Microplastic Pollution in The Topsoil of Hot And Dried Areas, Human Exposure and Source Assessment, Aghili Plain, Iran

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Abstract

Although the distribution of microplastic (MPs) has been studied in different environmental compartments during the last decade, there is still a knowledge gap in their distribution and abundances in soil. This study aimed to investigate the abundance, distribution, and type of MPs in the soil of Aghili plain as a hot and dry area in southwest of Iran. In this study, composite soil samples (n=102) were collected from the residential and agricultural areas in Aghili plain, Iran. A combination of visual observations, Scanning Electron Microscopy (SEM) – Energy-dispersive X-ray spectroscopy (EDS), and Raman micro-spectroscopy was conducted to quantify and characterize MPs in soil samples. The intakes of MPs in adults and children were estimated through two exposure EPA scenarios. The total MPs loading in the studies soil was 11.93 ± 0.9 items in Kg^{-1} of surface soil in Aghili plain. The MPs had various morphology (fiber, pellet, fragment, and spherule shapes), colors (white-transparent, yellow-orange, red-pink, blue-green and black-grey colors), and sizes (<100 µm up to 1000 ≤ µm). Black-grey fibers in size less than 100 µm were dominant MPs in soil samples using a binocular microscope. Trace amounts of C, N, O, Na, P, Si revealed by EDS suggested the organic and inorganic contaminations on the surface of identified MPs. Intake of MPs per day/year through ingestion of polluted soil was calculated. Mean normal and acute exposure was estimated at 0.435 and 0.871 MPs per year through ingestion by children and adults, respectively.

Introduction

Microplastics (MPs) generally are categorized as particles with nano (<100 nm in one dimension) and micro (100 nm to 5 mm) sizes, which can be uptake by many organisms due to their small size and ubiquitous distribution in the environment. There are considerable concerns about MPs' potential biomagnification and bioaccumulation in marine and terrestrial biotas (Ng et al., 2018b). Many studies in aquatic environments recognized the potentially harmful impacts of MPs on marine organisms (Cole et al., 2011, Costa and Barletta, 2015, Ding et al., 2018, Everaert et al., 2018, Abbasi et al., 2018, Galloway et al., 2017, Du et al., 2020) and translocation beyond the gut and transferred through the trophic level (Galloway et al., 2017, Kershaw, 2017, Kershaw and Rochman, 2015, Auta et al., 2017, Rochman et al., 2016). According to the studies, the soil ecosystem may probably receive much more microplastic waste than aquatic environments (Horton et al., 2017, Zhu et al., 2019). More recently, terrestrial ecosystems have been considered to study the MPs ingestion by soil organisms (Huerta Lwanga et al., 2016, Rodriguez-Seijo et al., 2017), effects on the biota, biodiversity, and ecosystem processes (Rillig, 2012, Horton et al., 2017, de Souza Machado et al., 2018). Ee-Ling Ng et al. (2019), Horton et al. (2017), and Duis and Coors (2016) reviewed microplastic and nano plastic pollution, transport, and fate in the terrestrial environments and their consequent impact on individual and ecosystem health. Rillig and et al. (2012) studied the effects of MPs on soil biota and ecology context pollutions. The results showed that different MPs have long-term effects on soil protist communities and functions (Rillig, 2012). Deposited MPs in urban soils may cause human exposure to MPs through ingestion of suspended solids and inhaling fine airborne particles (19). Abbasi et al. (2019) investigated distribution and potential health impacts OF MPs in air and street dust of Asaluyeh County (Abbasi et al., 2019) and Bushehr City (Abbasi, 2017) in Iran. Similar research by Dehghani et al. (Dehghani et al., 2017) in Tehran metropolitan demonstrated that high MPs exposures for children and workers through incidental ingestion. Furthermore, land application of sludge and organic fertilizer, plastic mulching and wastewater irrigation considered as pollution sources in agricultural areas (Zhu et al., 2019).

Moreover, the lack of evidence on the ecological impacts of MPs was addressed the gaps in our knowledge (Ng et al., 2018a, Horton et al., 2017, Duis and Coors, 2016). This research is essential due to highlight the role of impact...
of the urban and agricultural areas as one of the main sources of primary MPs in soil of Aghili plain with hot and
dried climate conditions.

