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Exposure to microplastics (<10 μm) associated to plastic bottles mineral water consumption: The first quantitative study

P. Zuccarello, M. Ferrante*, A. Cristaldi, C. Copat, A. Grasso, D. Sangregorio, M. Fiore, G. Oliveri Conti

Environmental and Food Hygiene Laboratories, Department "G.F. Ingrassia", University of Catania, Italy

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ABSTRACT

The uncontrolled introduction into the environment of plastic polymers have caused the dispersion of plastic fragments, known as Microplastics (MPs), that represent an important topic for public health. This study was the first to investigate the cause of the release of MPs in mineral waters and to estimate the concentration of MPs smaller than 10 μm both in number of particles and in mass unit. This study was carried out using a patent method regarding the extraction and analysis of MPs in more kind of matrix. Therefore, aims of this study were a) to assess the number of MPs with diameters of between 0.5 and 10 μm in mineral waters contained in plastic bottles, b) to evaluate if the physical-chemical properties of mineral waters and bottle quality could influence the release of MPs and, finally, c) to estimate the human daily exposure to MPs due to mineral water consumption.

The Mps were found in every sample. The main concentration of MPs was 656.8 μg/L ± 632.9 or 5.42E+07 p/L ± 1.95E+07. The main diameter of detected MPs was 2.44 μm ± 0.66 (where p/L, where p was the number of MPs). The Estimated Daily Intake (EDI) for adults and children were 1,531,524 p/kg/body-weight/day corresponding to 40.1 μg/kg/body-weight/day and 3,350,208 p/kg/body-weight/day corresponding to 87.8 μg/kg/body-weight/day, respectively.

The number of MPs contamination in bottled mineral waters was strongly correlated to the pH of waters and to plastic density of bottle. Otherwise, micrograms of MPs per liter and the MPs diameters were strongly affected by plastic thickness. The most mineral water brand contaminated by MPs was the one whose bottles were made from poor quality plastic.

In absence of reference values, it was no possible carried out a risk assessment for MPs exposure. It is fundamental to establish the reference method of analysis to monitoring every source of human intake.

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1. Introduction

Despite plastics support several daily human activities (e.g. food management, medical devise, etc.) there is an increasingly widespread concern about their abundance and the human health risks not fully assessed (Bergmann et al., 2015; Andrady, 2017). Plastic replaces many materials still in use, such as glass in the bottling of mineral water and soft drinks, as well as food packaging and storage container (Halden, 2010).

The uncontrolled introduction into the environment of these plastic polymers, together with their environmental persistence, have caused the dispersion of plastic fragments, known as Microplastics (MPs). MPs are by-products plastic materials stem from different environmental stress factors (Doyle et al., 2011; Eriksen et al., 2013a, 2013b; Thompson et al., 2009; Galgani et al., 2013).

Therefore, human health risks assessment associated to MPs daily intake represents an important topic for public health. The main human intake routes are inhalation, ingestion and skin perfusions (Wright and Kelly, 2017).

MPs have been detected in various natural habitats and in the digestive tracts of several species. These particles have also been reported as pollutants in commercially available seafood, salt or bottled water highlighting the importance of evaluating their potential implications for human health (Lancet Planet Health, 2017). The assessment of the MPs related risks needs to understand the really exposure concentrations and migration pathways in human

* Corresponding author.
E-mail address: marfer@unict.it (M. Ferrante).

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body. No certain data are yet available about MPs absorption and distribution in body districts. In fact, the absence of effective and efficient extraction methods of MPs from complex matrices (water, food, etc.) made the risk estimation and, the consequent assessment of the health impact associated with dietary exposure, difficult to perform. Currently a large literature reports data about MPs in food and environmental exposures (Bergmann et al., 2015; Prata, 2018), but they consider only fragment with diameter greater than 1 mm (Doyle et al., 2011). The World Health Organization (WHO) announced the need to investigate the potential effects of plastic on human health and launched its review in March after a separate study found MPs in 90% of 259 bottles (Mason et al., 2018).

