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Human exposure to microplastics: A study in Iran

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Abstract

Exposure of microplastics (MPs) to a cohort of adults of various demographics from different regions of Iran has been quantitatively assessed. Specifically, MPs were retrieved from filtered 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. 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 samples (about 650, or on average 0.33 MP per individual), and MPs about twice as high in 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 considerably lower in residents of a remote and sparsely populated area. Polyethylene-polyethylene terephthalate and polypropylene fibres of < 100 μm in length, likely derived from clothing and soft furnishings in the indoor setting and a wider range of sources in the exterior environment, were the most abundant type of MP in all body receptors. Daily sampling of receptors from six participants over a seven-day period revealed that, despite these broad 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 health are unknown.

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

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.

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.

2. Material and methods

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).

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 from head hair, hand skin, face skin and saliva were collected for microplastic analysis during 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. In addition, six people from Tehran (three male and three female of various occupations) were sampled daily for MPs from their hair, face, hands and saliva for a continuous period of seven days.

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

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.

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 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% H_2O_2 (Arman Sina, Tehran) added to the contents for 2 to 10 d to remove organic matter. Residual H_2O_2 solution was subsequently eliminated by further drying in the sand bath for about 12 h. Fifty mL of a solution of $ZnCl_2$ solution and of density 1.6 g cm^{-3} was then added to each bottle and the contents shaken for 5 min at 350 rpm before being allowed to settle for 90 min. The remaining supernatants were centrifuged in 50 mL polypropylene Falcon centrifuge tubes for 3 min at 4000 rpm and then vacuum-filtered through 2 µm S&S blue band filter papers before residues were rinsed with distilled water to prevent the formation of $ZnCl_2$ crystals. In order to capture all MPs, the process of density separation, centrifuging, and 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 but not chemically processed. All filters were air-dried at room temperature in a glass cabinet for a few days and subsequently transferred to Petri dishes for counting.

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, hardness, elasticity) that were associated with plastic and non-plastic materials (Abbasi et al., 2017). Thus, we employed binocular microscopy at up to 200 × magnification (Carl-Zeiss, Oberkochen, German), polarised light microscopy (Olympus BX41TF, Shinjuku, Japan) and fluorescence microscopy using ultraviolet light with 200 × magnification by the upright, (Olympus CX31, Shinjuku, Japan). The polymeric composition of these particles was determined using micro-Raman spectroscopy (µ-Raman-532-Ci, Avantes, Apeldoorn, Netherland) with a laser of 785 nm and Raman shift of 400-1800 cm^{-1}. Here, MPs were attached to microscope slides covered by double-sided adhesive tape.

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

3. Results

3.1. MP abundance and distribution

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 control filters). Thus, amongst the cohort of 2000 participants and 8000 samples, a total of over 16,000 MPs were counted according to the criteria above. Overall, MPs were most frequently 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 common amongst males than females (and in a ratio of about 2:1) with hair exhibiting the biggest discrepancy in numbers between the sexes (and in a ratio of about 7.5:1). The total number of MPs detected was considerably higher in residents from the urbanised regions (in the approximate range 4000 to 6000) than in the village (< 800), and amongst the cities the greatest number of MPs was encountered in Bushehr.

On an individual basis, there was considerable variability amongst participants. For instance, in many cases no MPs were observed, especially in saliva samples, while in the hair of two 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 samplings of six participants from Tehran (Figure 3). Thus, while the broad distributions and relative abundances between the different receptors are consistent with those reported above, some participants returned order of magnitude differences in the number of MPs in specific receptors on consecutive days. While some differences were associated with the onset of the weekend (days 6 and 7), others were observed without significantly altering lifestyle or any obvious source of exposure.

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).

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

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

The percentage size distributions of MPs in face and hand skin, hair and saliva, shown in Figure 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 the L < 100 μm and L = 100 - 250 μm ranges, respectively, with contributions of < 20% arising from larger particles. For head hair, about 40% and 30% of MPs are found in the L < 100 μm and L = 100 - 250 μm ranges, respectively, with remaining contributions resulting from larger particles. In saliva, between 76% and 94% of MPs were encountered in the L < 100 μm size fraction, with contributions from other individual size ranges never exceeding 13%.

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.

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 m^{-2} 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 polyethylene-polyethylene terephthalate and polypropylene, accounts for the widespread 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 hair and its propensity to acquire electrostatic charge are highly effective in intercepting and 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 to be readily transferred to hair when dressing or undressing or while leaning-resting on furnishings constructed of synthetic textiles.

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 MPs if constructed of natural material. Lower overall quantities of MPs observed in the head 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 the manufacture of contemporary Muslim veils. The removal of veils during time spent 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.

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.

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

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

Despite being in direct contact with multiple sources of MPs, hand skin displayed a relative 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.

