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Human exposure to microplastics: A study in Iran

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17 **Abstract**

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

35

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

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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).

Amongst the greatest concerns of MPs is human exposure and any consequent adverse impacts 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 during storage (Iniguez et al., 2017; Li et al., 2018; Welle and Franz, 2018) and the inhalation of fugitive atmospheric particles (Prata, 2018; Abbasi et al., 2019). Here, estimates of the quantities and types of MP that are taken in are based on measurements in dietary components like shellfish, salt and water and in interior and exterior air (Cox et al., 2019; Zhang et al., 2020). An alternative means of evaluating exposure, however, and one that could probe influences of demographics, working practices and climate, for example, would be to measure 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 individuals go about their daily activities.

66 In the present study, human cohorts of males and females from different regions of Iran have
67 been tested for MP exposure by counting particles associated with or accumulated by various
68 receptors (head hair, hands, faces and saliva). **The size and shape distributions of MPs have**
69 **amongst participants and receptors have also been determined microscopically** and the
70 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*

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

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

87

88 *2.2. MP sampling*

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

96 For head hair (**including head skin**) and face skin samples, participants were instructed to wash
97 their hair-head and face at night and collect samples 24 hours later. Here, collection was
98 accomplished with the assistance of a researcher by washing the face (with cleaned hands)
99 using filtered water into a 2 L bottle through a custom-built, 35-cm diameter stainless steel
100 funnel before likewise washing head hair and collecting the sample. Between different samples,
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*

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

111

112 For hand, face or hair samples that appeared turbid **because of soil contamination arising from**
113 **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
115 had decreased to about 5 mL, bottles were removed from the sand bath and 35 mL of 35%
116 H₂O₂ (Arman Sina, Tehran) added to the contents for 2 to 10 d to remove organic matter.
117 Residual H₂O₂ solution was subsequently eliminated by further drying in the sand bath for
118 about 12 h. Fifty mL of a solution of ZnCl₂ solution and of density 1.6 g cm⁻³ was then added
119 to each bottle and the contents shaken for 5 min at 350 rpm before being allowed to settle for
120 90 min. The remaining supernatants were centrifuged in 50 mL polypropylene Falcon
121 centrifuge tubes for 3 min at 4000 rpm and then vacuum-filtered through 2 µm S&S blue band
122 filter papers before residues were rinsed with distilled water to prevent the formation of ZnCl₂
123 crystals. In order to capture all MPs, the process of density separation, centrifuging, and
124 filtering (through the same filter) was repeated three times. For the majority of samples where
125 contamination was not visible, and including the controls, bottle contents were vacuum-filtered
126 but not chemically processed. All filters were air-dried at room temperature in a glass cabinet
127 for a few days and subsequently transferred to Petri dishes for counting.

128
129 The contents of a random selection of filters (*n* = 50) were examined microscopically in order
130 to evaluate the visual and physical characteristics of particles (e.g. shape, form, colour, gloss,
131 hardness, elasticity) that were associated with plastic and non-plastic materials (Abbasi et al.,
132 2017). Thus, we employed binocular microscopy at up to 200 × magnification (Carl-Zeiss,
133 Oberkochen, German), polarised light microscopy (Olympus BX41TF, Shinjuku, Japan) and
134 fluorescence microscopy using ultraviolet light with 200 × magnification by the upright,
135 (Olympus CX31, Shinjuku, Japan). The polymeric composition of these particles was
136 determined using micro-Raman spectroscopy (µ-Raman-532-Ci, Avantes, Apeldoorn,
137 Netherland) with a laser of 785 nm and Raman shift of 400-1800 cm⁻¹. Here, MPs were attached
138 to microscope slides covered by double-sided adhesive tape.

139

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 μm .
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 μm probe and ImageJ software, size in terms of length or primary diameter as follows (L
145 $\leq 100 \mu\text{m}$; $100 < L \leq 250 \mu\text{m}$; $250 < L \leq 500 \mu\text{m}$; $L > 500 \mu\text{m}$).

146

147

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
151 in terms of both numbers and percentages (note that no MPs were observed in the various
152 control filters). Thus, amongst the cohort of 2000 participants and 8000 samples, a total of over
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
155 least abundant in saliva (about 650, or on average 0.33 MP per individual). MPs were more
156 common amongst males than females (and in a ratio of about 2:1) with hair exhibiting the
157 biggest discrepancy in numbers between the sexes (and in a ratio of about 7.5:1). The total
158 number of MPs detected was considerably higher in residents from the urbanised regions (in
159 the approximate range 4000 to 6000) than in the village (< 800), and amongst the cities the
160 greatest number of MPs was encountered in Bushehr.

