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2	Human exposure to microplastics: A study in Iran
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17 Abstract

Exposure of microplastics (MPs) to a cohort of adults of various demographics from different 18 regions of Iran has been quantitatively assessed. Specifically, MPs were retrieved from filtered 19 20 washes of the hand and face skin, head hair and saliva of individuals (n = 2000) after exposure periods of 24 h and were counted and characterised for shape-form and size microscopically. 21 22 A total of over 16,000 MPs were recorded in the study, with head hair returning the most samples (> 7000, or, on average, >3.5 MP per individual per day), saliva returning the least 23 samples (about 650, or on average 0.33 MP per individual), and MPs about twice as high in 24 25 males than females. The number of MPs was similar amongst residents of different urbanised regions but with evidence of greater quantities captured in more humid settings, and was 26 considerably lower in residents of a remote and sparsely populated area. Polyethylene-27 polyethylene terephthalate and polypropylene fibres of $< 100 \,\mu\text{m}$ in length, likely derived from 28 clothing and soft furnishings in the indoor setting and a wider range of sources in the exterior 29 environment, were the most abundant type of MP in all body receptors. Daily sampling of 30 receptors from six participants over a seven-day period revealed that, despite these broad 31 32 trends, both inter- and intra-individual exposure was highly heterogeneous. Although the present study has demonstrated the ubiquity of MP exposure the resulting impacts on human 33 health are unknown. 34

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36 Keywords: Microplastics; Human; Exposure; Hair, Skin; Saliva

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44 **1. Introduction**

Microplastics (MPs) have received considerable attention over the past two decades because of their presence in a wide variety of environments, including rivers and lakes, groundwater, the ocean, soils, the atmosphere and the household (Dris et al., 2017; Chae and An, 2018; Boucher et al., 2019; Kane and Clare, 2019; Panno et al., 2019). Ubiquitous contamination results from the wide use of plastics in society and industry and the persistence and ready transport of primary and secondary particles of sub-mm dimensions (Rezaei et al., 2019; Waldschläger et al., 2020).

52

53 Amongst the greatest concerns of MPs is human exposure and any consequent adverse impacts 54 on human health. Exposure may result from a variety of pathways but most attention has focused on the consumption of food and drink contaminated by MPs in the environment or 55 during storage (Iniguez et al., 2017; Li et al., 2018; Welle and Franz, 2018) and the inhalation 56 of fugitive atmospheric particles (Prata, 2018; Abbasi et al., 2019). Here, estimates of the 57 quantities and types of MP that are taken in are based on measurements in dietary components 58 like shellfish, salt and water and in interior and exterior air (Cox et al., 2019; Zhang et al., 59 2020). An alternative means of evaluating exposure, however, and one that could probe 60 61 influences of demographics, working practices and climate, for example, would be to measure 62 MPs in human body receptors, like hair and skin. These receptors can act as passive samplers that capture MPs from multiple sources and different pathways over a specific timeframe as 63 64 individuals go about their daily activities.

In the present study, human cohorts of males and females from different regions of Iran have been tested for MP exposure by counting particles associated with or accumulated by various receptors (head hair, hands, faces and saliva). The size and shape distributions of MPs have amongst participants and receptors have also been determined microscopically and the polymeric makeup of selected samples has been established by Raman spectroscopy.

71

72 **2. Material and methods**

73 2.1. Study area and sample cohort

In the current study, four contrasting regions in Iran were considered (see Figure 1). Namely,
the continental cities of Tehran and Shiraz (population ~ 8.7 million and 2 million, respectively,
climate cold and semi-arid and mild and semi-arid, respectively), the coastal port of Bushehr
(population 160,000, climate hot semi-arid), and the remote, agricultural village of Ghazghan
(population 2000, climate cold and dry).

79 Occupants of several thousand households were contacted and after sufficient positive responses were received research teams were deployed in each region. A total of 8000 samples 80 from head hair, hand skin, face skin and saliva were collected for microplastic analysis during 81 82 the dry season (August 2019). Specifically, 500 adults (250 males and 250 females and mostly working six to eight hours per day) from each region were sampled for the different receptors. 83 In addition, six people from Tehran (three male and three female of various occupations) were 84 sampled daily for MPs from their hair, face, hands and saliva for a continuous period of seven 85 86 days.