Our study is the first that assess, with high accuracy, the presence and the quantity of MPs $\leq$10 μm in the mineral water using a new Italian patented method.

So, aims of this study were:

a) to assess the number of MPs with diameters of between 0.5 and 10 μm in mineral waters contained in plastic bottles;
b) to evaluate if the physical-chemical properties of mineral waters and bottle quality could influence the release of MPs;
c) to estimate the human daily exposure to MPs due to mineral water consumption.

2. Materials and methods

2.1. Samples collection and selection

Ten nationally distributed brands of still or sparkling mineral waters contained in PET plastic bottles (with packaging of 500 ml) were purchased in different supermarkets in the province of Catania (Italy). Physical-chemical parameters of the sampled mineral waters were taken from the labels. For each brand, three different bottles of different lots were analyzed. Every sample has shelf-life between August and October of the year 2019. So, results of the study will not be influenced by variability of the shelf-life. At the same time, three reagents blank were analyzed as controls.

2.2. MPs extraction and analytical method

This study was carried out using a new method of extraction and subsequent analysis according to a patent for industrial invention to the Italian Ministry of Economic Development number no. 102018000003337 of March 7 of 2018 entitled “Method for the extraction and determination of MPs in organic and inorganic matrix samples".

Nitrile gloves and laminar flow hoods were used to minimize the cross contamination of samples during extractions of MPs by airborne MPs in environmental dusts. During all phases of samples treatment, no plastic material was used. All laboratory's equipments used was preventively washed with water grade LC-MS and, subsequently, with organic solvents (dichloromethane, ether, etc. were all of grade LC-MS). After specific extraction of water samples, qualitative and quantitative detection analysis of MPs were carried out by Scanning Electron Microscopy (SEM) Cambridge Stereoscan S360 coupled with an Energy Dispersive Detector (SEM-EDX) Oxford Inca Xstream with settings according to those described in the above-mentioned patent.

2.3. Plastic density

Supposing that MPs derived exclusively from the plastic bottles, the determination of the density of the constituent plastics of each bottle is fundamental to calculate the concentration of MPs in microgram per liter. The density of a material is defined as the relationship between the mass of the body and its own volume; it is calculated as $\rho = m/V$. In the International System (S.I.) the density is expressed as g/cm^3 (www.goldbook.iupac.org).

After having cut out regular one cm^2 surface sections from each bottle of the various brands of water in exam, each section was weighed using a precision analytical balance. Subsequently, to estimate the thickness of the sections and calculate the exact volume and the density, small strips of plastic were transferred on the surface of an aluminum stub with a diameter of 25 mm, metallized with gold and were analyzed using the cited SEM. The sections of the bottles analyzed to the SEM for the determination of the thickness are reported in Fig. 1.

2.4. Statistical analysis

Statistical analysis was performed using “*R environment for statistical computing*” software. A one-way ANOVA was performed to investigate the differences of the results compared to the blank reagents and among the brands. A p-value of <0.05 was considered significant.

The Analysis of the Principal Components (PCA) on the distance matrix was used to identify which brand of mineral waters was higher affected on MPs release. Data were standardized before analysis and the results were displayed in a biplot distance (Legendre and Legendre, 2012).

The Analysis of the Principal Components (PCA) on the correlation matrix was instead used to evaluate the trend between the average MPs concentration and the physical-chemical parameters of mineral waters. Data were standardized before analysis and the results were visualized in a biplot correlation (Turchetti et al., 2013).

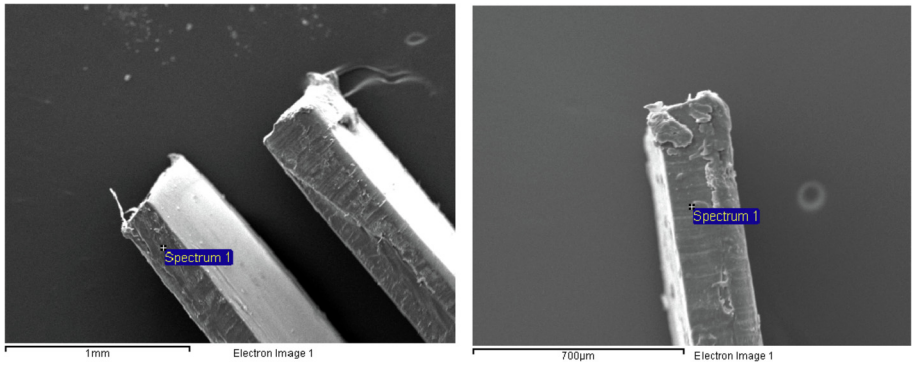


Fig. 1. SEM images of plastic bottle sections.

