Original Article

Quantitative and Qualitative Evaluation of Microplastics in Different Salts from Iran

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Abstract

Aim: In this study, the microplastic (MP) concentration in several brands of salts was investigated. **Materials and Methods:** Fifteen samples of crystallized salt, refined sea salt, unrefined sea salt, and rock salt were purchased from local markets and analyzed for MPs concentration. The salts were digested with the Catalytic Wet Peroxide Oxidation method first, and the MPs were floated based on density difference. Then, MPs were counted by scanning electron microscopy and nature was confirmed by using micro-Raman spectroscopy. **Results:** The MP concentrations in crystallized salt, refined sea salt, unrefined sea salt, and rock salt were 151.4 ± 48.8, 406.7 ± 93.3, 1417.4 ± 203.3, and 283.4 ± 97.0 MPs/kg, respectively. The most abundant polymers were polyethylene, polypropylene, and polyethylene terephthalate. The fiber was the dominant shape of MPs in all salt samples. **Conclusions:** This study reveals the presence of MPs in crystallized salt, refined sea salt, unrefined sea salt, and rock salt. Therefore, the consumption of salts can expose humans to MPs.

Keywords: Microplastic, refined salt, rock salt, salt, sea salt, unrefined salt

Introduction

The mass production of plastics began in the 1950s, and today, plastic play an essential role in human life.[1] Microplastics (MPs) have entered the environment and have been found in the terrestrial and aquatic environment, air, sea, river, urban runoff, raw and treated wastewater, compost, and food, due to increasing human use of plastic materials.[2-4] MPs are defined as synthetic plastic particles <5 mm and more than 1 μm in diameter.[5] MPs are divided into two categories based on the origin of production. Primary MPs are manufactured in micro- and nano-sized, and secondary MPs result from the fragmentation of larger plastic particles in the environment.[6-8] The small size of these particles causes them to be ingested by aquatic organisms, and so, MPs in marine environments are potentially more hazardous than larger plastic wastes.[9] Due to their hydrophobic nature, MPs tend to absorb and transfer drugs, personal care products, and persistent organic contaminants, such as polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls.[10,11] These particles can also carry microbial contaminants and pose a risk to human health.[12] MPs can cause cancer and affect liver and brain function.[9] The first step to understand the importance of MPs to human health is to find the exposure ways and quantification of them, and one of the major ways to this exposure is through diet.[13] Since salts are mainly produced by the evaporation of saline waters such as the sea, lakes, wells, and rock salts that contain MPs, this pollutant is also found in salts.[13,14] Consequently, daily consumption of salts can expose humans to a large number of MPs in the longtime.[8] Lee *et al*. found that 94% of the salts from different countries contained MPs. They also reviewed seven different studies and found that there is 140.2 MPs/kg in salt on average, and according to the annual consumption of 3.75 kg of salt per person, several hundred MPs enter the individual's body annually.[15] Due to the importance of this issue, MP concentrations in several brands of crystallized salt,

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refined and unrefined sea salts, and rock salts from Iran were investigated in this study.

Materials and Methods

No standard method for sampling and analyzing of MPs has been published by any organization yet. However, National Oceanic and Atmospheric Administration has developed laboratory methods for the separation and quantification of MPs in water and sediments.[16] After all, the measurement steps of MPs in various studies usually include sampling and sieving, pretreatment (digestion), separation based on density difference, counting, and identification of the chemical structure of MPs, which have also been used in this study. Each step is described below:

Fifteen samples were purchased from the local markets (five samples of crystallized salt [S_1], four samples of refined sea salt [S_2 food grade], two samples of unrefined sea salt [S_3], and four samples of rock salt [S_4]), and analyzed for MPs concentration.

The first step was digestion and MPs extraction that was performed according to the study of Masura *et al*. with a little change[16] using the Catalytic Wet Peroxidation Oxidation method. Two 250 g subsamples from each sample were transferred to a 1 l beaker for quantitative and qualitative analyses. Then, 20 ml of 0.05 M ferrous sulfate solution (Merck, Germany) and 20 ml of H_2O_2.35% (Dr. Mojallali, Iran) were added to each sample and kept at room temperature for 5 min. They were then covered with a watch glass and placed on a hot plate at 75°C. After observing the reaction bubbles, they were removed from the hot plate and were placed under the hood. Then, they were placed on the hot plate for another 30 min. Further, before this step, another 20 ml of H_2O_2.35% was added to the S_2, S_3, and S_4, which contained a lot of organic matter. The samples were then cooled at room temperature.[16] To prepare the 0.05 M ferrous sulfate solution, 7.6 g of $FeSO_4$ powder was added to 1 l of distilled water. Further, 3 ml of sulfuric acid was added to completely dissolving of the ferrous sulfate powder.[17] 600 ml of distilled water was then added to each sample and completely mixed, and the samples were placed at room temperature for 24 h to density separation of MPs. Then, the supernatant was decanted to a beaker, and the density separation was repeated two more times for the sediment.[18] A series of each salt type was then passed through a fiberglass filter (Whatman, GF-3, 125 mm, 0.6 µm) for qualitative analysis, and another series was passed through a hydrophilic PTFE filter (FILTERBIO, PTFE-L, 0.47 mm, 0.45 µm) for quantitative analysis, using a glass vacuum set. The filters dried at room temperature and transferred to a clean glass Petri dish.

