

## Investigation of Microplastic Contamination in Vietnamese Sea Salts Based on Raman and Fourier-Transform Infrared Spectroscopies

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## Abstract

Microplastics (MPs) are plastic items smaller than 5 mm as a result of the degradation of plastic products and wastes in the environment. They have been encountered in a variety of sea products, including sea salts. The aim of this research is to investigate the microplastic contamination in Vietnamese sea salts based on Raman and Fourier-Transform infrared (FT-IR) spectroscopies. A total of 14 salt brands and no-name salt bags collected randomly at six urban and suburban groceries in Ho-Chi-Minh city were analyzed. The results showed the presence of polymers in the decreasing percentages - PET, PE, PP, PVC, PS, PA-6, and PMMA. there was a higher fluctuation in MPs amount in non-branded salts compared with branded salts. However, the average of MPs was fairly similar – 133.62 items/kg, corresponding to 487.71 items entering the human body per year via salt consumption. All these obtained results revealed MPs pollution in Vietnam sea salts may come from seawater. This study supplied more information about MPs pollution in the sea and for environmental research and food safety.

*Keywords:* Anthropogenic microfibers; Sea salt contamination; Raman and Fourier-Transform infrared spectroscopies; Can Gio Vietnam

## **1. Introduction**

Plastic pollution has been one of the major global environmental concerns due to the triple increase in amount of the global plastic waste (Lebreton and Andrady, 2019). Vietnam is in world-top 5 countries with the estimated amount of plastic waste discharged into the sea from 0.28 to 0.73 million tons/year (Le, 2019). Plastic waste experienced degradation in silent ways in the environment by breaking into tiny pieces called micro plastics (MPs). They are classified into primary meso plastics (<5 cm) such as beads from cosmetics, pellets used in material manufacture, and secondary micro-plastics as

a result of physical, chemical and biological fragmentation of larger plastics (Urbanek *et al.*, 2018). The later form can be seen in a heterogeneous mixture of diverse shaped plastic materials such as pellets, fragments, fibers and other shapes in the range of  $0.1 - 5000 \,\mu\text{m}$  (Hidalgo-Ruz *et al.*, 2012). They have a variety of colors and shapes commonly particles and fibers depending on their sources and relevant degradations (Rezania *et al.*, 2018). Light micro debris can spread over long distances and distribute in water levels by aquatic wind-wave-driven circulation, leading to pollution in places far

away from pollution sources (Urbanek *et al.*, 2018). Hence, an exact identification of micro plastic pollution sources and their behaviors is still an extreme challenge.

Although MPs have been found in various types of samples, it is difficult to compare their abundance exactly owing to no standardized MPs analysis procedures at this moment. A typical workflow comprises physical and spectroscopic qualification. The anthropogenic debris could be directly seen under a stereomicroscope, or a dissection microscope after stained with Rose Bengal (Kosuth et al., 2018), or a fluorescence microscope after incubated with fluorescent dye Nile Red. Although Nile Red method is highly sensitive, biogenic materials such as lipids and chitin creating fluorescent signals will interfere the plastic detection (Erni-Cassola et al., 2017). Thus, this method is preferable for environmental than biota samples. In the meanwhile, the Fourier Transform infrared (FT-IR) spectroscopies have become a conventional tool to determine MPs in bivalves, honey and sugar, tap water and beer (Kosuth et al., 2018), drinking water, table salt and air (Zhang et al., 2020). Recently, Raman has attracted more attention in the literature due to more advantages than FT-IR. Raman is considered to replace for FT-IR thanks to better resolution, wider spectral coverage and lower water interference (Ribeiro - Claro et al., 2017), and was employed to identify 16 - 84 items/kg MPs in table salt of Turkey (Gündoğdu, 2018) and other countries (Karami et al., 2017).

