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Detection of Various Microplastics in Human Stool

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ABSTRACT/BACKGROUND

Microplastics are ubiquitous in natural environments. It has been documented that marine organisms consume microplastics, allowing particles to infiltrate the food chain.

Objectives: To examine human defecation for the presence of microplastics in order to determine if humans ingest them voluntarily.

Design: Prospective case series in which participants followed step-by-step instructions to complete a dietary diary and collect stool samples.

Setting: India.

Participants: Eight healthy volunteers between the ages of 33 and 65.

Measurements: After chemical digestion, stool samples were analyzed using Fourier-transform infrared microspectroscopy to determine the presence and morphology of 10 common categories of microplastic.

Results: All eight stool samples proved positive for the presence of microplastics. There were a median of 20 microplastics (50 to 500 m in size) per 10 g of human faeces. In total, nine varieties of plastic were identified, with polypropylene and polyethylene terephthalate being the most prevalent.

Limitations: Few participants were included, and each provided only one sample. The origin and fate of microplastics in the digestive system have not been studied.

Conclusion: Multiple microplastics were detected in human stool, indicating accidental ingestion from multiple sources. There is a need for additional research on the extent of microplastic ingestion and its potential effects on human health.

Keywords: Microplastics; Plastic Particulates; Marine Animals

INTRODUCTION

The global production of plastics has increased exponentially over the past century to more than 350 million tons per year, a portion of which winds up as debris[1,2]. The definition of microplastics is plastic particles smaller than 5 mm[3]. They are either manufactured in these sizes or are fragments of larger plastic structures. Microplastics are worrisome because they continue to contaminate aquatic[4], terrestrial[5], and atmospheric[6] environments. In addition, there have been multiple reports of microplastics in food [7], specifically in seafood [8,9], sea salt[10 -12], and potable water[12-14]. In the field, microplastics are primarily detected in the gastrointestinal tract of marine animals[15], whereas in the laboratory, cellularabsorption and tissue accumulation of microplastics and, more notably, nanoplastics have been demonstrated[16-19]. Plastic particulates are viewed as foreign entities within tissue and can provoke local immune responses[20]. In addition, microplastics can act as a vector for other compounds, such as environmental contaminants or plastic additives, which can leach out and cause exposure to dangerous substances[18,21]. Concerns have been voiced by scientists and public authorities regarding the presence of microplastics in food, their potential ingestion by humans, and their health effects[7,22,23], but data are scarce. There are reports of humans inhaling microplastics[6,24], but the gastrointestinal burden has not yet been studied in humans, despite the discovery of microplastics in food and in the gastrointestinal tracts of marine animals.

METHODS

Student Participants and Procedures

Eight individuals from sikkim, Punjab; Chandigarh, Harayana; Rajasthan, Kerela; Madhyapradesh, Nepal were recruited to represent various geographical regions and dietary patterns. They were given a stool sampling device and asked to record their food intake (with no dietary restrictions) six to seven days prior to sampling. The components of toothpaste, cosmetics, and chewing gum were documented. Participants collected feces in accordance with predetermined instructions to avoid contamination with plastics or synthetic fibers. Stool samples were collected with metal spoons and deposited in labeled, pseudonymized, and preweighed glass vials containing an antibacterial aqueous solution. The materials were chemically pretreated to dissolve naturally occurring organic matter. Microplastics and indigestible material remnants were filtered through a 50-m metal sieve. Following resuspension in ultrapure water, an aliquot was vacuum-transferred to a filter and desiccated. Using imaging mode Fourier-transform infrared (FT-IR) microspectroscopy (Spotlight 400 [PerkinElmer]), the composition of microparticles (>50 m) was determined. The acquired IR map of 1 scanned filter contained approximately one million IR spectra, which were compared to an internal database. Figure [panels A to C]; sections 1.6 and 1.7 of the Supplement. We concentrated on ten prevalent polymers[2]: polyethylene, polypropylene, polyvinyl chloride, polystyrene, polyethylene terephthalate, polyamide, polyurethane, polycarbonate, polymethyl methacrylate, and polyoxymethylene. The quality control sample (1 procedural blank without stool) was processed and analyzed alongside the stool samples to cover all potential sources of contamination, including sample containers, laboratory equipment, chemicals, sample digestion and filtration, and analytical measurements. The results are depicted as medians and interquartile ranges (IQRs) after beingprocessed with Prism 7.00 (GraphPad Software).