87

88 2.2. MP sampling

Samples were collected in wide-necked, screw-capped, silica glass bottles or jars that had been pre-cleaned by triplicate washes with vacuum-filtered tap water (in the laboratory or on site through 2 µm S&S blue band filters). For hand skin samples, participants were instructed to rinse their hands every six-eight hours over a period of 24 h in a few hundred mL of filtered water supplied in a 500 mL glass jar (Figure 2). For saliva samples, participants were instructed to rinse their mouths every six-eight hours over a period of 24 h using filtered water supplied in a glass bottle into a 250 mL jar.

For head hair (including head skin) and face skin samples, participants were instructed to wash 96 their hair-head and face at night and collect samples 24 hours later. Here, collection was 97 accomplished with the assistance of a researcher by washing the face (with cleaned hands) 98 using filtered water into a 2 L bottle through a custom-built, 35-cm diameter stainless steel 99 funnel before likewise washing head hair and collecting the sample. Between different samples, 100 101 funnels were washed with filtered water and during transportation between different 102 households were wrapped in aluminium foil. As controls (n = 30), 250 mL aliquots of filtered 103 water were collected in glass jars after processing them likewise.

104

105 2.3. Extraction and counting of MPs

In order to prevent MP contamination during sample manipulation in the laboratory, all reagents and water were filtered through 2 µm S&S blue band filters, working surfaces were thoroughly wiped with ethanol, and all glassware and plastic-ware were cleaned with filtered water. Windows and doors remained closed and white cotton laboratory coats, single-use latex gloves and facemasks were worn throughout.

111

For hand, face or hair samples that appeared turbid because of soil contamination arising from
agricultural practices, for example, bottles were opened and covered loosely with aluminium

114 foil before being transferred to a sand bath at 80°C. When the volume of water in each bottle had decreased to about 5 mL, bottles were removed from the sand bath and 35 mL of 35% 115 H₂O₂ (Arman Sina, Tehran) added to the contents for 2 to 10 d to remove organic matter. 116 Residual H₂O₂ solution was subsequently eliminated by further drying in the sand bath for 117 about 12 h. Fifty mL of a solution of ZnCl₂ solution and of density 1.6 g cm⁻³ was then added 118 to each bottle and the contents shaken for 5 min at 350 rpm before being allowed to settle for 119 90 min. The remaining supernatants were centrifuged in 50 mL polypropylene Falcon 120 centrifuge tubes for 3 min at 4000 rpm and then vacuum-filtered through 2 µm S&S blue band 121 filter papers before residues were rinsed with distilled water to prevent the formation of ZnCl₂ 122 crystals. In order to capture all MPs, the process of density separation, centrifuging, and 123 124 filtering (through the same filter) was repeated three times. For the majority of samples where contamination was not visible, and including the controls, bottle contents were vacuum-filtered 125 but not chemically processed. All filters were air-dried at room temperature in a glass cabinet 126 for a few days and subsequently transferred to Petri dishes for counting. 127

128

129 The contents of a random selection of filters (n = 50) were examined microscopically in order to evaluate the visual and physical characteristics of particles (e.g. shape, form, colour, gloss, 130 hardness, elasticity) that were associated with plastic and non-plastic materials (Abbasi et al., 131 2017). Thus, we employed binocular microscopy at up to $200 \times \text{magnification}$ (Carl-Zeiss, 132 Oberkochen, German), polarised light microscopy (Olympus BX41TF, Shinjuku, Japan) and 133 fluorescence microscopy using ultraviolet light with $200 \times \text{magnification}$ by the upright, 134 (Olympus CX31, Shinjuku, Japan). The polymeric composition of these particles was 135 determined using micro-Raman spectroscopy (µ-Raman-532-Ci, Avantes, Apeldoorn, 136 Netherland) with a laser of 785 nm and Raman shift of 400-1800 cm⁻¹. Here, MPs were attached 137 to microscope slides covered by double-sided adhesive tape. 138

140 Based on these characteristics, all filters were subsequently examined by binocular microscopy 141 in order to quantify the abundance of MPs with an approximate lower size limit of 5 μ m. 142 Particles were also classified according to colour (white-transparent, yellow-orange, red-pink, 143 blue-green or black-grey), shape (fiber, film, fragment or regular shape) and, with the aid of a 144 250 μ m probe and ImageJ software, size in terms of length or primary diameter as follows (L 145 $\leq 100 \mu$ m; $100 < L \le 250 \mu$ m; $250 < L \le 500 \mu$ m; L > 500 μ m).

