of feces (μg/g) to 16.55 μg/g. Of the six types of microplastic analyzed (PET, PS, PP, PE, HDPE, and LDPE), four types were detected in the stool samples, with PP being the most abundant (average concentration of 10.19 μg/g in the positive samples) and prevalent (prevalence rate of 0.36). The other types of microplastics found in the stool samples were HDPE, PS, and PET, with prevalence rates of 0.27, 0.18, and 0.09, respectively, and average concentrations of 9.97, 3.73, and 6.94 μg/g, respectively (Figure 1 and Supplementary Table S1). There is considerable variation in the microplastic compositions of the participants' stool samples, suggesting that they might be exposed to microplastics through different sources.

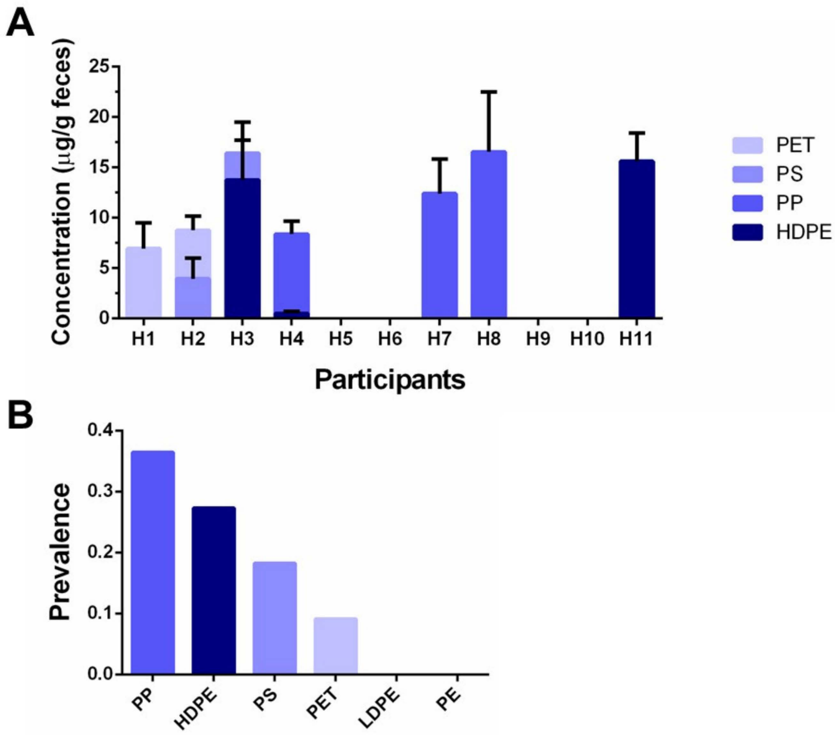

Figure 1. Microplastic concentration and prevalence in stool samples collected from a farming community in Pacet, Mojokerto, Indonesia: (**A**) Concentrations of the different microplastics in each of the 11 study participants; (**B**) Prevalence of different microplastics found in the stool samples.

The ratio of positive samples (63.64%) in this study is lower than the ratios previously reported for human stool samples from Beijing (95.8%) [24], New York (100%) [25], and different cities across Europe and Asia (100%) [23]. Our samples were collected from a farming community in a rural village of Indonesia, whereas in the aforementioned studies, the samples were collected from city dwellers. In an extensive study involving 2000 participants from different regions in Iran, Abbasi and Turner (2021) reported microplastic detection in water rinsed from head hair, face skin, hand skin, and saliva [30]. In accordance with this study, they reported that the abundance of microplastics was similar in urban populations but was considerably lower in rural populations [30].

The difference in the prevalence between rural and urban populations might be due to the different lifestyles and dietary habits of the two populations, in which the rural population consumed less processed and plastic-packaged products than the urban population did. Note that the participants in this study acquired their drinking water directly from natural springs that were not contaminated by microplastics (Table 2), whereas city dwellers commonly acquire their drinking water from a centralized water supply or bottled water. Several studies have reported microplastic contamination in tap water and bottled water [11,46,47], and the consumption of contaminated water might cause the higher prevalence of microplastic exposure in city dwellers. In addition, a previous study reported that the airborne microplastic concentration in urban areas is much higher than that in rural areas [48], this might also contribute to the higher prevalence of microplastic contamination in urban populations. Looking at microplastic composition, PP was observed as the most abundant and prevalent type of microplastic in the stool samples. PP was also the most common microplastic observed in human stools from Beijing [24] and from different cities across Europe and Asia [23]. PP was also detected in colectomy samples from Northeastern Peninsular Malaysia [26] and in skin surface, hair, and saliva samples from urban and rural regions of Iran [30]. Together, these results suggest an abundance of PP contamination in both urban and rural populations.

Table 2. Microplastics content in staple foods and drinking water.

Samples	Type of Microplastic	Content (μg/g)
	Food	
Marine Milkfish	nd	nd
Freshwater Tilapia	nd	nd
Freshwater catfish	nd	nd
Mussel	nd	nd
Shrimp	nd	nd
Tofu Brand 1	nd	nd
Tofu Brand 2	nd	nd
Tempeh Brand 1	LDPE	11.08 ± 2.72
Tempeh Brand 2	nd	nd
Rice Brand 1	nd	nd
Rice Brand 2	nd	nd
Preserved marine fish 1	nd	nd
Preserved marine fish 2	nd	nd
	Drinking water	
Spring Water 1	nd	nd
Spring Water 2	nd	nd
Spring Water 3	nd	nd

3.2. The Abundance and Characteristic of Microplastics in Drinking Water and Staple Foods

Previous studies have reported the detection of microplastics in drinking water and food [11,49]. Therefore, involuntary exposure of the human body to microplastics through water and food seems certain. To evaluate whether the microplastics found in the stools of the participants can be associated with contamination in water and food, we measured the microplastic concentrations in the water and staple foods that were frequently consumed by the participants. However, we did not find microplastic contamination in the drinking water or in most of the staple foods frequently consumed by the study participants. The only staple food contaminated with microplastics was tempeh, a popular staple source of protein for Indonesians made from fermented soybeans. LDPE was detected in one of the tempeh brands analyzed, with an average concentration of 11.08 μg/g. There was no statistically significant correlation between the frequency of consuming products packaged in plastic and seafood with the occurrence of microplastics in the stool samples (Supplementary Table S2).