RESULTS

Three men and five women between the ages of 33 and 65 participated in the study (Table). None of the participants were vegetarian during the observation period, and six of them consumed seafood. Typically, food was wrapped, packaged, or stored in plastic. Seven participants (87.5%) drank daily from plastic bottles, and three used cosmetics containing synthetic polymers (such as shower gel, face cleanser, and hand moisturizer). Each participant provided one stool sample weighing a median of 34 g (IQR: 8 to 39 g), of which a median aliquot of 7 g (IQR: 3 to 11 g) was analyzed using FT-IR. All eight samples contained microplastics between 50 and 500 m in size. No plastic particles larger than 500 m were detected, and particles smaller than 50 m were not investigated due to methodological constraints. The identified microplastics were predominantly fragments and films and infrequently spherical or fibrous. The median microplastic concentration per 10 g of stool was20 pieces (IQR: 18 to 172 pieces). In each stool sample, 3 to 7 plastic types were identified, and 9 plastic types (out of 10 analyzed) were found overall (Supplement Table 1, available at Annals.org). All eight samples contained polypropylene and polyethylene terephthalate at relative frequencies of 62.8% and 17.0%, respectively. The only plastic not detected in any sample was polymethyl methacrylate. Panel D of Figure displays the relative abundances of allmicroplastics detected. Notably, the quality control sample contained none of the ten investigated plastics.

DISCUSSION

Emerging environmental concern, microplastics have already infiltrated the food chain[7]. This is the first investigation that, to our knowledge, provides evidence of the presence of microplastics in human faeces, indicating ingestion. The average daily stool excretion of an adult is approximately 100 g[25], and in our study, wefound 20 microplastic particulates per 10 g of feces. We found polypropylene and polyethylene terephthalate in all stool samples, accounting for nearly 80% of the total microplastic burden. In addition, we detected seven other categories of microplastic, indicating that there are numerous potential sources of ingestion. In addition to food and water, microplastics can originate from food processing, packaging, and preparation, as well as airborne debris. The estimated annual ingestion of microplastics from shellfish is between 123 and 11,000 particles[8, 9, 26], 37 to 1,000 particles from salt[10–12], and 4,400 to 5,800 particles from potable water [12]. Whereas airborne pollution accounts for between 13,731 and 68,415 particles ingested annually [26]. A recent meta-analysis[27] concluded that the annual consumption of microplastics ranges from 39,000 to 52,000 particles. In addition, bottled water appears to be a significant contributor to microplastic ingestion, withan average of 118 to 325 particles per liter[13,14], for a total of 90,000 microplastics annually if the prescribed water intake comes exclusively from bottled sources[27]. In our survey, we sought to characterize the participants' ordinary domestic life. However, seafood was Food and beverages that were seldom consumed were frequently stowed in plasticcontainers. In contrast, none of the 10 investigated plastics were found in any of the reported personal care products. Our study is limited by its small sample size, and larger studies are required to establish correlations between categories and quantities of fecal microplastics and geographic region, food consumption, and other potential sources of microplastic ingestion. To analyze microplastics in human feces, it was necessary to develop new techniques. Stool sampling for this type of analysis is not standardized and may be subject to contamination. The participants were given spotless instruments and detailed instructions to collect approximately 30 g of excrement. There were no sampling difficulties reported, but we received two low-weight samples from which we analyzed reduced aliquots. These two samples contained the greatest concentrations of microplastics. Future trials must determine the optimal sample weight to reconcile analytic feasibility with the need to avoid sampling bias. The current analytic standard, FT-IR microspectroscopy, was used to identify the type and number of plastic particles[28]. Although this is a highly accurate detection method, our studyis likely biased toward a type II error because a number of factors may have led to an underestimation of the microplastics concentration. First, chemical pretreatment of samples can degrade specific microplastics and reduce their rate of recovery[29]. The particles were only counted as microplastics if the spectral similarity with reference microplastics exceeded 70% and the IR expert confirmed the presence of distinctive IR peaks. Thirdly, after sample preparation, residual particulates may obscure plastic particles during FT-IR image analysis. In the microplastic testing laboratory, great care was taken to avoid sample contamination during all analytical stages, as evidenced by the negative quality control sample. The potential health effects of ingested microplastics and nanoplastics, which (at least in animals) may translocate into gastrointestinal tissues or other organs and cause detrimental effects[15–19], are the subject of ongoing discussion. Along with particulate size, the intestinal mucosal barrier likely plays a role in microplastic absorption. Although there are insufficient data on microplastic or nanoplastic absorption from the human gastrointestinal tract, patients with increased intestinal permeability (for example, as a result of chronic inflammatory bowel disease) may be more susceptible to microparticle absorption[30] and potential damage[31]. In conclusion, this small prospective case series demonstrated the presence of multiple microplastics in human stool, and that no sample was devoid of microplastics. Larger investigations are needed to validate these findings. In addition, there is an urgent need for research on the origins of microplastics ingested by humans, their potential intestinal absorption, and their effects on human health.