2.5. Estimated Daily Intake

The Estimated Daily Intake (EDI) of MPs concentrations in mineral water (μg/L or p/L, where p was the number of MPs) (μg/kg bw/day) through drinking water was calculated using the following equation:

$$EDI = (C \times IR)/BW$$

where IR, the Ingestion Rate, is assumed to be 2 L/day for adults and 1 L/day for children; C is the microplastic concentration (μg/L and p/L) and BW is the body weight assumed to be 70 kg for adults and 16 kg for children (Arena et al., 2015).

3. Results

3.1. Physical-chemical parameters and density

Data reported in the labels of the bottled mineral water, listed in Table 1, were considered for our results. The estimated plastic densities are reported in Table 2.

The density of plastics ranged between 1.12 g/cm^3 and 2.36 g/cm^3 with a main value of 1.54 ± 0.35 g/cm^3.

3.2. MPs concentrations

The MPs were found in every sample. Fig. 2 shows the SEM images of some MPs detected and their microanalysis. Results showed a wide variability of MPs concentration in all the samples compared to the blank reagents. Specifically, Fig. 3 reports in graph for each brand the MPs concentrations expressed both in micrograms per liter (μg/L) and number of Microplastics per liter (p/L).

The concentration (μg/L) of MPs ranged between 100 μg/L and 3000 μg/L with a main value of 656.8 μg/L (SD = ± 632.9 μg/L) (see Fig. 3). The univariate ANOVA shows no significant difference with blank reagent ($\rho > 0.05$). However, Tukey's test shows a significant difference for the brands 5 and 6 ($\rho < 0.05$).

The concentration (p/L) of MPs ranged between 3.16E+07 p/L and 1.1E+08 p/L with a main value of 5.42E+07 p/L (SD = ± 1.95E+07 p/L) (Fig. 4). Univariate ANOVA shows a significant difference with blank reagent ($\rho < 0.05$). Tukey's test shows a significant difference for the brands 1 ($\rho < 0.05$), 4 ($\rho < 0.05$), 6 ($\rho < 0.05$) and 7 ($\rho < 0$).

The diameters of detected MPs ranged between 1.28 μm and 4.2 μm with a main value of 2.44 μm (SD = ± 0.66 μm) (Fig. 5). Univariate ANOVA shows a significant difference with blank reagent($\rho < 0.05$). Tukey's test shows a significant difference for the brand 4 ($\rho < 0.05$).

The distance biplot (Fig. 6) (PC1 and PC2 represent the 62% and 19% of variance, respectively) showed that the brand **2, 4** and **7** were the most associated to the number of MPs (p/L) and to pH. Otherwise, the brand **5** was strongly affected by the microgram of MPs per liter, by plastic thickness and MPs diameters, while the brands **1, 3** and **6** were weakly associated. Finally, **8, 9** and **10** were the least interested to presence of MPs (Fig. 6).

Correlation biplot (PC1 and PC2 represent the 62% and 19% of variance, respectively) showed that the number of MPs in mineral waters was rather related to pH and plastic density. Otherwise, micrograms of MPs per liter and the MPs diameters were strongly affected by plastic thickness. Finally, Mg^{2+}, Ca^{2+}, conductibility and hardness were strongly related together (Fig. 7).

3.3. Human exposure

The Estimated Daily Intakes for adults and children were 1,531,524 p/kg/body-weight/day corresponding to 40.1 μg/kg/body-weight/day and 3,350,208 p/kg/body-weight/day corresponding to 87.8 μg/kg/body-weight/day, respectively.