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
165 amongst individuals, and on the same individual, is evident in the results of the seven-day
166 samplings of six participants from Tehran (Figure 3). Thus, while the broad distributions and
167 relative abundances between the different receptors are consistent with those reported above,
168 some participants returned order of magnitude differences in the number of MPs in specific
169 receptors on consecutive days. While some differences were associated with the onset of the
170 weekend (days 6 and 7), others were observed without significantly altering lifestyle or any
171 obvious source of exposure.

172

173 *3.2. MP characteristics*

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

180 **Fibres were the most abundant type of MP observed overall (91.6%), with regular (primary)**
181 **and irregular (secondary) MPs constituting 5.2% and 3.2% of the total count, respectively. In**
182 head hair and saliva, fibres constituted more than 97% of MPs counted in each location and for
183 both sexes; lower percentages were observed for hand and face samples, and in particular for
184 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

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

201

202 **4. Discussion**

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

207

208 Regarding the indoor setting, common sources of synthetic microfibrinous particles include soft
209 furnishings and items of clothing, with a recent study showing that the release of fibres to air
210 from garment wear is of equal importance to fibre emission to water during laundering
211 activities (De Falco et al., 2020). In the exterior setting, MP deposition from the atmosphere
212 has been reported to be as high as $1000 \text{ m}^{-2} \text{ d}^{-1}$ in urban settings, with the dominant type of a

213 fibrous nature and likely to be derived from textile clothing (Liu et al., 2019; Wright et al.,
214 2020). In more remote regions, there are fewer direct sources of airborne MPs but there may
215 be important contributions from fine (e.g., urban) particulates that have been transported long
216 distances with air masses (Allen et al., 2019). This suggests that, more generally, exposure to
217 exterior, atmospheric MPs may be significant from local, regional and inter-regional sources.

218

219 The ubiquity of airborne MPs of a fibrous nature, and constructed principally from
220 polyethylene-polyethylene terephthalate and polypropylene, accounts for the widespread
221 occurrence of microfibers retrieved from the hair of participants throughout the current study.
222 Presumably, the horizontal orientation of the head and the high surface area and tortuosity of
223 hair and its propensity to acquire electrostatic charge are highly effective in intercepting and
224 trapping microfibers of a range of sizes from both interior and external settings. These
225 properties, coupled with fibres that are readily shed from certain garments, also enable fibres
226 to be readily transferred to hair when dressing or undressing or while leaning-resting on
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
230 source of MPs to head hair if constructed of synthetic material or as a shield from airborne
231 MPs if constructed of natural material. Lower overall quantities of MPs observed in the head
232 hair of females than in head hair of males observed throughout the present study (see Table 1,
233 and $p < 10^{-3}$ according to an independent *t*-test) likely reflects the dominant use of cotton in
234 the manufacture of contemporary Muslim veils. The removal of veils during time spent
235 indoors at weekends also accounts for the highest concentrations of MPs in female head hair
236 observed on days 6 and 7 of the timed data in Figure 3.

237

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

244

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

253

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

263

264 Despite being in direct contact with multiple sources of MPs, hand skin displayed a relative
265 abundance of MPs that was lower than that for head hair but similar to that returned by face
266 skin. This is because typical hand activities are unlikely to result in a net accumulation of MPs
267 but rather their transfer between body receptors or between handled surfaces. Overall, hand
268 skin returned the lowest percentage of fibrous particles amongst the receptors, presumably
269 because of the larger diversity of MP-generating materials handled both indoors and outdoors
270 than is in suspension in and intercepted from the atmosphere.

271

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

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

290 Despite heterogeneous exposure to environmental, consumer and cosmetic MPs by different
291 pathways, acute and chronic effects, from transit through the digestive tract and entrapment in
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
295 be compounded by the mobilisation of toxic chemicals, including metals, metalloids and
296 hydrophobic organic pollutants, from MPs seated in the lung. These chemicals may form an
297 intrinsic component of the polymer itself, like unreacted monomers, additives or catalytic
298 residues (e.g. antimony trioxide in polyester), or have been acquired from the external
299 environment (e.g. vehicular emissions) or the interior setting (e.g. brominated flame
300 retardants).