There are no salt mines in Vietnam and the salt manufacture relies on water evaporation and salt crystallization from seawater under the prolonged exposure to strong sunlight, favorable wind and moisture on thousands of hectares of open ponds along with coastal provinces from Northern, Central Coast to Southern (Bac Lieu, Ben Tre, Vung Tau and Can Gio). Thus, table salt is one of the main transport pathways of substances including MPs from aquatic environment to human. In addition, airborne, refinery, packaging and transportation are highly feasible contamination sources since sea-salt manufacturing processes are open. Sever studies have reported the presence of MPs in 94% of salts tested worldwide (Lee *et al.*, 2019). The MPs abundance in table salts varied with countries, from 9.7 MPs/kg (PP, PE, PET) in Taiwan salts (Lee *et al.*, 2019), 56-103 MPs/kg (polyesters, PET, PP, PA, PE and PS) in Indian salts (Seth and Shriwastav, 2018) to 50-280 MPs/kg (mostly PE and PP) in Spain (Iñiguez *et al.*, 2017).

There are three salt types on Vietnamese market: crystalized salt coarse, fine salt refined by companies, and iodized salt. An average of 5 g per day for a healthy adult is recommended by The World Health Organization (Peixoto et al., 2019), and MPs entered in the human body with a large amount,  $(0-7.3) \times 10^4$  items annually via salt consumption (Zhang et al., 2020). The trend in salty food consumption is increasing in several nations including Vietnam with up to 10 g/day (Jensen et al., 2018) because salts are the main component in almost Vietnamese kinds of spices. Though, the research of MPs in Vietnamese marine salts is still very rare. Up to now, only study of Kim et al. (2018) used FT-IR to detect MPs in two salt brands of Vietnam. Focus on these points, this study aims to quantify the MPs in all salt types of branded and non-branded salts in Ho-Chi-Minh city by Raman microscope and FT-IR for further prediction of MPs accumulation in the human body via salt digestion.

## 2. Materials and Methods

### 2.1 Sampling

A total of 14 brands were purchased from urban and suburban markets of Ho-Chi Minh city. The commercial names are kept confidential and marked by alphabetically letters. Branded products are labeled with ingredients, production place and expiration date. The salts were categorized into two types: coarse (P), refined (R) including non-iodized and iodized (Table S1). In addition, refined salts are manually packaged in 1 - kg plastic bag without any descriptions and sold in local markets (Figure 1). They are regarded as non-branded salts.



Figure 1. Vietnamese commercial sea salts branded (a) and non-branded (b)

#### 2.2 Sample preparation procedure

A weight of 200 g of salts was transferred into a glass bottle. The sample in one bottle was a replicate, and three replicates were processed to receive representative results of each brand. A particular volume of filtered water was added and stirred until obtaining the homogenized brine solution. Then 30% hydrogen peroxide was added and stirred at 70 - 80°C until the solution was discolored, and stored in dark at room temperature  $(30 \pm 5^{\circ}C, 60 \pm 10\% \text{ moisture})$  overnight.

The extraction was carried out on the funnel so that micro-particles could be captured in the center of filter papers. The matter in the solution was captured on 1.6-µm pore size, 47-mm diameter glass fiber (GF) membrane (GE Healthcare Whatman). It depends on the capacity of filter paper, for each sample, the filtration was divided into stages: (i) light and floated MPs; (ii) heavy MPs mixed with undissolved impurities. All filter membranes were kept in Petri dishes, carefully wrapped and let for drying at room temperature for further analysis.

#### 2.3 Microplastic analysis

The library included GF membrane, polyethylene terephthalate (PET) 300  $\mu$ m, low-density polyethylene (LDPE) 300  $\mu$ m, un-plasticized polyvinyl chloride (uPVC) 250  $\mu$ m, poly(methyl methacrylate) (PMMA) 85  $\mu$ m, and nylon-6 (PA-6) 15 – 20  $\mu$ m purchased from Goodfellow (UK); and, high-density PE (HDPE), polypropylene (PP), polystyrene (PS) and acrylonitrile butadiene styrene (ABS) pellets from Vietnam plastic production companies.

The MPs analysis is subject to determine individual particles by using a Raman microscope (XploRA Horiba Scientific). The microscopic mode was used to observe shape and color and their sizes were manually calculated. The number of pieces was noted down and classified into shape, color categories (Hidalgo-Ruz et al., 2012). The Raman spectrometer included a single beam laser operating at 532 nm of wavelength coupled with a charge-coupled device (CCD) detector. Every individual particle was analyzed in a wavelength range of 50 to 3600 cm<sup>-1</sup> with an acquisition time of 15 seconds and a grating of 900 lines per mm.