Aghili plain is located in Khuzestan Province, southwest of Iran. Since 2004, the study area has been experiencing
heavy dust storm events, the so-called “Middle Eastern Dust (MED) event” (Shepherd et al., 2016), which lead to
considerable air dust pollution and health effects on residents. Furthermore, Aghili plain is one of the most
important agricultural centers in the north of Khuzestan province. It is irrigated by Karun and Shoor rivers as two of
the main rivers in Iran. Rivers pollution by several anthropogenic sources and local pollution sources release
different environmental pollutants, including MPs, into the soil with hot and dry climate. According to our best
knowledge, the abundance of MPs in Iranian plain soil has not yet been studied. This study aimed to investigate the
frequency, characterization, and distribution of MPs contamination in the soil of Aghili plain in Iran.

Materials And Methods

Study area

Aghili plain is located in Khuzestan province in the southwest of Iran (Figure 1) with an average elevation of 70
meters above global sea level. This plain has hot and dry (semi-desert) climate conditions with average rainfall and
temperature of 326 mm/y and 33 °C, respectively. The top layer of the soil in the study area is characterized by
quaternary unconsolidated alluvial sediment (Ahmadi et al., 2019). The main land use of the studied area belongs
to agricultural activities (80%), followed by national lands (12%) and residential and industrial areas (8%)(Ahmadi
et al., 2019). The population of the city is about 65000 people. There are two rivers in this region; Karun and Shoor
Rivers, which can be considered two primary sources of agricultural activities and MPs pollution in the soil.

MPs sampling

For the sampling process, firstly, the study area was divided into 14 sampling sites by a GPS locator (MG868S) (Fig.
1). A systematic sampling procedure collected a total of 102 topsoil samples from urban and agricultural areas
during the dry season (March 2021) (Fig. 1). Five soil samples determined by calipers were mixed from the surficial
soil layer (20 cm) in an area of 5×5 m². Approximately 1 ± 0.25 kg of mixed topsoil sample was gathered and
stored in a stainless still container and transferred to the laboratory. During sampling process, all facilities washed
with filtered distilled water and oven dried.

Extraction and counting of MPs in soil

According to the author’s previously published paper, the density separation methodology was conducted for
microplastic debris extraction with modifications (Abbasi et al., 2019). All samples transferred to the glass beakers,
air-dried for seven days, and then to remove stones, vegetation, and other debris, a 5-mm stainless steel sieve was
used. Organic matter was removed by adding 35 mL of H₂O₂ (30%) to a soil sample for eight days until bubble
formation finished. The vacuum filtration (chm filter papers F2024 with a pore size of 2μ) applied to remove the
remaining H₂O₂. 55 mL of saturated ZnCl2 (Arman Sina, Tehran) solution with density of 1.6 to 1.8 g cm⁻³ added to
the soil samples and shake for 5 min at 350 rpm. After a settling time of 90 min, the supernatant was centrifuged (3
min; 4000 rpm) and vacuum-filtered (chm filter papers F2024 with a pore size of 2μ). All applied papers rinsed with
distilled water. To remove all MPs, the processes repeated for three times through the same filter. Filter papers dried
at room temperature in a non-plastic cabinet and moved to glass petri dishes for further identification and
observation.
Binocular microscopy (Carl-Zeiss) was used to identify and isolate MPs and determine typical features such as form, gloss, color, and hardness by visual methods (Abbasi et al., 2019, Abbasi, 2017, Stolte et al., 2015, Hidalgo-Ruz et al., 2012). The suspected MPs were classified into blue-green, black-grey, red-pink, yellow-orange, white-transparent colors based on the color. Moreover, MPs were classified into different shapes, including fiber, film, fragment, and Spherule. ImageJ software and a 250 µm stainless steel probe were applied to categorize MPs into the following sizes: \( L \leq 100 \, \mu \text{m} \); \( 100 < L \leq 250 \, \mu \text{m} \); \( 250 < L \leq 500 \, \mu \text{m} \); \( 500 < L \leq 1000 \, \mu \text{m} \); \( 1000 < L < 5000 \, \mu \text{m} \) (Abbasi et al., 2019).