301

302 **5. Conclusions**

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

309

310

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317

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435 **Figure 1.** Locations of the four study areas in Iran.

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448 **Figure 2.** An illustration of the sampling protocols for head hair, face and hand skin and saliva.

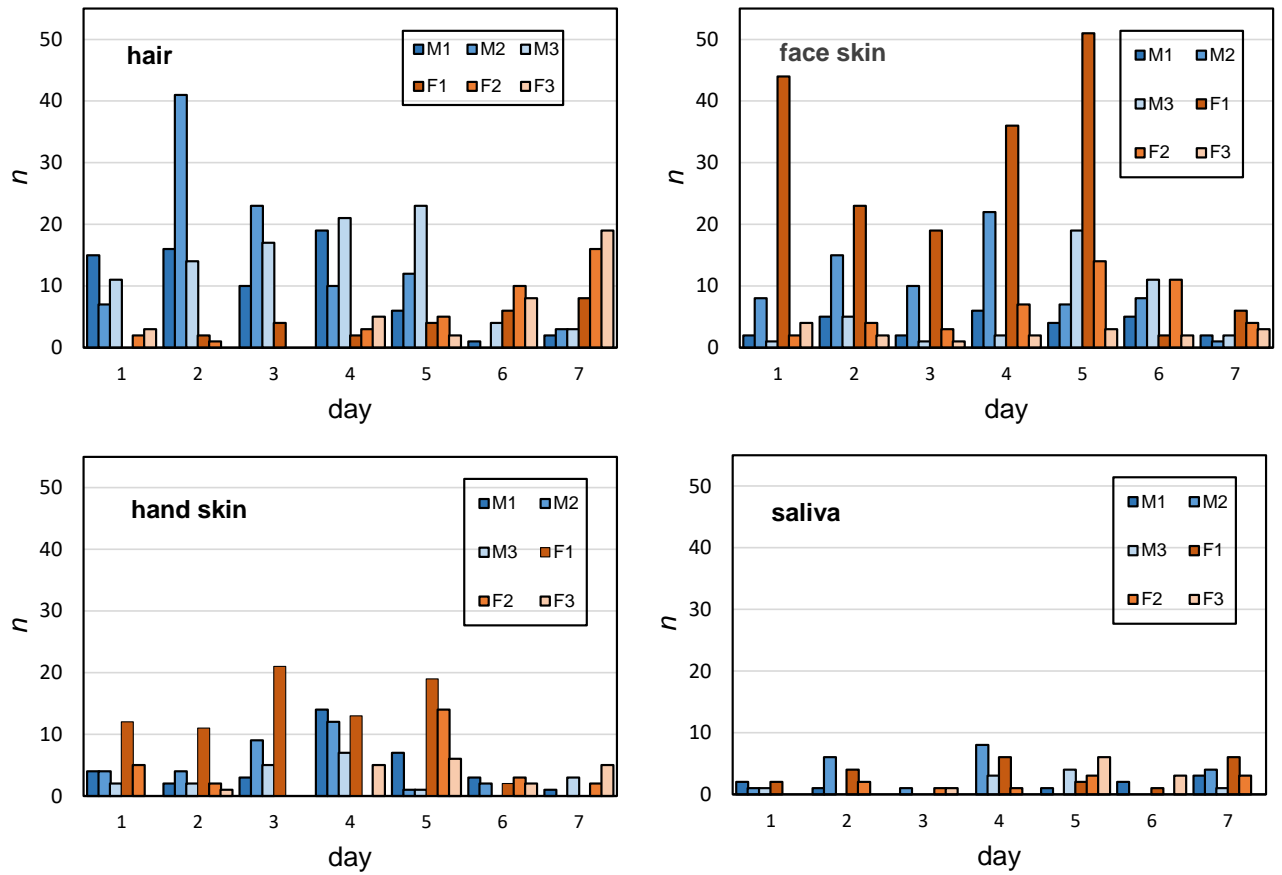
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453 **Figure 3.** The number of MPs recorded in the different receptors of six individuals from Tehran
 454 (three male, M, and three female, F) over a continuous seven day period.