For larger pieces with complex Raman spectra, the FT-IR spectroscopy (PerkinElmer Frontier, USA) was used to reaffirm the polymer type. The visible item was transferred onto KBr membrane and its IR spectrum was recorded in the range of mid-infrared wavelength ( $450 - 4000 \text{ cm}^{-1}$ ) in both transmission and absorbance modes.

#### 2.4 Quality control

Two types of blank samples were tested simultaneously to correct the potential procedural contamination. The air controls were clean filter membranes on glass Petri dishes around the working area. The water controls were processed by the same sample procedure with filtered water.

#### 2.5. Statistical analysis

One-way analysis of variance (ANOVA) was chosen to establish differences in MPs abundance (items/kg) between products. A Turkey's multiple comparison test was used analyze the mean differences. All statistical analyses were performed at 95% confidence interval on SPSS v22 software.

## 3. Results and Discussion

### 3.1 Evaluation of sample handling process

There are different sample treatment procedures to extract MPs from sea salts. The whole handling process is generally so time - consuming and, in some cases, relatively complicated. In fact, the steps were based on properties of each particular sample. Depending on the cleanness of salt samples, hydrogen peroxide is necessary to oxidase dissolved organic compounds and discolored the solution mixture to a large extent, which is beneficial for the following MPs floatation and filtration. The solution discoloration and matter distribution was clearly observed after overnight static incubation (Figure 2c of G - branded salt), compared with the status once peroxide was added (Figure 2b). The results show that the oxidation and amount of oxidants varied by samples, particularly large amount of hydrogen peroxide was required for non-branded and some of branded products.



**Figure 2.** The oxidation steps in the samples preparation: (a) before oxidation, (b) once adding H<sub>2</sub>O<sub>2</sub>, (c) after oxidation – brand G



**Figure 3.** Microplastics found in the supernatant of brand G (a, b), and in the sediment of brand N (c, d). Pink circles are visible stone grains, black circles are visible undissolved salt particles, blue circle is invisible fiber which was observed at the magnification x10 under Raman microscope

The insoluble impurities are the main interference. The remaining products such as algae fibers floating on the surface could be removed easily after oxidation, while others tend to experience temporal accumulation, commonly sedimentation in the water column (Wang et al., 2016). The MPs distribution in the mixture has changed over time and their movements are easily affected by physical forces during the incubation with hydrogen peroxide and filtration process. In addition, smaller MPs preferentially aggregate with other matter due to strong Brownian motion to form larger and heavier precipitated particles (Sun et al., 2020). Thus, long time oxidation is not recommended to minimize the aggregation of MPs with impurities. To avoid missing MPs, the extraction were carried out by stages depending on the capacity of filter membranes. The first stage called supernatant, and the last stage so-called sediment should be paid attention to. Filter membranes of supernatant filtrate had brighter (Figure 3a) with clearer micro plastic observation at x10 magnification (Figure b) than the sediment's membranes (Figure 3c, d). As many particles were on the membranes, physical methods were firstly applied for fast characterization. The suspected particles were analyzed with Raman spectroscopy.

### 3.2 Microplastic characterizations

MPs could be recognized by the physical appearance. Many impurities with certain shapes similar to MPs might be distinguished by the color. The impurities usually appeared in dim black color and unclear shapes (Figure 4a) while the color of MPs should be clear and homogenous (Figure 4b) (Hidalgo-Ruz et al., 2012). Since plastic products were produced with various colors, colored particles such as yellow or pink would be highly potential MPs. The color was categorized in 4 groups - (i) light color, (ii) mid color, (iii) dark color (Hidalgo - Ruz et al., 2012). If the filter membrane was seen through homogenous pieces, the pieces were called colorless (group iv). Brown debris was investigated carefully to avoid the false identification with sand or mud particles which tended to gather and overlap plastic particles (Figure 4c). A melting test was applied to separate sand, salt particles from visible whitish and brown MPs (De Witte et al., 2014)

and in some cases, the real colors were slightly different from visible colors (Figure 4d). A very hot needle pointed directly at particle bundles and plastic debris will produce melting odor or get curl especially fibers. If bundles were broken, they were not plastics. If they changed the cellular structure, twisted and stuck around the needle, they would be microalgae filaments.