**MPs characterizations**

The topography and elemental composition of MPs were analyzed by SEM/EDX (high vacuum scanning electron microscopy/energy-dispersive X-ray microanalysis with a Tescan VEGA 3 microscope) by resolution of 2 nm at 20 kV and an Oxford Instruments X-Max 50 with silicon drift detector 150 and software of AZtec and INCA. The brushed MPs were transferred to the Al SEM stubs on the double-sided adhesive carbon tabs carefully. The polymeric construction of MPs was determined using a micro-Raman spectrometer (laser: 785 nm and Raman shift of 400-1800 cm\(^{-1}\), acquisition times: 20 - 30 s) (made in Japan, LabRAM HR, Horiba,).

**Microplastic intake via ingestion**

The number of Microplastic intakes via ingestion was calculated based on a normal exposure scenario of USEPA with a mean particle ingestion rate of 100 and 200 mg day\(^{-1}\) for adults and children (1-6 years old), respectively (EPA, 2008). The acute exposure scenario suggested 1 g of particle ingestion per outdoor day for children. Also, based on the guidance values to calculate the intake of microplastics in occupational exposure, it is suggested that 330 mg day\(^{-1}\) of ingested soil for outdoor workers (29) and 200 days per year is considered the mean value of working days.

**QA/QC control**

During this research, different control procedures were implemented. All sampling instruments and containers were washed with deionized water before sampling. Soil samples were taken with a metallic pan and kept in the aluminum foil. All the experiments were conducted under a clean laboratory environment. So, all working surfaces cleaned with ethanol, all reagents and distilled water filtered through chm filter papers (F2024 with a pore size of 2µ), all glassware was cleaned with distilled water to prevent plastic and fiber cross contamination. Moreover, all windows and doors keep closed to avoid the impact of air turbulence and airborne and MPs pollution during the lab workings. Cotton laboratory coats, face masks and single-use latex gloves were used. Simultaneously, three replicates of blanks were conducted to check the possible suspended airborne contamination.

**Statistical analysis**

SPSS software (version 16.0) was applied to analyze all data and expressed as mean ± standard deviation of the mean (SDM).

**Results And Discussion**

**Characteristics of the MPs in soil**
The average mean of MPs was identified as 11.93 ± 0.9 items in Kg⁻¹ of surface soil in the Aghili plain, Iran. Identified MPs size was ranged from < 100 up to 1 mm. The abundance of MPs with a particle size of less than 0.25 mm accounts for about 86 % (Table 1, Figure. 2a). MPs with > 250 µm occupied 14 % of total MPs in topsoil samples. The total fraction of MPs with the 100–250 µm was (85%) that approximately is six times greater than the rest of the studied fractions. The total number of MPs in the size of <100 µm was 1.5 times greater than MPs with 100-250 µm in size. The proportion of different sizes were in the following order: (less than 100 µm) > (100-250 µm) > (250 – 500 µm) > (500-1000) > (higher than 1000 µm) which were consistent with other results reported decreasing abundancy of MPs with higher particle sizes (Du et al., 2020, Nor and Obbard, 2014). There were significant differences in MPs sizes among sampling sites. The sizes of MPs in sites S1 (85%), S2 (93%), S3(74%), S4 (40%), S6(43%), S7(45%), S8(67%), S11(62%) were mainly less than 100 µm (figure. 2a, Table.1). Land-use patterns and physio-chemical properties of the soil may impact the MPs abundancy in the soil. It may be related to land-use type that mostly contains farm and agricultural lands (Du et al., 2020). However, there are different natural and anthropogenic pollution sources in the study area. The highest MPs were observed at Site 9 followed by sites 6, 3, and 11. These sampling sites were located in residential areas with a high population density and vehicle traffic. The statins with low MPs abundances were situated in the areas with lower populations and dominant agricultural activities. The agricultural usage of sewage sludge containing high MPs concentrations is considered as a significant MPs source in agricultural lands (34). It may suggest the direct relation of social activities, urbanization level, solid waste disposal in landfills, and traffic load with the MPs pollution studied are soils. Moreover, fertilizers (including dried sludge of wastewater and chemical fertilizers), pesticides, and fossil fuel combustion are considered the main anthropogenic sources of soil pollution in the study area(Ahmadi et al., 2019).