Overall, in the studied population, there is no strong association between the consumed food and water and the microplastic contamination in their gastrointestinal tracts. Even though microplastics have been previously detected in Indonesian water supply and seafood [14–18,50], we did not detect microplastic contamination in the drinking water and in most of the staple foods regularly consumed by the study participants (Table 2). The only positive sample came from tempeh (fermented soybeans), a popular source of protein in Indonesia. It is estimated that Indonesians consume 0.139 kg of tempeh per week. Here, we detected 11.08 µg/g of microplastics in tempeh. Therefore, as much as 1.54 mg of microplastics could be ingested every week via tempeh consumption. The contamination in tempeh most likely arises from the packaging because the analyzed brand was wrapped in plastic packaging. One study reported that microplastics can be generated by simply opening plastic containers with scissors, hand tearing, or by cutting with knives. These processes can produce 0.46–250 microplastics/cm depending on the properties of the containers [51]. This result highlights the fact that microplastic contamination could originate from any source, even a plant-based food, as long as the food is wrapped using plastic-based packaging.

3.3. High Abundance of Microplastic in Table Salts and Oral Hygiene Products

In addition to staple foods and drinking water, microplastics could enter the human body through food seasonings, such as table salts, or oral hygiene products, such as toothpaste. Previous studies have reported microplastic contamination in sea salts produced in Indonesia [52,53], and contamination in toothpaste has been reported in other countries [43], but no data were available for Indonesia. We found high concentrations of microplastics in two brands of table salt and toothpaste regularly used by the participants. On average, 2.06 and 5.55 µg/g of PP and PE, respectively, were detected in the two table salt brands. These results agree with a study by Kim et al. (2018), who reported PP and PE contamination in Indonesian sea salts [53]. Conversely, 15.42 and 17.80 µg/g of PP and HDPE, respectively, were observed in two of the toothpaste brands analyzed (Table 3). Because we found that PP and HDPE were the main microplastics found in the participant's stool samples in this study, these results suggest that salt and oral hygiene products might be an important source of microplastic contamination in the studied population. Salt has been recognized as an important source of microplastic contamination in the human body, but there is limited information regarding contamination and intake from oral hygiene products such as toothpaste. The high concentrations of microplastics in the toothpaste observed in this study suggest that toothpaste might be an important source of human contamination.

Table 3. Microplastics content in table salt and toothpaste.

Samples	Type of Microplastic	Content (µg/g)
	Table Salt	
Brand 1	PP	2.06 ± 0.43
Brand 2	PE	5.55 ± 2.27
	Toothpaste	
Brand 1	PP	15.42 ± 5.57
Brand 2	HDPE	17.80 ± 5.16

The most common type of microplastic found in this study was PP. This was anticipated because PP is reported to be the most prevalent type of microplastic detected in environmental, food, and animal samples from Indonesia, with 90% of studies analyzing microplastic pollution in Indonesia detecting PP in their samples. PP is particularly abundant in Indonesian aquatic organisms [18]. It has been suggested that PP contamination can cause disruptions in the immune system, particularly by inducing proinflammatory cytokines that cause local inflammation in tissues and organs and by causing cytotoxicity in human peripheral blood lymphocytes [31,32,54]. Because PP polymers appear to be prevalent in the human body and daily consumables, further studies and an immediate focus are required to assess their potential effect on human health and to mitigate their incorporation into the human body.

4. Conclusions

In this pilot study, we detected microplastics in human stools and daily consumables collected from a farming community in a rural village of Indonesia. As much as 63.64% of the participants have microplastic contamination in their gastrointestinal tracts, with PP and HDPE observed as the most common contaminants. This is the first report on human exposure from Indonesia and one of the few studies involving a population from a rural area. The microplastic content of consumables frequently used by the study participants was also analyzed. Even though no microplastics were detected in the drinking water and most of the staple foods, high abundances of PP and HDPE were found in tempeh (a staple protein source), table salts, and toothpaste, suggesting that these products might contribute to microplastic exposure in the studied population. Note that the sample size in this study was limited, making it impossible to establish statistically sound correlations and causalities. Studies with larger numbers of samples covering different populations in Indonesia are required to evaluate the full extent of the microplastic contamination in Indonesia to determine factors that could affect the exposure rate and to formulate mitigation strategies. Nevertheless, this study provides preliminary data regarding the microplastic contamination rate in Indonesian rural populations and their possible sources. Information from this study can be used as a basis for further studies with larger and more diverse populations.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su132212840/s1: Table S1 Microplastic concentration found in participants' stool samples and Table S2 Summary of data collected from interview with participants and the total of microplastics found in each stool sample.

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Institutional Review Board Statement: The collection of human stool samples was approved by the Health Research Ethic Committee of Universitas Surabaya (No. 005-OL/KE/III/2021). Stool samples were obtained from 11 adult participants from the highland population of Pacet, Mojokerto, Indonesia. All samples were anonymized and obtained with written consent from the participants.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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