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148 **3. Results**

149 *3.1. MP abundance and distribution*

150 Table 1 summarises the distribution of MPs counted according to region, sex and body receptor in terms of both numbers and percentages (note that no MPs were observed in the various 151 control filters). Thus, amongst the cohort of 2000 participants and 8000 samples, a total of over 152 153 16,000 MPs were counted according to the criteria above. Overall, MPs were most frequently 154 observed in hair samples (> 7000, or, on average, >3.5 MP per individual per day) and were least abundant in saliva (about 650, or on average 0.33 MP per individual). MPs were more 155 common amongst males than females (and in a ratio of about 2:1) with hair exhibiting the 156 biggest discrepancy in numbers between the sexes (and in a ratio of about 7.5:1). The total 157 number of MPs detected was considerably higher in residents from the urbanised regions (in 158 the approximate range 4000 to 6000) than in the village (< 800), and amongst the cities the 159 greatest number of MPs was encountered in Bushehr. 160

161

162 On an individual basis, there was considerable variability amongst participants. For instance, 163 in many cases no MPs were observed, especially in saliva samples, while in the hair of two 164 males and in the face skin of two females counts exceeded 50 per individual. The variability amongst individuals, and on the same individual, is evident in the results of the seven-day 165 samplings of six participants from Tehran (Figure 3). Thus, while the broad distributions and 166 relative abundances between the different receptors are consistent with those reported above, 167 some participants returned order of magnitude differences in the number of MPs in specific 168 receptors on consecutive days. While some differences were associated with the onset of the 169 170 weekend (days 6 and 7), others were observed without significantly altering lifestyle or any obvious source of exposure. 171

172

173 *3.2. MP characteristics*

Figure 4 exemplifies the types of MPs that were observed in the study and as captured by optical microscopy. Fibres ranged from small and relatively thick strands to thinner, longer and curled threads, some of which existed as coiled structures, and were usually black, white or transparent in colour. Regular shapes, including spheres and granular structures that are likely to be 'primary' in origin, and irregular shapes, consisting of flakes, fragments and films that are likely 'secondary' in origin, exhibited a broader range of colours.

Fibres were the most abundant type of MP observed overall (91.6%), with regular (primary) and irregular (secondary) MPs constituting 5.2% and 3.2% of the total count, respectively. In head hair and saliva, fibres constituted more than 97% of MPs counted in each location and for both sexes; lower percentages were observed for hand and face samples, and in particular for females where values of around 70% were returned for Tehran and Shiraz (Table 1).

185

186 Of the samples analysed by micro-Raman spectroscopy, 62 were fibres and were constructed 187 of polyethylene or polyethylene terephthalate (n = 35), polypropylene (n = 23), polystyrene (n

188 = 3) or polyvinyl chloride (n = 1), eight were primary particles of a spherical or hexagonal 189 shape and were constructed of polyethylene-polyethylene terephthalate (n = 2), polypropylene 190 (n = 5) or polystyrene (n = 1), and six were secondary fragments and were constructed of 191 polyethylene-polyethylene terephthalate (n = 3) or polypropylene (n = 3).

192

The percentage size distributions of MPs in face and hand skin, hair and saliva, shown in Figure 193 194 5 for each region sampled, reveal a decrease in MP abundance with increasing size range in all cases. For hand and face skin, pooled together here, about 60% and 25% of MPs are found in 195 196 the L < 100 μ m and L = 100 - 250 μ m ranges, respectively, with contributions of < 20% arising 197 from larger particles. For head hair, about 40% and 30% of MPs are found in the L $< 100 \mu m$ and $L = 100 - 250 \mu m$ ranges, respectively, with remaining contributions resulting from larger 198 particles. In saliva, between 76% and 94% of MPs were encountered in the $L < 100 \mu m$ size 199 fraction, with contributions from other individual size ranges never exceeding 13%. 200

201

202 **4. Discussion**

The findings of the present study are perhaps not surprising given the ubiquity of MPs in the indoor and exterior environments and in commodities that are widely used or worn. Nevertheless, the results are significant in demonstrating both the nature and heterogeneity of human exposure to MPs from different routes.

207

Regarding the indoor setting, common sources of synthetic microfibrous particles include soft furnishings and items of clothing, with a recent study showing that the release of fibres to air from garment wear is of equal importance to fibre emission to water during laundering activities (De Falco et al., 2020). In the exterior setting, MP deposition from the atmosphere has been reported to be as high as $1000 \text{ m}^{-2} \text{ d}^{-1}$ in urban settings, with the dominant type of a fibrous nature and likely to be derived from textile clothing (Liu et al., 2019; Wright et al., 2020). In more remote regions, there are fewer direct sources of airborne MPs but there may be important contributions from fine (e.g., urban) particulates that have been transported long distances with air masses (Allen et al., 2019). This suggests that, more generally, exposure to exterior, atmospheric MPs may be significant from local, regional and inter-regional sources.