Microplastics were classified into two shape groups: (i) fibers and (ii) non-fibers. In the former group, pieces are fibrous or thin uniform plastic strands, but not segmented (Zhang et al., 2019). The fibers should be equally thick throughout their entire length so that Raman spectra could be exactly produced (Figure 4e). If the fibers were too thin and segmented, they would be organic fibers (Figure 4f). In later group, MPs were observed in pellets (rounds with solid structure, cylindrical, flat, sphere, ovoid), fragments (round, sub - round), or other shapes (rectangle, triangle, angular, sub-angular). The last care is overlapping between particles. Invisible MPs, especially microfibers were encountered on the surface of larger MPs (Figure 4g).

The polymer identification is based on specific bands caused by vibrations of the functional groups (Table 2). An example is taken in Figure 5. In the initial observation, it is a pink non-fiber fragment. As the piece was seen by the naked eyes (1 mm in length), it could be analyzed by both FT-IR and Raman microscope. In Raman spectrum (Figure 5a), a medium band at 1440.82 cm<sup>-1</sup> is subjected to -CH<sub>2</sub>- bending. The last signal is a strong band at 2852.09 cm<sup>-1</sup>, which assigns to stretching vibrations of C - H bonds. The second intensive band at 830.103 cm<sup>-1</sup> is in the border of 550 - 800 cm<sup>-1</sup> range of vibrations of C - Cl bonds. However, a different study assigned stretching vibrations of C - Cl bonds to bands at 635 and 695 cm<sup>-1</sup> (Solodovnichenko et al., 2016). The comparison with the library shows its spectrum looks like the spectrum of PVC and PS. Hence, it is necessary to use an additional method to confirm the polymer type. Three regions should be taken into account in FT - IR spectrum (Figure 5b). Firstly, 1200-1400 cm-1 is assigned to C - H bonds, particularly medium peak at 1426.57 cm<sup>-1</sup> in bending structure of CH<sub>2</sub>-, 1254.87 cm<sup>-1</sup> (Ul-Hamid *et al.*, 2015)

and 1322.96 cm<sup>-1</sup> of C-H bending (Jung *et al.*, 2017). This medium band of CH<sub>2</sub>was also observed in Raman spectra at 1440.82 cm<sup>-1</sup>. The CH<sub>2</sub>- rocking is present at 961.65 cm<sup>-1</sup> (Ul-Hamid *et al.*, 2015). A relative small band at 1094 cm<sup>-1</sup> is assigned to C - C stretching. The most important band is 614.30 cm-1 corresponding to C - Cl stretching (Ul - Hamid *et al.*, 2015; Jung *et al.*, 2017). This fragment is now believed to be PVC as only PVC has C - Cl bonds amongst the plastic types within this study scope.



Figure 4. The recognition of micro plastics by naked eyes and stereomicroscope: (a) Blurred items were discarded, and b) Shaped items were highly potential MPs; (c) Distiguish mud and sand particles with microplastics under microscope; (d) Distinguish white salt particles and non-plastic items with visible MPs by smelting test and observe color of MPs under the stereomicroscope; (e) Anthropogenic microfibers and f) Organic bundle of fibers; (g) The overlap between micro plastics.