Amongst the receptors, saliva was found to contain the fewest number of MPs, the greatest percentage of fibrous material and, according to a Kruskal-Wallis test and an α value of 0.05, the smallest sized particles. MPs can enter the oral cavity through inhalation, intake of food and drink that is contaminated in the environment (Seth and Shriwastav, 2018), by processing, packaging or storage (Ossmann et al., 2018) or from atmospheric deposition during preparation and consumption (Schwabl et al., 2019; Zhang et al., 2020), and hand-to-mouth activities involving food or resulting from habit (Hauptman and Woolf, 2017). It is also possible that, in 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 plastic-ceramic composite dental fillings (Borrero-Lopez et al., 2019). Regardless of the origins of MPs observed in this receptor, our quantitative data provide only a snapshot of abundance as saliva is continuously produced and swallowed. However, the detection of MPs here is significant as it confirms that ingestion is an important route of human exposure (Cox et al., 2019; Schwabl et al., 2019) and one that appears to be independent of age, sex, environment and working practices. Moreover, a size distribution in saliva that is distinctly different to that representative of exposure to other receptors suggests that there is some means of selectively ingesting smaller, fibrous MPs, or that larger particles are more readily eliminated from the oral cavity into the digestive tract.

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 the deep lung, for example, are unknown. Regarding the latter, at sufficiently high levels it is anticipated that lung inflammation would occur, and that this in turn could lead to formation 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 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 residues (e.g. antimony trioxide in polyester), or have been acquired from the external environment (e.g. vehicular emissions) or the interior setting (e.g. brominated flame retardants).

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 μm) 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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Figure 1. Locations of the four study areas in Iran.

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

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

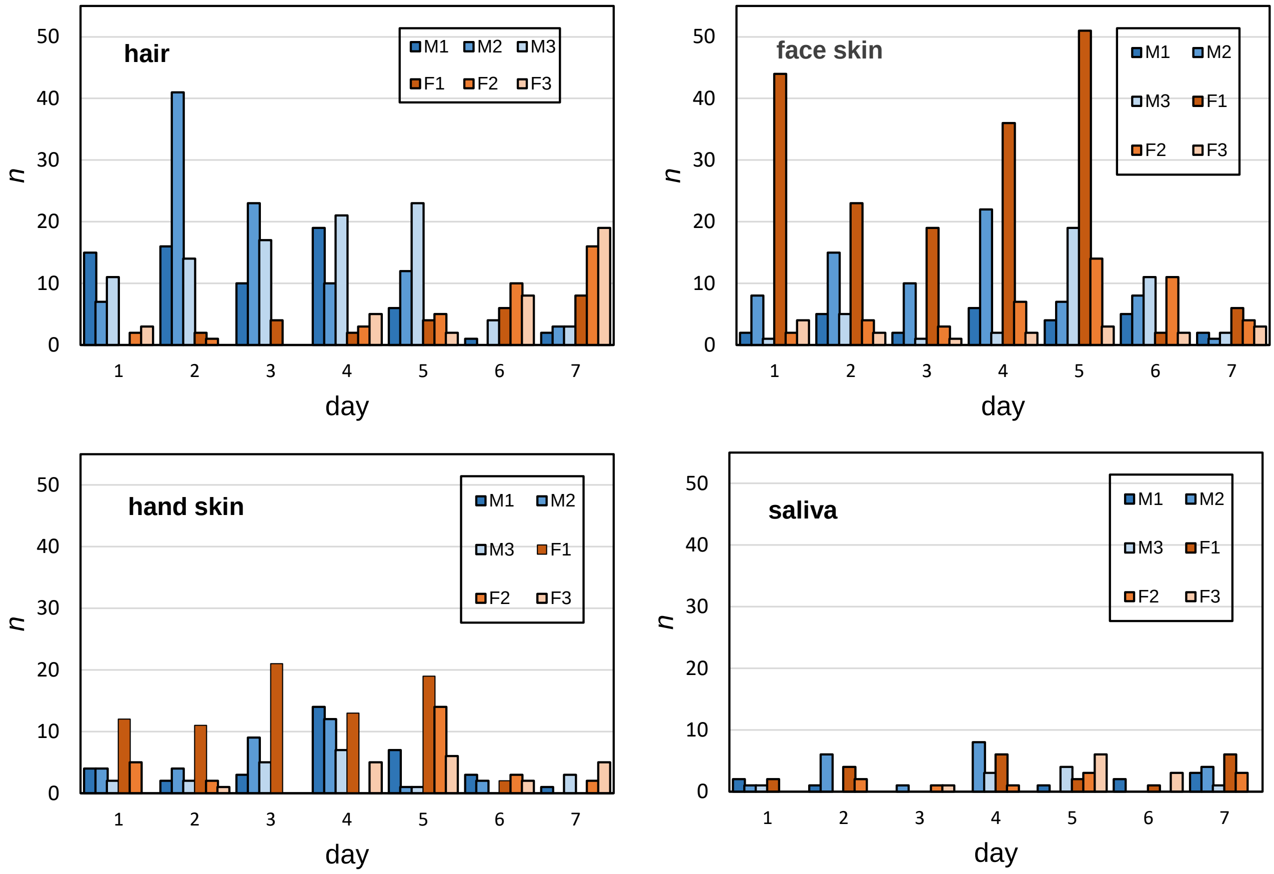

Figure 4. Microscopic images of various fibrous MPs, primary MPs and secondary MPs recovered from individuals in the present study.