The ubiquity of airborne MPs of a fibrous nature, and constructed principally from 219 polyethylene-polyethylene terephthalate and polypropylene, accounts for the widespread 220 221 occurrence of microfibers retrieved from the hair of participants throughout the current study. Presumably, the horizontal orientation of the head and the high surface area and tortuosity of 222 hair and its propensity to acquire electrostatic charge are highly effective in intercepting and 223 224 trapping microfibers of a range of sizes from both interior and external settings. These properties, coupled with fibres that are readily shed from certain garments, also enable fibres 225 to be readily transferred to hair when dressing or undressing or while leaning-resting on 226 227 furnishings constructed of synthetic textiles.

228

229 The wearing of headgear, and in particular veils by Muslim women, may act either as a direct source of MPs to head hair if constructed of synthetic material or as a shield from airborne 230 MPs if constructed of natural material. Lower overall quantities of MPs observed in the head 231 232 hair of females than in head hair of males observed throughout the present study (see Table 1, and $p < 10^{-3}$ according to an independent *t*-test) likely reflects the dominant use of cotton in 233 the manufacture of contemporary Muslim veils. The removal of veils during time spent 234 235 indoors at weekends also accounts for the highest concentrations of MPs in female head hair observed on days 6 and 7 of the timed data in Figure 3. 236

237

The more general heterogeneity of the results reflects variations among individuals and families regions that include daily activities and habits, places of work, clothing type, and household furnishings and cleaning frequency. Climatic factors may also play a role in regional differences of MP concentrations in head hair. Specifically, the greatest number of particles reported for residents of Bushehr may be attributed to the more humid conditions encountered here that promote the adhesion of MPs to hair and other human receptors.

244

The size range of particles examined in the present study (above a few µm) is too large to 245 enable penetration through human skin via hair follicles or exits of sweat glands (Schneider et 246 al., 2009). However, and despite a different orientation to the nose and mouth, a similar height 247 means the capture of MPs on the head could be a proxy for exposure to MPs that have the 248 potential to be inhaled. Significantly, fibrous particles of a few tens of µm in length and towards 249 the lower end of the size range reported in this study appear to be able to avoid mucociliary 250 clearance and deposit in the deep lung (Pauly et al., 1998; Gasperi et al., 2018), with larger 251 252 particles cleared in the upper airways and exposed the digestive tract.

253

Using the reasoning above, the vertical orientation of the face and (usually) lower coverage of 254 hair than on the head results in lower quantities of fibrous MPs in this receptor. However, in 255 female participants there was a higher percentage of relatively small (L $< 100 \mu$ m) non-fibrous 256 (primary and secondary) particles on the face. This observation is consistent with the 257 application of facial exfoliates by many female participants (including F1 in Figure 3) that 258 contain high concentrations of more regularly shaped (e.g. granular) microplastic abrasive 259 agents of dimensions typically less than a few hundred µm (Cheung and Fok, 2017; Praveena 260 et al., 2018). Other potential sources of non-fibrous facial MPs include glitters and various 261 decorative polyesters that are added to specialist contemporary make-ups (Yurtsever, 2019). 262

264

abundance of MPs that was lower than that for head hair but similar to that returned by face skin. This is because typical hand activities are unlikely to result in a net accumulation of MPs but rather their transfer between body receptors or between handled surfaces. Overall, hand skin returned the lowest percentage of fibrous particles amongst the receptors, presumably because of the larger diversity of MP-generating materials handled both indoors and outdoors than is in suspension in and intercepted from the atmosphere.