| Polymer  | IR bands (cm <sup>-1</sup> )                                    | Raman bands (cm <sup>-1</sup> )   |  |  |  |
|--|---|---|--|--|--|
|  | and bond assignment   | and bond assignment   |  |  |  |
|  | 2852.89: C–H stretch (a)  | 2901.35: -CH <sub>2</sub> - stretch (b)   |  |  |  |
| Polv-  | 1467.56: -CH <sub>2</sub> - bend (a)                            | 1438.09: -CH <sub>2</sub> - wag (b)   |  |  |  |
| ethylene   | 720.47: $-CH_{2}$ rock (a)                                      | $1292.38: -CH_2 + twist (b)$  |  |  |  |
| (PF)   | 720.17. CH2 HOOR (u)  | 1124 35: C-C stretch (b)  |  |  |  |
| $(\mathbf{I} \mathbf{L})$  |   | 1061.20; C C stretch (b)  |  |  |  |
|  | (E:- C0)  | $(E_{in} \subseteq I)$  |  |  |  |
|  | (Fig S8)  | (Fig SI)  |  |  |  |
|  | 2919.02: $-CH_2$ - stretch (c),                                 | 2879.46; 2882.60; 2885.75:  |  |  |  |
|  | 2838.94: C–H stretch (a)  | $-CH_3$ stretch (c)   |  |  |  |
|  | 1462.76: –CH <sub>2</sub> – bend (a, c)                         | 1454.4: $-CH_2$ - deformation (i)   |  |  |  |
| Poly-  | 1376.5:CH <sub>3</sub> bend (a, c)                              | 1293.92: C–C stretch (c),   |  |  |  |
| propylene  | 973.02: C-C stretch (a)   | C–H deformation (i)   |  |  |  |
| (PP)   | 840.81: $-CH_2 - rock(a)$                                       | 1125.93:CH3 rock (c)  |  |  |  |
|  | C-CH <sub>2</sub> stretch (a_c)                                 | 836.96: $-CH_{2}$ -rock (i)   |  |  |  |
|  | e elli, sueten (u, e)   | $C_{-CH_2}$ stretch (i)   |  |  |  |
|  | $(E_{i\alpha} \in C_{i\alpha} \in F_{i\alpha} \in C_{i\alpha})$ | (Eia S2)  |  |  |  |
|  | (Fig 39a, Fig 39b)  | (Fig.52)  |  |  |  |
|  | 2854.06: C-H stretch (e)  | 2852.09: C-H stretch (d)  |  |  |  |
|  | $1426.57: -CH_2 - bend (a, d)$                                  | 1440.82: $-CH_2$ - bend (d, f)  |  |  |  |
| Polyvinyl  | 1254.87: C-H bend (a),  | 1302.88: (d)  |  |  |  |
| chloride   | 1094: C–C stretch (a)   | 830.10: C–Cl (f)  |  |  |  |
| (PVC)  | 961.65: C–H wag (e)   | 355.95: (d)   |  |  |  |
|  | 614.30: C-Cl stretch (a)  |   |  |  |  |
|  | (Fig S10)   | (Fig S3)  |  |  |  |
|  |   | 1725.63: C=O stretch (g, h)   |  |  |  |
|  |   | (g, n)  |  |  |  |
| Poly-<br>ethylene  |   | 1611.09: 🖙 C=C, stretch of 1,4-para   |  |  |  |
|  |   | di-substituted benzene ring (h)   |  |  |  |
|  | Not applicable  | 1446.05: C-O (g)  |  |  |  |
| taraphthalat   |   |   |  |  |  |
| terephinaiat   |   | 855.727; C=C (g)  |  |  |  |
| e (PET)  |   | 855.727: C=C (g)  |  |  |  |
| e (PET)  |   | 855.727: C=C (g)<br>632.97: (g)   |  |  |  |
| e (PET)  |   | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )  |  |  |  |
| e (PET)  |   | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)   |  |  |  |
| e (PET)  |   | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: -CH deformation (i)   |  |  |  |
| e (PET)  | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: -CH <sub>2</sub> - deformation (i)<br>997.74: aromatic ring breathing (i)   |  |  |  |
| e (PET)<br>Polystyrene<br>(PS)   | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: -CH <sub>2</sub> - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616 01: aromatic C LI deform (i)   |  |  |  |
| e (PET)<br>Polystyrene<br>(PS)   | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: -CH <sub>2</sub> - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)  |  |  |  |
| e (PET) Polystyrene (PS)   | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>(Fig S4)<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>(Fig S5)  |  |  |  |
| e (PET) Polystyrene (PS)   | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: -CH <sub>2</sub> - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)   |  |  |  |
| Polystyrene<br>(PS)<br>Poly  | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)  |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl   | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)  |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl<br>methacrylat  | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)   |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl<br>methacrylat<br>e) (PMMA)                                       | Not applicable<br>Not applicable                                | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>601.49: O-C=O deformation (i)  |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl<br>methacrylat<br>e) (PMMA)                                       | Not applicable<br>Not applicable                                | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>601.49: O-C=O deformation (i)<br>( <i>Fig S6</i> )   |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl<br>methacrylat<br>e) (PMMA)                                       | Not applicable<br>Not applicable                                | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>601.49: O-C=O deformation (i)<br>( <i>Fig S6</i> )<br>2903.09: $-CH_2$ - stretch (f)   |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl<br>methacrylat<br>e) (PMMA)                                       | Not applicable<br>Not applicable                                | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>601.49: O-C=O deformation (i)<br>( <i>Fig S6</i> )<br>2903.09: $-CH_2$ - stretch (f)<br>1441.6: $-CH_2$ - (i, f)  |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl<br>methacrylat<br>e) (PMMA)<br>Polyamide-<br>6 (PA-6)             | Not applicable  | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>601.49: O-C=O deformation (i)<br>( <i>Fig S6</i> )<br>2903.09: $-CH_2$ - stretch (f)<br>1441.6: $-CH_2$ - (j, f)<br>1437.79: $-CH_2$ - bend (f, i)  |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl<br>methacrylat<br>e) (PMMA)<br>Polyamide-<br>6 (PA-6),<br>nylon 6 | Not applicable<br>Not applicable                                | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>601.49: O-C=O deformation (i)<br>( <i>Fig S6</i> )<br>2903.09: $-CH_2$ - stretch (f)<br>1441.6: $-CH_2$ - (j, f)<br>1437.79: $-CH_2$ - bend (f, j)<br>928.406: CH - twict (i)                           |  |  |  |
| Polystyrene<br>(PS)<br>Poly<br>(methyl<br>methacrylat<br>e) (PMMA)<br>Polyamide-<br>6 (PA-6),<br>nylon-6 | Not applicable<br>Not applicable                                | 855.727: C=C (g)<br>632.97: (g)<br>( <i>Fig S4</i> )<br>1604.24: aromatic C=C stretch (i)<br>1450.09: $-CH_2$ - deformation (i)<br>997.74: aromatic ring breathing (i)<br>616.91: aromatic C-H deform (i)<br>( <i>Fig S5</i> )<br>1723.71: C=O stretch (i)<br>1447.51: $-CH_2$ - deformation (i)<br>1181.31: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>808.67: C-C-C-C stretch (i)<br>601.49: O-C=O deformation (i)<br>( <i>Fig S6</i> )<br>2903.09: $-CH_2$ - stretch (f)<br>1441.6: $-CH_2$ - (j, f)<br>1437.79: $-CH_2$ - bend (f, j)<br>928.406: $-CH_2$ - twist (j)<br>( <i>Fig S7</i> ) |  |  |  |