4. Discussion

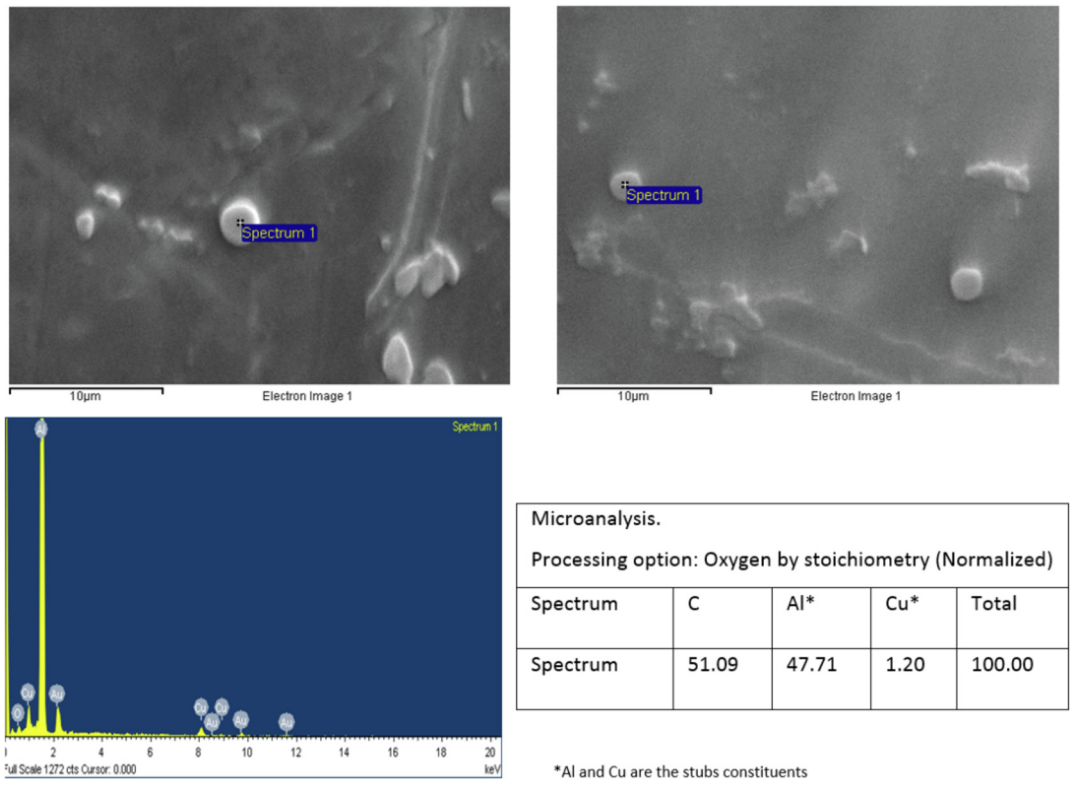
Microplastics (MPs) represent one of the emerging issues in environmental and nutrition hygiene (The Lancet Planetary Health, 2017). More data are necessary to support public health decisions

Table 1
Physical-chemical parameters of mineral waters extracted from the label.

Brand	Fixed residue 180 °C mg/L	Conductivity 20 °C μS/cm	pH	Ca^{++} mg/L	Mg^{++} mg/L	Hardness °F
1	174	276	7.6	5.7	0.32	3
2	370	483	7.3	9.1	0.53	224
3	313	493	7.2	8.6	1.9	29
4	80	118	7.8	2.0	0.17	6
5	152	212	6.8	2.6	0.77	10
6	745	1104	5.8	173	25	534
7	458	610	7.2	80	10	242
8	1390	1830	6.1	400	24	1096
9	145	187	7.6	21	7.5	83
10	1308	1780	6.2	234	38	741

Table 2
Weight, thickness, calculated volumes of sections and estimated plastic density by brand.