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

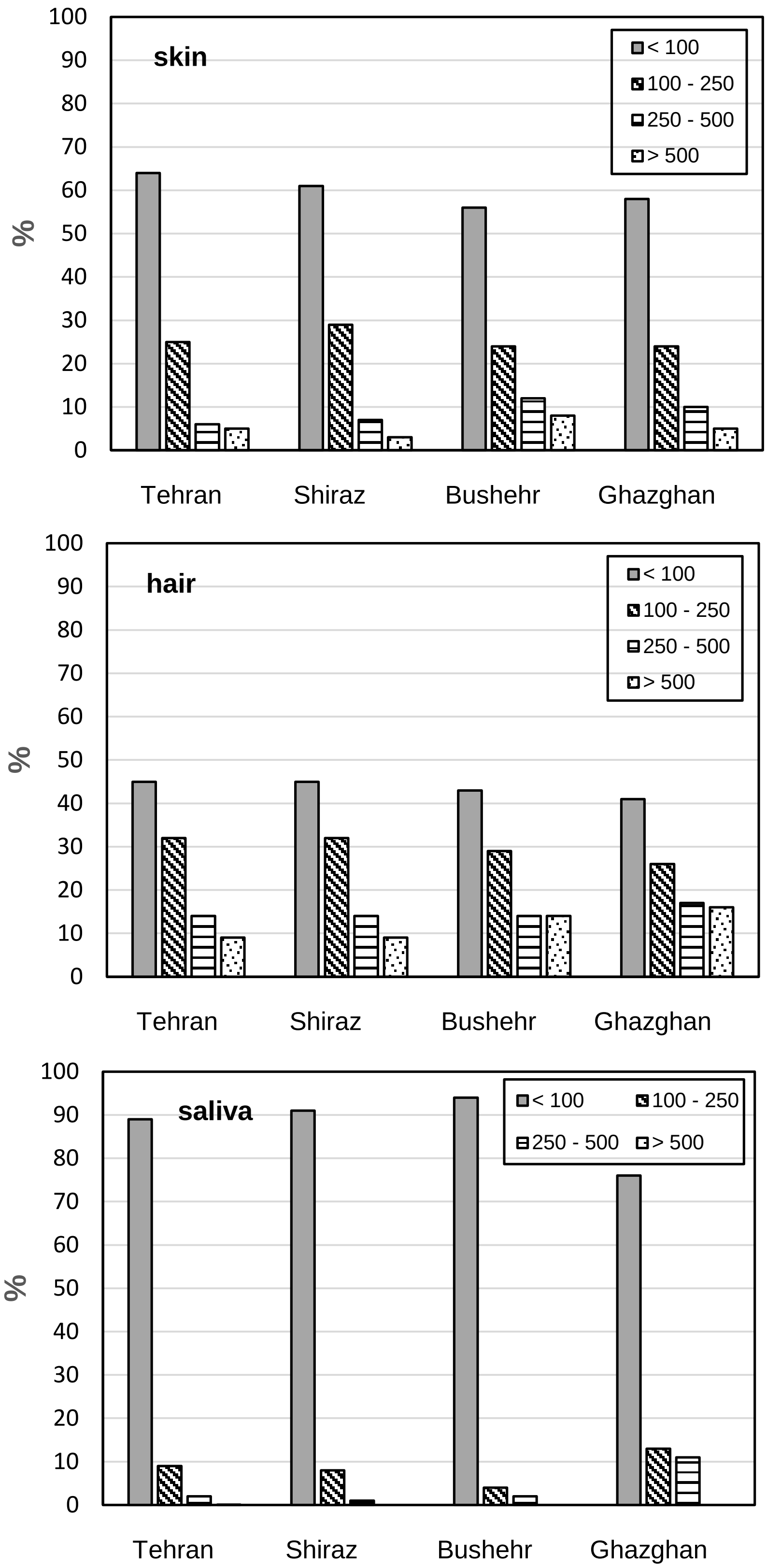
skin
hair
saliva
%
< 100
100 - 250
250 - 500
> 500
Tehran
Shiraz
Bushehr
Ghazghan
0
10
20
30
40
50
60
70
80
90
100

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	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