UniRAM Raman spectrometer (South Korea) equipped with a solid-state laser with an excitation wavelength of 785 nm and power of 200 mW was used for qualitative analyses. Two cut-outs (1 cm × 1 cm) from each fiberglass filter were attached to the Au-coated glass holder, and the Raman spectra (surface-enhanced Raman spectroscopy) were recorded. Three spectra were taken from each cut-out, and the spectra

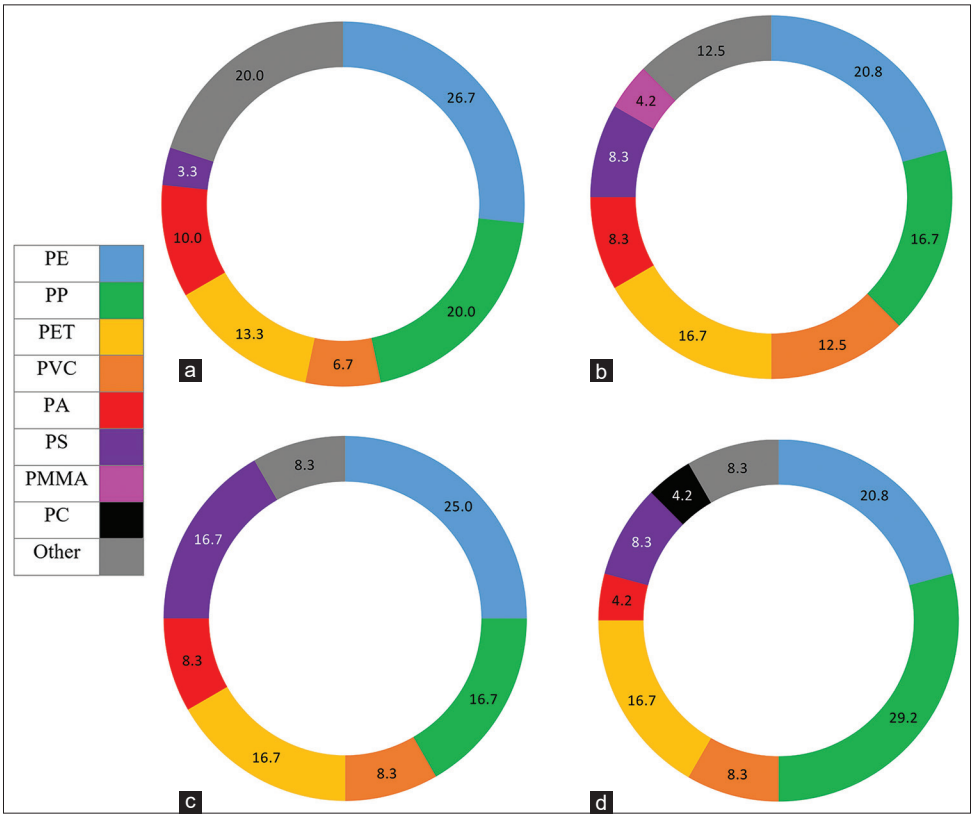

Figure 1: Distribution of microplastics polymer types in crystallized salt (a), refined sea salt (food grade) (b), unrefined sea salt (c), and rock salt (d)