Brand	Weight (g)	Thickness (cm)	Volume of section (cm^3)	Plastic density (g/cm^3)
1	0.032	0.0213	0.0213	1.50
2	0.024	0.0184	0.0184	1.30
3	0.027	0.0162	0.0162	1.67
4	0.016	0.0100	0.0100	1.60
5	0.026	0.0156	0.0156	1.67
6	0.033	0.0267	0.0267	1.24
7	0.012	0.0073	0.0073	1.64
8	0.017	0.0152	0.0152	1.12
9	0.074	0.0314	0.0314	2.36
10	0.025	0.0187	0.0187	1.34



Microanalysis.

Processing option: Oxygen by stoichiometry (Normalized)

Spectrum	C	Al*	Cu*	Total
Spectrum	51.09	47.71	1.20	100.00

*Al and Cu are the stubs constituents

Fig. 2. SEM images of MPs and related microanalysis.

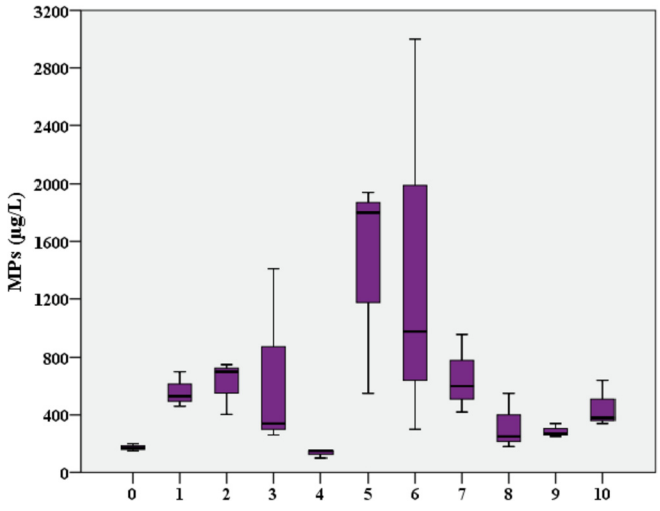


Fig. 3. Distribution of MPs (µg/L) by mineral water brand (0 is the blank reagent).

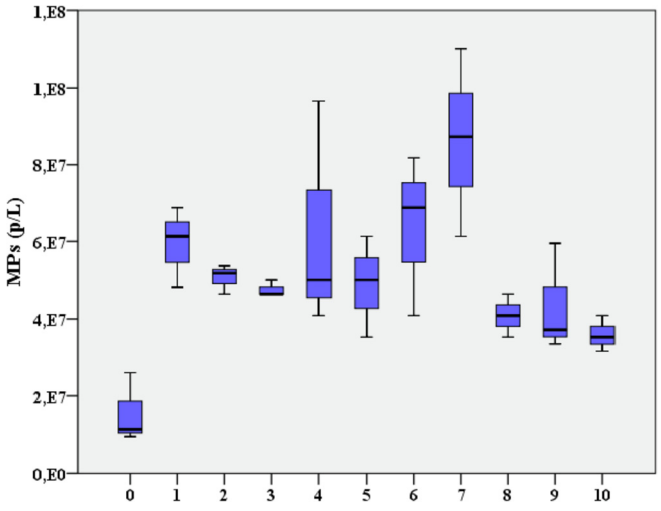


Fig. 4. Distribution of MPs (p/L) by mineral water brand (0 is the blank reagent).

and policies. MPs were found in oceans (Anderson et al., 2016; Browne et al., 2011), estuaries (Browne et al., 2010. Lima et al., 2014. Zhao et al., 2014), in freshwater bodies (Sanchez et al., 2014) and in polar ice (Zarfl and Matthies, 2010; Hubard et al., 2014). Currently, ingestion of fish products seems to be the main route of MPs exposure for humans (Bergmann et al., 2015).

In various studies, it was assigned different diameters range to MPs as shown below:

- <10 mm (Graham and Thompson, 2009)
- <5 mm (Barnes et al., 2009; Betts, 2008)
- 2–6 mm (Derraik, 2002)
- <2 mm (Ryan et al., 2009)
- <1 mm (Browne et al., 2007; 2011; Claessens et al., 2011).