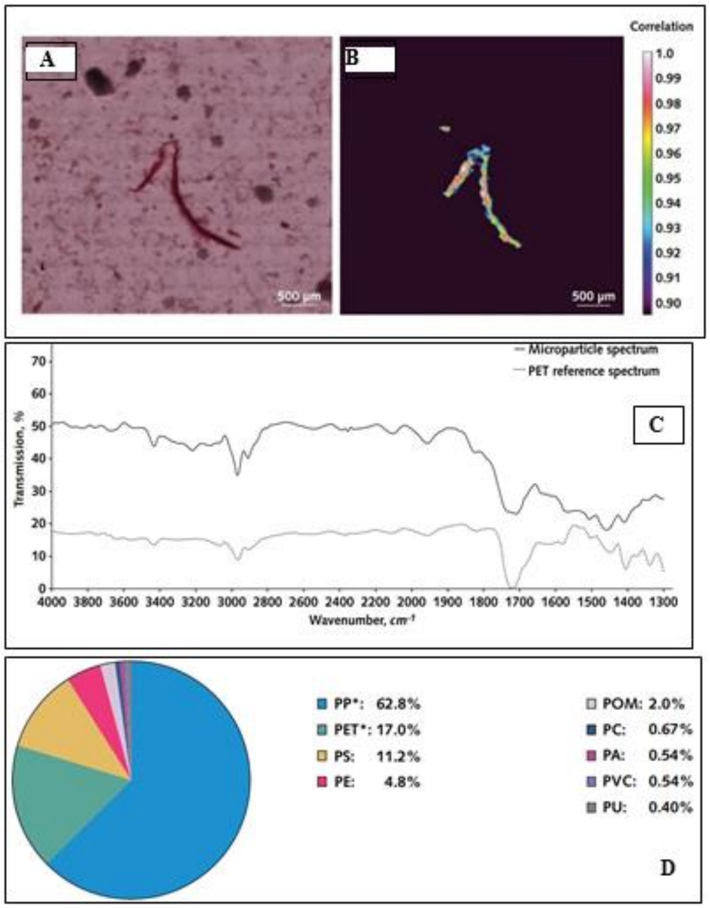

Figure 1: FT-IR microspectroscopy and spectral correlation analysis were used to characterize microplastics.

A. A microscopic image of random particles and a fiber that provides context.

B. The chemical composition of solids was determined using imaging mode FT-IR microspectroscopy. The correlation value with PET is displayed as a heat map, indicating the presence of 1 microplastic fiber and 1 fragment.

C. There was a strong correlation between the acquired IR spectrum and the PET reference spectrum.

D. Relative frequencies of nine microplastics detected in eight stool samples. * Present in all samples and accounting for nearly 80% of microplastics detected.

FT-IR- Fourier Transform Infrared; PA- Polyamide; PC- Polycarbonate; PE- Polyethylene; PET- Polyethylene Terephthalate; POM- Polyoxymethylene; PP- Polypropylene; PS- Polystyrene; PU- Polyurethane; PVC- Polyvinyl Chloride.

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