| MPs Size (µm) | L<100 | 100≤L<250 | 250≤L<500 | 500≤L<1000 | ≥ 1000 |
|---------------|-------|-----------|-----------|------------|-------|
| Abundance (%) | 52.096| 33.54     | 8.38      | 2.99       | 2.99  |
Table 2
Normal exposure (NE), acute exposure (AE), and related daily and annually ingested MPs by adults and children at the different sampling sites.

| Sampling site | MPs abundance (MPs Kg⁻¹ soil) | Number of ingested MPs | adults | adults | AE | AE | AE | children | children |
|---------------|--------------------------------|------------------------|--------|--------|----|----|----|----------|----------|
|               |                                |                        | NE     | NE     | AE | AE | AE | NE       | NE       |
|               |                                |                        | (day⁻¹) | (year⁻¹) | (day⁻¹) | (year⁻¹) | (day⁻¹) | (year⁻¹) | (day⁻¹) | (year⁻¹) |
| S1            | 14                             |                        | 0.0014 | 0.511  | 0.0046 | 0.0017 | 0.0028 | 1.022    | 0.014    | 5.11     |
| S2            | 15                             |                        | 0.0015 | 0.548  | 0.0050 | 0.0018 | 0.003  | 1.095    | 0.015    | 5.475    |
| S3            | 19                             |                        | 0.0019 | 0.694  | 0.0063 | 0.0023 | 0.0038 | 1.387    | 0.019    | 6.935    |
| S4            | 15                             |                        | 0.0015 | 0.548  | 0.0050 | 0.0018 | 0.003  | 1.095    | 0.015    | 5.475    |
| S5            | 3                              |                        | 0.0003 | 0.11   | 0.0010 | 0.0004 | 0.0006 | 0.219    | 0.003    | 1.095    |
| S6            | 21                             |                        | 0.0021 | 0.767  | 0.0069 | 0.0025 | 0.0042 | 1.533    | 0.021    | 7.665    |
| S7            | 9                              |                        | 0.0009 | 0.329  | 0.0030 | 0.0011 | 0.0018 | 0.657    | 0.009    | 3.285    |
| S8            | 9                              |                        | 0.0009 | 0.329  | 0.0030 | 0.0011 | 0.0018 | 0.657    | 0.009    | 3.285    |
| S9            | 22                             |                        | 0.0022 | 0.803  | 0.0073 | 0.0027 | 0.0044 | 1.606    | 0.022    | 8.03     |
| S10           | 2                              |                        | 0.0002 | 0.073  | 0.0007 | 0.0002 | 0.0004 | 0.146    | 0.002    | 0.73     |
| S11           | 16                             |                        | 0.0016 | 0.584  | 0.0053 | 0.0019 | 0.0032 | 1.168    | 0.016    | 5.84     |
| S12           | 1                              |                        | 0.0001 | 0.037  | 0.0003 | 0.0001 | 0.0002 | 0.073    | 0.001    | 0.365    |
| S13           | 9                              |                        | 0.0009 | 0.329  | 0.0030 | 0.0011 | 0.0018 | 0.657    | 0.009    | 3.285    |
| S14           | 12                             |                        | 0.0012 | 0.438  | 0.0040 | 0.0015 | 0.0024 | 0.876    | 0.012    | 4.38     |
| Median        | 13                             |                        | 0.0013 | 0.475  | 0.0043 | 0.0016 | 0.0026 | 0.949    | 0.013    | 4.745    |
| Mean          |                                |                        | 0.0012 | 0.435  | 0.0039 | 0.0014 | 0.0024 | 0.8710   | 0.0119   | 4.353    |
| SD            |                                |                        | 0.0007 | 0.247  | 0.0022 | 0.0008 | 0.0013 | 0.4947   | 0.0068   | 2.473    |

The dominant color in studied MPs belonged to black/grey group with 31.74% of abundance, followed by white/transparent (29.94%), yellow/orange (19.16%), red/pink (12.58%), and blue/green (6.59%) colors (Fig. 2b). Moreover, different fiber, pellet, fragment, and Spherule shapes were identified MPs within various colors and diameters (Figure. 2c). The most abundant MPs shapes in the soil samples were as fallow: fibrous (47%), fragment (34%), Spherule (16%), and pellet (6%). Fibrous MPs are more abundant form in the soil. It was estimated that 70% of all textile items globally are synthetic, considered an important source of fibrous MPs in soil (Razeghi et al., 2021). Also, plastic microfibers (<5mm) and nanofibers (<100nm) can persist for decades in soils treated with sludge from wastewater treatment plants (Browne et al., 2013, Yan and Peng, 2021).