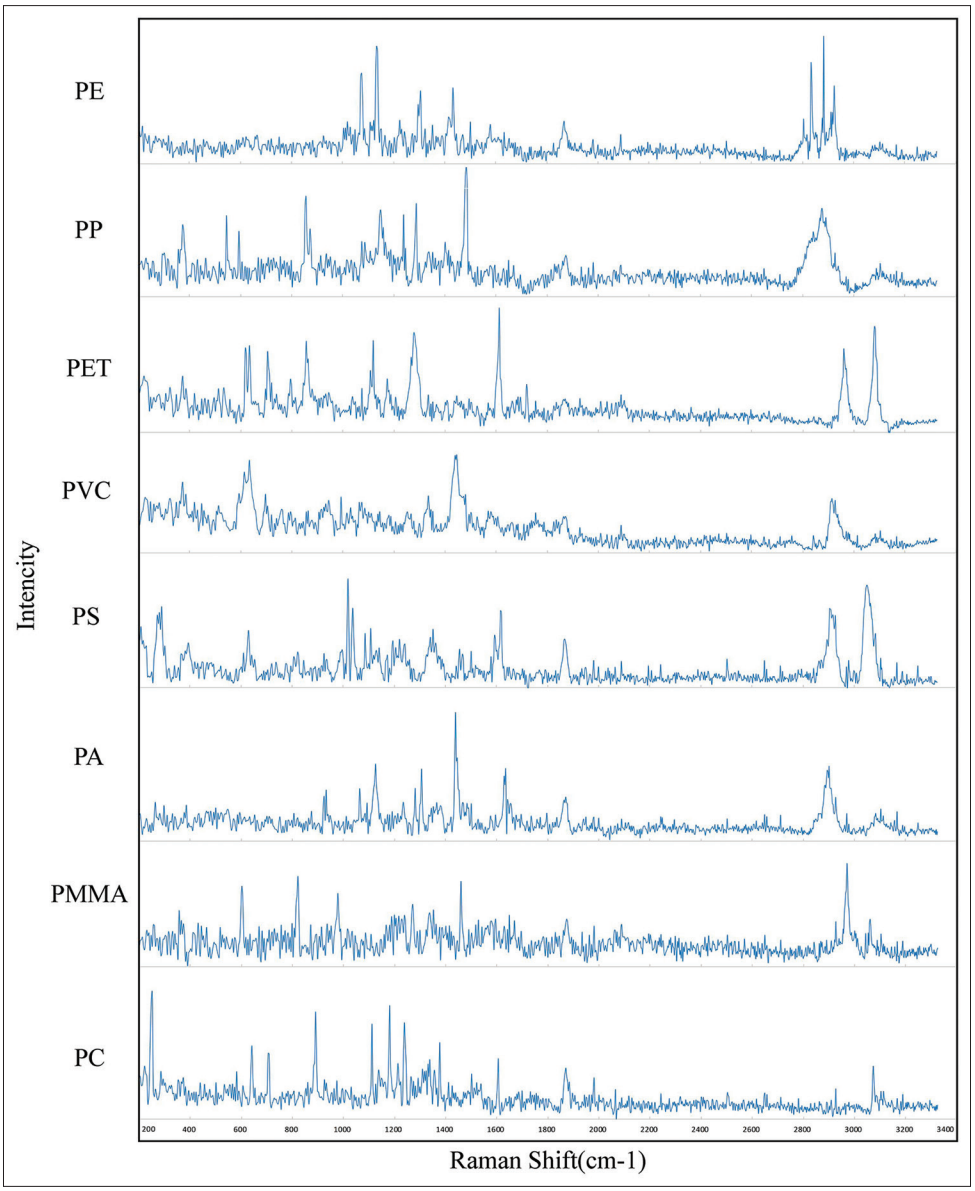

Figure 2: Identified polymers spectra

were baseline corrected using the origin 2019 software. Then, the spectra were compared with reference spectra, and the MPs were identified. Four cut-outs (≈5 mm × 8 mm) from each PTFE filter were analyzed and photographed using a scanning electron microscope (SEM, Philips XL30 ESEM, Netherlands). A layer of conductive gold was sputtered onto the filters before analyzing. MPs were counted based on size (<10, 10–50, 50–100, and >100) and shape distribution (fiber and fragment). The exact dimension of each filter was measured by SEM, and then the MP concentration in 250 g of salts was calculated by comparing the cut-out with the total filter area. Then, the MP concentration was quadrupled and corrected using the results of qualitative analysis and control samples.

Controls

The results related to the MP concentration were corrected using the blank samples, and the percentage of nonplastic materials determined by micro-Raman spectroscopy. All sampling and analyzing equipment, including sampling containers, beakers, vacuum set, and Petri dishes, were glass types and acid-washed to ensure that they were not contaminated. In addition, all equipment was rinsed three times with distilled water and covered with aluminum foil. Air movement in the laboratory was minimized by closing all windows and doors, and all analyses were performed under the hood with a laminar flow. The working surface was cleaned with ethanol 70%.[5,12]

Results

A total of 90 spectra were obtained from the samples using micro-Raman and used for characterization of MPs. The most abundant polymer identified were polyethylene, polypropylene, and polyethylene terephthalate, respectively.