These byproducts should be defined Macroplastics, differently from those fragments with diameters about the micron and correctly defined as MPs. Only one study is available currently reporting data about MPs with diameters smaller than 10 µm (Mason et al., 2018). Mason et al. (2018) had used an optical microscope after treating the samples with Nile Red solution. No digestion of organic and inorganic carbon was carried out. The single particle was no examined by microanalysis to confirm its chemical composition. Finally, this method can analyze only

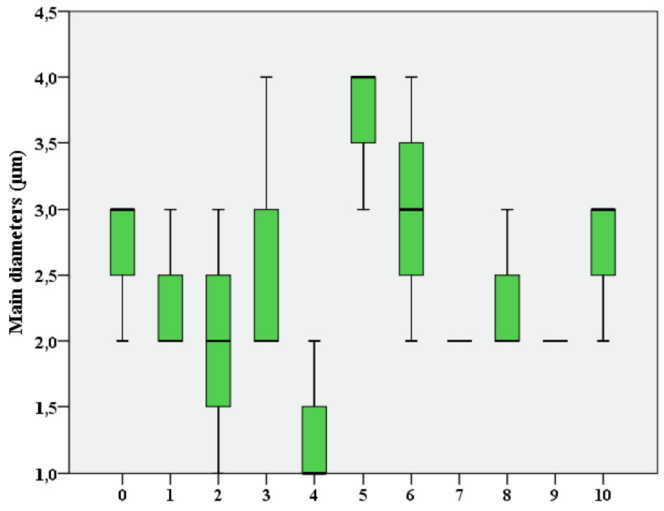


Fig. 5. Distribution of the diameters of MPs (µm) by mineral water brand.

particle bigger than 6.5 µm. Other studies, on the other hand, use MPs extraction methods through filtrations in polycarbonate filters, a polymer that is also classified among plastics and the subsequent analyzes conducted by Scanning Electron Microscopy do not allow the analyses to be distinguished with certainty from interferences (Endo et al., 2001).

Analysis of the smaller MPs is fundamental since they could be up-taken from the gastrointestinal tract via endocytosis by the M cells of the Peyer's Patches and could be adsorbed in hematic circle (Powell et al., 2010; Wright and Kelly, 2017; Song et al., 2009).

Thanks to the extraction procedure developed by the Environmental and Food Hygiene Laboratory, it was possible to isolate the plastic particles smaller than 10 µm from the aqueous matrix, minimizing external contamination. In our study, the ANOVA showed that the number of particles and their main diameters in the blank reagents were significantly different ($\rho < 0.05$) rather than those of the samples. It shows how the used method is reliable to isolate the MPs from water matrix. The microanalysis is the best method to discriminate a carbon particle from others. Avoiding the use of filters and other plastic materials guarantee the minimal contamination from process and minimize analytical interferences.

ANOVA on concentration expressed as µg/L was no significant. It could be explained by the incorrect conversion from the number of MPs and their estimated average volume using the same density for each brand of water. In fact, the MPs could not exclusively derived from packaging of waters but they could be a contamination of water before their bottling.

Principal Component Analysis (PCA) showed that the number of MPs contamination in bottled mineral waters was strongly correlated to the pH of waters but only weakly to plastic density of bottle. Otherwise, micrograms of MPs per liter and the MPs diameters were strongly affected by plastic thickness. It seems that hard plastics cause the release biggest fragments but in a minor number. Instead, the more deformable plastics and weakly alkaline pH would increase the number of smallest MPs.

The brand of mineral water most contaminated by MPs was the 7, whose bottles were made macroscopically and microscopically by poor quality plastic, with the smallest thickness among the bottles examined (0.073 mm), characterized by high deformability.