Figure 2d shows the total MPs items per Kg⁻¹ of topsoil of the sampling sites in the studied area. 167 MPs were found in soil samples with a mean of 11.9 ± 6.78 items kg⁻¹ of topsoil in Aghili plain. The maximum and minimum MPs abundance were observed at site 9 (N = 22 items Kg⁻¹) and site 12 (N = 1 items kg⁻¹), respectively. The total
number of items Kg\(^{-1}\) per sites were observed as the following trend: S9 (22) > S6 (21) > S3 (19) > S11 (16) > S2 and S4 (15) > S1 (14) > S14 (12) > S13, S7 and S8 (9) > S5 (3) > S10 (2) > S12 (1). Yan and et al. reported higher abundance of MPs in the soil of eastern coastal zones; China ranged from 1.3 to 14712.5 MPs kg\(^{-1}\) of soil. Particle sizes less than 1mm were accounted for about 60%. (Yan and Peng, 2021). However, the farmland soil in Shanghai (16.1±3.5 MPs kg\(^{-1}\)) and a surface layer of greenhouse soil (78.00±12.91 MPs kg\(^{-1}\)) showed the higher MPs items (Yan and Peng, 2021). Weber et al. showed the impact of land use and fluvial processes on the spatial patterns of mesoblastic (2.06 ±1.55 in kg\(^{-1}\)) and coarse microplastics (1.88 in kg\(^{-1}\)+1.49 kg\(^{-1}\)) in floodplain soils (Weber and Opp, 2020).

SEM-EDX analytical technique used to report the morphology and the composition of microplastic's surface. It was conducted on the representative samples to determine the chemical verification of MPs.

Selected samples from identified MPs were analyzed by Raman spectrometry to reveal the kind of plastics. The results indicated that six, four, two, and four number of identified MPs were constructed from polyethylene terephthalate (PET), polystyrene (PS), polypropylene (PP), and Nylon, respectively. One sample contains two kinds of plastics, including PP and PET (Figure .3). So, the identified types of MPs in this area were polyethylene terephthalate, which accounted for about 39% of the detected MPs, followed by Polystyrene and Nylon (22%) and Polypropylene (16%). The current study demonstrated the dominance of fibrous shape and PET (a polyester) consistent with the other studies (Henry et al., 2019, Razeghi et al., 2021). Liu et al. found a positive correlation between PET concentration and MP bers (50µm-2 mm) abundance(Liu et al., 2019).

SEM imagined selected samples (Figure 4), revealed that a considerable number of identified MP consisted of single straight, curved, or spiral bers that most of them were smooth and clean. Furthermore, fragments shapes of MPs consisted of different sizes and colors with broken and sharp angles. The long-time of MPs retention in the soil inevitably led to weathering and degradation of MPs. Moreover, the MPs' surface could be impacted by the local and regional air currents, such as dust storm events. The weathered MPs showed the cracked surface and breaking into more minor MPs or even nano plastic. Moreover, the morphology of microplastics changed by weathering degradation and led to forming of active oxygen and persistent free radicals on the MPs surface (Yan and Peng, 2021). The results of EDX analysis confirmed the organic and inorganic contaminations on the surface of identified MPs (e.g., C, N, O, Cl Na, P, Si, Zn). Figure 5: SEM images and EDX spectra for two selected samples. (a) A black-gray PET fiber shows contamination by extraneous PM containing C, O, Na, P, and Cl (b) a white-transparent Nylon fragment with evidence of contamination by extraneous PM including C, N, O, Na, Si, Cl, Zn.