The proportion of different polymers in crystallized salt, refined sea salt (food grade), unrefined sea salt, and rock salt is shown in Figure 1. Twenty percent of the particles in crystallized salt, 12.5% of particles in refined sea salt (food grade), 8.3% of particles in unrefined sea salt, and 8.3% of particles in rock salt had unknown spectra, which were reduced from the MP concentration in salts. Examples of micro-Raman spectra are shown in Figure 2.

The MP concentrations in crystallized salt, refined and unrefined sea salt, and rock salt were 151.4 ± 48.8, 406.7 ± 93.3, 1288.6 ± 184.9, 283.4 ± 97.0 MPs/kg, respectively. Blank samples contaminated with 22 ± 3.56 MPs and MP concentrations in salt samples were corrected using blank samples. Fiber and fragment were the MPs shapes, and the fiber was the dominant shape of MPs in all samples. The percentage of MPs based on sizes and shapes is shown in Figure 3, and the microscopic images are shown in Figure 4.

Discussions

This study demonstrated that all salt samples from Iran were contaminated with MPs, like all salts from other countries such as China, India, New Zealand, Germany, Denmark, and Indonesia.[9] The MP concentration and the analytical methods used to identify and characterize the MPs in other studies are presented in Table 1. Since there are no standard methods for MPs analysis,[19] the methods for measuring and identification of MPs have been varied in studies. The different methods can affect the number of identifiable MPs[20] and make it difficult to compare the study's results. As can be seen in Table 1, the MP concentrations have been varied from 1 MPs/kg in Karami *et al*.'s study[21] to 31,680 MPs/kg in Renzi *et al*.'s study.[22] As mentioned, this difference may be due to the different methods and salts used in the studies.

Selvam *et al*. noted that 60% of MPs in sea salts were smaller than 100 µm.[14] In our study, we found that 94% and 84% of refined and unrefined sea salts were smaller than 100 µm, respectively. This may be due to the use of a small pore size filter (0.45 µm) in our study. Using such small pore size

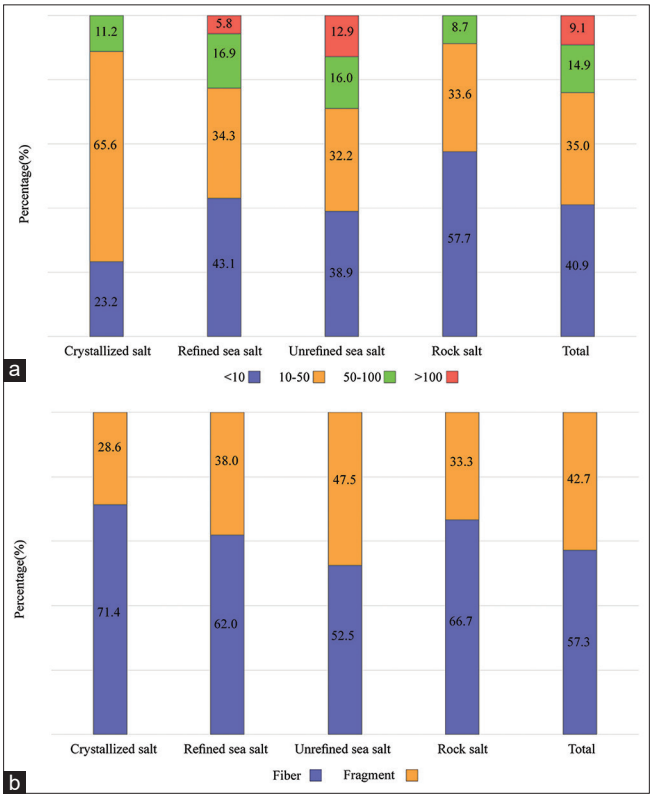

Figure 3: The percentage of microplastics based on sizes (a) and shapes (b) in crystallized salt, refined sea salt (food grade), unrefined sea salt, and rock salt

Table 1: Abundance and measuring methods of microplastics in other studies related to salts