The Estimated Daily Intake (EDI) highlights the high amount of MPs ingested daily by bottled mineral water. If these data will be

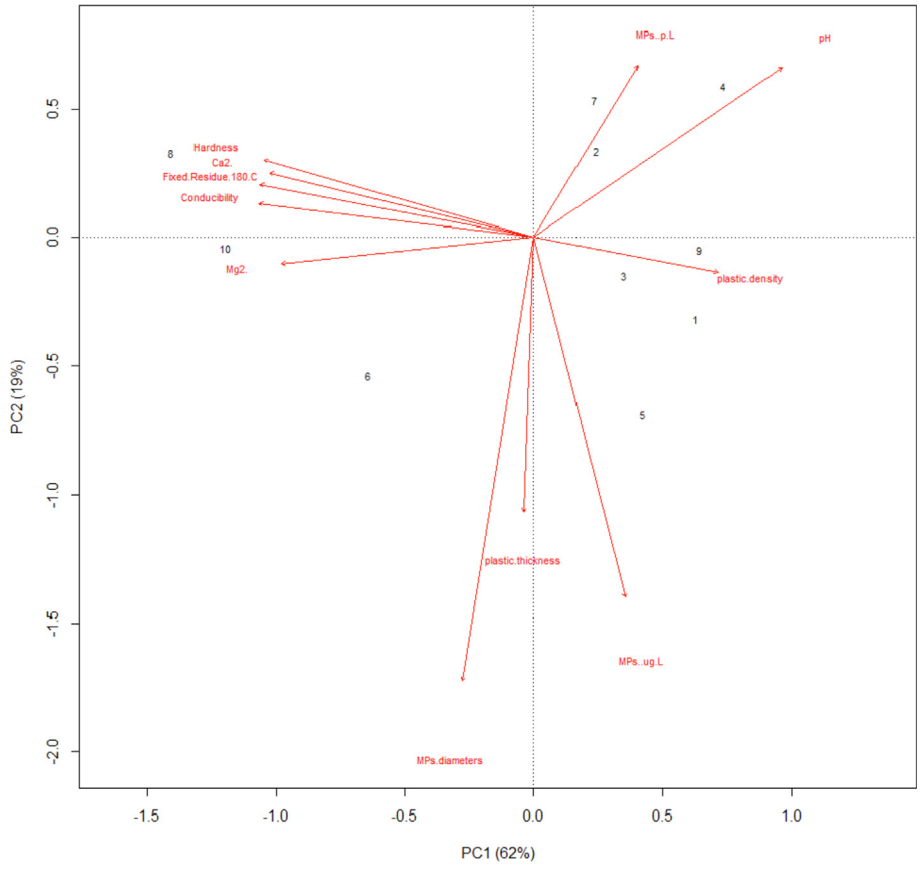


Fig. 6. PCA on distance matrix.