**Human intake of MPs by soil ingestion**

The soil ecosystems might be polluted by MPs as small, heterogeneously mixed plastics by several sources such as landfills, agricultural mulching films, sewage irrigation, and more. MPs can migrate in the soil body specially in the topsoil and damage the soil's health, function, and structure. This changes in the soil ecosystem led to high adsorption capacity for hazardous contaminants, that deteriorates soil pollution and rises the adverse effects to organisms and human health. Furthermore, MPs are ingested by soil organisms and are transferred via the food chain. Also, plant growth is impacted by MPs. The accumulation and transportation of MPs in plants increase the potential effects on plants. Based on the exposure scenarios (EPA, 2012, Abbasi et al., 2019), the estimated normal intakes of MPs in soil ranged from 0.0365 to 0.803 MPs year\(^{-1}\) (median =0.475 MPs year\(^{-1}\)) and 0.073 to 1.606 MPs year\(^{-1}\) (median = 0.949 MPs year\(^{-1}\)) in adults and children, respectively. The acute exposure led to intake of the higher number of MPs in adults ranged from 0.00012 - 0.0027 MPs year\(^{-1}\) (median= 0.0014 MPs year\(^{-1}\)) and
in children ranged from 0.365 - 8.03 MPs year$^{-1}$ (median= 4.354 MPs year$^{-1}$). It can be observed that the acute exposure to MPs through the ingestion route is 3.3 and 5 times higher than the normal exposure in adults and children, respectively.

It is stated that MPs size fractions could significantly increase the impact of MPs risk assessments. Fine MPs (<250 µm) tended to adhere to the hand's skins more, and finer MPs (<50 µm) tended to be ingested involuntarily (Choate et al., 2006, Siciliano et al., 2009). In the current study, MPs with sizes less than 250 µm corresponded to more than 85 % of size class, which related with the high probability of MPs intake by inhabitants through hands contamination and direct ingestion. Also, the surface pollutions of MPs by organic and non-organic debris must be considered regarding health risk assessments. However, more study is needed to assess compressively the adverse health effects of MPs on the human body.

**Conclusions**

Microplastics in human environments are considered as a potential health threat which have been received little attention. The average abundance of MPs in the surface soil of Aghili plain is 11.93 ± 0.9 items in Kg$^{-1}$ of surface soil of Aghili plain. MPs have different colors, shapes, and sizes that may be originated from various sources, including domestic, agricultural, dust storm, and vehicular sources. The most abundant size fraction that appears to be ingested was MPs less than 100 µm. The daily and annually intake of MPs by adults and children was estimated in normal and acute exposure scenarios. The results confirmed the probability of the health risks due to MPs exposure. However, additional research is required to assess the rates of MPs exposure, ingestion toxicokinetic information, and adherence to skin. It was revealed that the human intake of MPs by ingesting soil contaminated particles must be considered a significant health issue in cities.

**Declarations**

**Ethical Approval:** All Author confirm that the manuscript is the authors' own original work, which has not been previously published elsewhere, and reflects the authors' own research and analysis in a truthful and complete manner. The paper properly credits the meaningful contributions of co-authors and co-researchers. The results are appropriately placed in the context of prior and existing research. All sources used are properly disclosed. All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

**Consent to Participate:** Not applicable

**Consent to Publish:** Not applicable

**Authors Contributions:** Professor Jaafarzadeh and Dr Abbasi conceived of the presented idea. Mrs Ravanbakhsh developed the theory and performed the computations. Dr Dehbandi and Dr Sharifi verified the analytical methods. Dr Zahedi and Mrs Ravanbakhsh helped in lab working and sampling. All authors discussed the results and contributed to the final manuscript.

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Figures

Figure 1

Studied area and sampling sites in Aghili plain
Figure 2

microplastics in the sampling sites; (a) size, (b) color, and (c) shape, and (d) the total number of MPs per site.
Figure 3

Four MPs samples spectra analyzed by Raman spectrometry
Figure 4

SEM images and EDX spectra for two selected samples. (a) A black-gray PET fiber shows contamination by extraneous PM containing C, O, Na, P, and Cl (b) a white-transparent Nylon fragment with evidence of contamination by extraneous PM including C, N, O, Na, Si, Cl, Zn.