Study	Salt type	Digestion	Density separation	Particle size (µm)	Detection	MPs concentration (MPs/kg)
Yang *et al*.[28]	Sea salt	WPO	NaCl	>5	Visual/FTIR	550-681
	Lake salt					43-364
	Rock/well salt					7-204
Karami *et al*.[21]	-	-	NaCl	>149	Visual/micro-Raman	1-10
Kim *et al*.[29]	Sea salt	WPO	-	100-5000	Visual/FTIR	674
Renzi and Blašković[22]	Table salt from Italian marine	-	-	4-2100	Visual/FTIR	1570-8230
	Table salt from Croatian marine			15-4628		27,130-31,680
Lee *et al*.[15]	Table salt	-	-	-	Visual/FTIR	9.77
Renzi and Blašković (2019)[30]	Table salt from Italian marine	-	-	10-150	Visual/FTIR	170-320
	Table salt from Croatian marine					70-200
Sathish *et al*. (2020)[23]	Sea salt	WPO	-	>0.8	Visual/SEM/FTIR	35±15-72±40
	Bore-well salt					2±1-29±11
This study (2021)	Crystallized salt	CWPO	NaCl	>0.45	SEM/micro-Raman	151.4±48.8
	Refined sea salt (food grade)					406.7±93.3
	Unrefined sea salt					1288.6±184.9
	Rock salt					283.4±97.0

MPs: Microplastics, WPO: Wet peroxide oxidation, CWPO: Catalytic WPO, FTIR: Fourier transform infrared, SEM: Scanning electron microscopy, NaCl: Sodium chloride

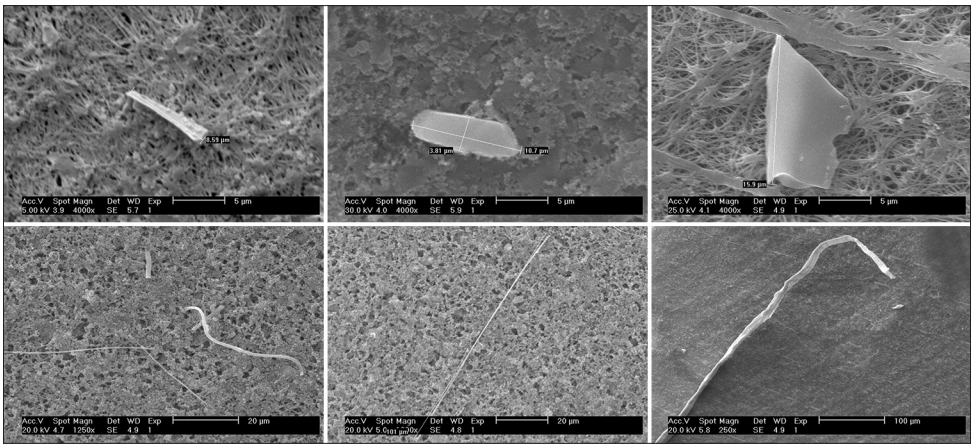

Figure 4: Microplastic images captured by scanning electron microscopy

filters improves results since MPs contain particles as small as 1 μm. MPs 10–50 μm were dominant in crystallized salt, despite other salts which MPs <10 μm were dominant. To the best of our knowledge, a sieving step is performed in the crystallization process of salt, which can reduce small-sized MPs in the crystallized salt.

The most abundant polymer identified in this study were Polyethylene, polypropylene, and polyethylene terephthalate, which is consistent roughly with the production volumes of these plastics,[12] and approximately similar results were obtained by Sathish *et al*.[23] Since micro-Raman spectroscopy can identify small material as small as 1 μm,[24,25] it seems that using this method is better than other methods.

We found four MP shapes in our other studies on drinking water, including fiber, fragment, oval, and spherical, but we did not found any oval and spherical MPs in this study, and fiber and fragment were the only MPs shapes that we found. The fiber was the dominant shape of MPs in all samples. Similar results have been obtained by Sathish *et al*.[23] Fadare *et al*.[26] found that 93.8% of MPs in salts were fibers. In our study, the highest percentage of fibers was observed in crystallized salts, and on average, 57.6% of MPs in all samples were fibers and others (42.4%) were fragments.

Despite the high concentration of MPs in all salt samples, due to the lower concentration of MPs in crystallized salts, it seems that crystallized salts are safer to use than others. It should be noted that there is no an established standard value for MPs in food,[27] and efforts should be made to determine the effect of MPs on human health and to standardize MPs measuring methods.

Conclusions

This study reveals the presence of MPs in crystallized salt, refined sea salt (food grade), unrefined sea salt, and rock salt. The concentrations of MPs in these salts were 151.4 ± 48.8, 406.7 ± 93.3, 1417.4 ± 203.3, 283.4 ± 97.0 MPs/kg, respectively. SEM photographs showed that two different shapes of MPs were fiber and fragment. The fiber was the dominant shape of MPs in all samples. The most abundant polymers were polyethylene, polypropylene, and polyethylene terephthalate, respectively.

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Conflicts of interest

There are no conflicts of interest.

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