271

Amongst the receptors, saliva was found to contain the fewest number of MPs, the greatest 272 percentage of fibrous material and, according to a Kruskal-Wallis test and an α value of 0.05, 273 the smallest sized particles. MPs can enter the oral cavity through inhalation, intake of food 274 and drink that is contaminated in the environment (Seth and Shriwastav, 2018), by processing, 275 packaging or storage (Ossmann et al., 2018) or from atmospheric deposition during preparation 276 and consumption (Schwabl et al., 2019; Zhang et al., 2020), and hand-to-mouth activities 277 278 involving food or resulting from habit (Hauptman and Woolf, 2017). It is also possible that, in 279 some participants, non-fibrous fragments of MPs are sourced from polyethylene particles in toothpaste (Ustabasi and Baysal, 2019) or derived from the wearing down of plastic-resin or 280 plastic-ceramic composite dental fillings (Borrero-Lopez et al., 2019). Regardless of the origins 281 of MPs observed in this receptor, our quantitative data provide only a snapshot of abundance 282 as saliva is continuously produced and swallowed. However, the detection of MPs here is 283 significant as it confirms that ingestion is an important route of human exposure (Cox et al., 284 2019; Schwabl et al., 2019) and one that appears to be independent of age, sex, environment 285 and working practices. Moreover, a size distribution in saliva that is distinctly different to that 286 representative of exposure to other receptors suggests that there is some means of selectively 287

ingesting smaller, fibrous MPs, or that larger particles are more readily eliminated from theoral cavity into the digestive tract.

290 Despite heterogeneous exposure to environmental, consumer and cosmetic MPs by different pathways, acute and chronic effects, from transit through the digestive tract and entrapment in 291 292 the deep lung, for example, are unknown. Regarding the latter, at sufficiently high levels it is 293 anticipated that lung inflammation would occur, and that this in turn could lead to formation 294 of reactive oxygen species and secondary effects (Gaspari et al., 2018). Any impacts could also be compounded by the mobilisation of toxic chemicals, including metals, metalloids and 295 296 hydrophobic organic pollutants, from MPs seated in the lung. These chemicals may form an intrinsic component of the polymer itself, like unreacted monomers, additives or catalytic 297 residues (e.g. antimony trioxide in polyester), or have been acquired from the external 298 environment (e.g. vehicular emissions) or the interior setting (e.g. brominated flame 299 300 retardants).

301

302 **5. Conclusions**

This study has shown that the exposure of MPs to humans is ubiquitous but heterogeneous in both space and time, with the hair, skin and mouth all acting as important passive receptors. The majority of MPs are fine (< 100 μ m) fibres constructed of polyethylene-polyethylene terephthalate and polypropylene that appear to be derived from both textiles (clothing and furnishings) and a range of sources in the exterior environment. Despite their pervasiveness, however, the acute and chronic health impacts of these particles is unknown.

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310

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435	Figure 1	1. Locations	of the fo	ur study	areas in	Iran.
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Figure 2. An illustration of the sampling protocols for head hair, face and hand skin and saliva.



453 Figure 3. The number of MPs recorded in the different receptors of six individuals from Tehran454 (three male, M, and three female, F) over a continuous seven day period.

461 Figure 4. Microscopic images of various fibrous MPs, primary MPs and secondary MPs

463

⁴⁶² recovered from individuals in the present study.

- **Figure 5.** Size distribution (in μ m) of MPs in skin (face-hand), hair and saliva in the different
- 466 geographical regions sampled.



Table 1: Numbers (n) and percentages (%) of total MPs for the different body receptors
amongst the 250 male (M) and 250 female (F) participants from each region of Iran. Also
shown is the percentage of MPs that were fibrous in nature (% fibres).

		face skin			hand skin		hair			saliva			total	
		n	%	% fibres	n	%	% fibres	n	%	% fibres	n	%	% fibres	n
Tehran	Μ	524	16.2	96.4	745	23.0	89.1	1896	58.6	97.8	72	2.2	99.8	3237
	F	851	47.7	72.6	557	31.2	71.3	235	13.2	98.1	142	8.0	98.1	1785
Shiraz	М	463	16.3	97.2	695	24.5	88.5	1598	56.2	98.1	86	3.0	99.9	2842
	F	633	46.4	69.9	369	27.1	71.4	199	14.6	97.3	162	11.9	97.2	1363
Bushehr	М	765	17.2	98.1	874	19.6	86.2	2754	61.8	98.3	62	1.4	100.0	4455
	F	874	44.5	87.3	624	31.8	83.2	415	21.1	97.7	51	2.6	98.2	1964
Ghazghan	М	121	18.7	99.2	142	21.9	91.3	342	52.9	96.9	42	6.5	100.0	647
	F	34	26.2	94.1	45	34.6	90.3	23	17.7	97.9	28	21.5	99.9	130
total	М	1873	16.8	97.5	2456	22.0	88.0	6590	58.9	98.0	262	2.3	99.9	11181
	F	2392	45.6	77.6	1595	30.4	76.5	872	16.6	97.7	383	7.3	97.9	5242