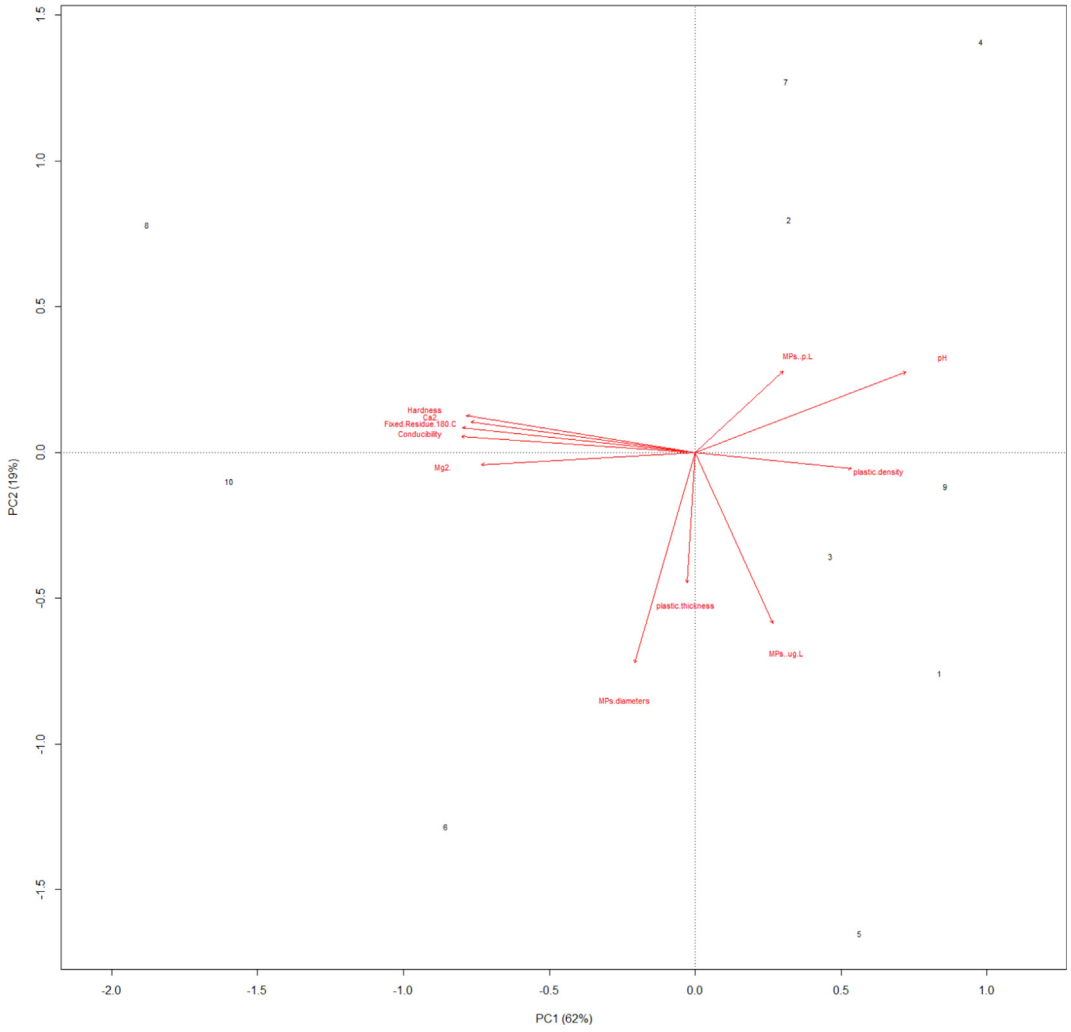


Fig. 7. PCA on correlation matrix.

confirmed by future studies, it would represent an important risk to public health that should not be underestimated. In addition to the embedding of such particles, their entry into the human body could involve the introduction of any substances associated with them such as additives, plasticizers, opacifiers, stabilizers, flame retardants, antistatic and conductive additives and medical additives (Murphy, 2001; Vethaak and Leslie, 2016; Zuccarello et al., 2018; Fiore et al., 2019), or substances considered endocrine disruptors. These substances, incorporated into plastics, can migrate from the matrix due to de-polymerization and leaching processes (Cole et al., 2011) and may increase cytotoxicity and inflammatory response (Xu et al., 2004). Many of them are hydrophobic and they are widely distributed in the most lipophilic tissues and slowly eliminated from the body (Kwon et al., 2017). For example, PVC can cause greater damage due to the prolonged release of vinyl chloride, one of the main risk factor for the onset of certain tumors (Mastrangelo et al., 2003). In addition to the constituents of the plastic matrix, the MPs can adsorb and convey other environmental contaminants (Teuten et al., 2009; Wang et al., 2016) and numerous microorganisms that lurk in the porosity of the matrix (Kirstein et al., 2016).

5. Conclusions

This study was the first to investigate the cause of the release of MPs in mineral waters and to estimate the concentration of MPs smaller than 10 μm both in number of particles and in mass unit.

In absence of reference values, it was no possible carried out a risk assessment for MPs exposure. It needs to increase quickly the number of the studies about intake from other food, pharmaceutical products and from environment, as well as the studies on epidemiology, toxicity and carcinogenicity. It is fundamental to establish the reference method of analysis to monitoring every source of human intake.

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We declare that our research group do not have any conflict of interest such as economic, political interests or national affinities, family or emotional ties, or any other relevant connection or shared interest.

In addition, we declare that our study did not require any ethical approval because there are not ethical issues related to consent of patients or experimentation on animals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watres.2019.03.091.

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