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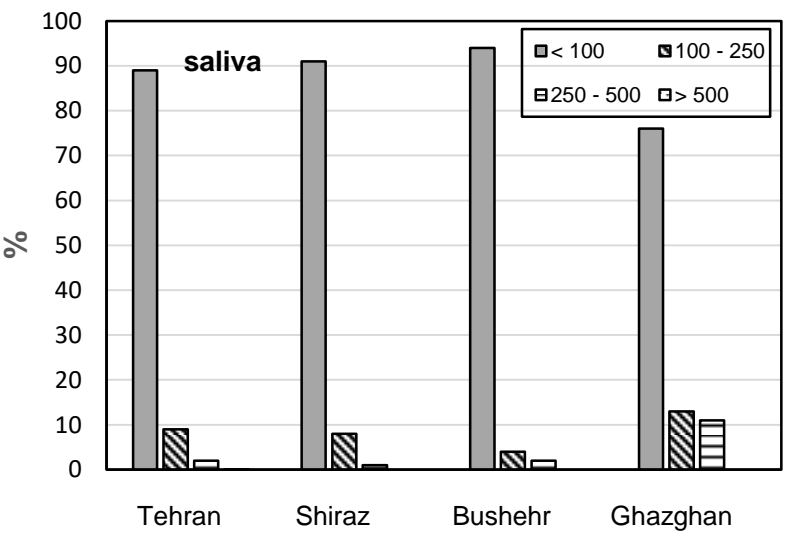
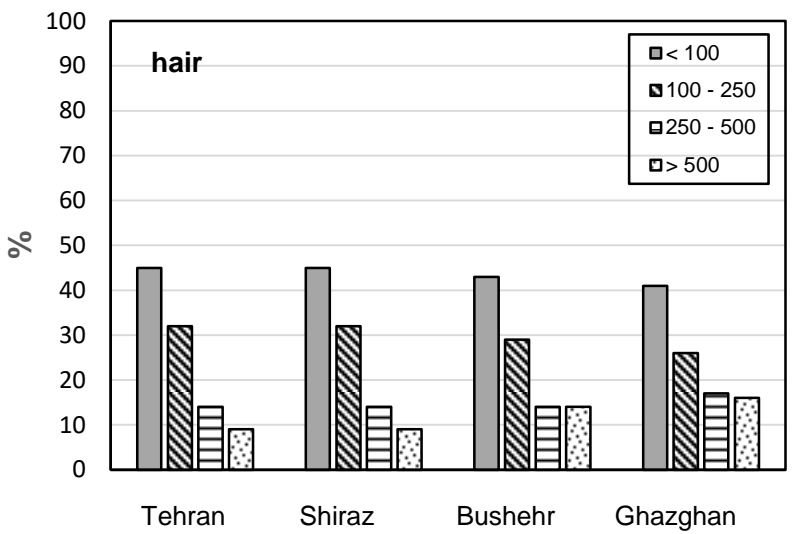
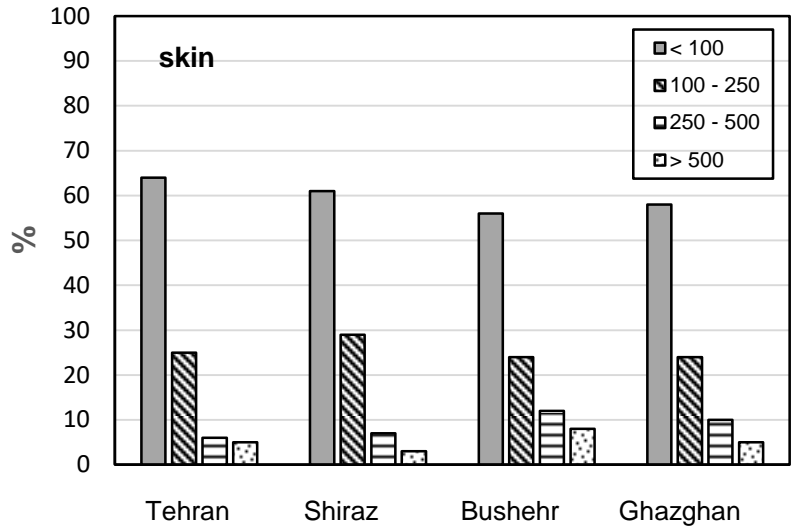
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461 **Figure 4.** Microscopic images of various fibrous MPs, primary MPs and secondary MPs
462 recovered from individuals in the present study.

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



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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
		n	%	% fibres	n	%	% fibres	n	%	% fibres	n	%	% fibres	
Tehran	M	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	M	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	M	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	M	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	M	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

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