Table 1. IR and Raman band assignments of seven investigated polymers

(a) Ung et al (2017); (b) Daniel and Hélio (2019); (c) Gopanna et al (2018);

(d) Solodovnichenko et al., 2016; (e) Anwar Ul-Hamid et al. (2015);

(f) Gündoğdu (2018); (g) Käppler et al. (2015); (h) Alexiou et al. (2020);

(i) Bruckmoser et al. (2015); (j) Milani (2015)



Figure 5. Polymer identification of a visible potential microplastic: Raman spectrum (a) and FT-IR spectrum (b).

## 3.3. The microplastics abundance in commercial sea salts

As seen in Figure 6, the proportions of MPs were generally below 50% of the total number of potential plastics identified by microscopic observation in individual sediment and supernatant parts. More importantly, the majority of MPs were partitioned in the supernatant. For instance, the percentage of MPs was only 3 - 5% sediment in G - brand salts, and no micro plastics were found in the sediment of other brands (E, F, J, K L, M). However, MPs accounted for high proportions in the sediment of several brands (A, B, I and N). Although the number of particles depositing in the sediment of non-branded products was higher than those of branded salts, the most of them were grain stones. These stone particles had similar appearances to plastic particles, for example, homogenous black, pink and yellow particles (Figure 3). Brand B is a special case. The vast sum of MPs was encountered in the sediment of salt coarse particle (B.P), while they tended to distribute in the supernatant of refined type (B.R sample).

The ANOVA analysis shows statistically significant differences in MPs amount between non-branded salts (n = 6, Sig. = 0.00185, Table S2b), possibly because contaminations came from different sources. The refined salt is more widely used in daily cooking and meals. Therefore, non-branded fine salts were manually produced on the household scales or local factories.

For this reason, the quality of non - branded salts was affected by not only the seawater where their pellets were crystalized but also the quality of local water sources, production environment where the salt coarse were ground and minced salts were packaged. As a result, the MPs quantities varied in the markets. Can Gio nonbranded salts contained a low level of MPs, only  $56.98 \pm 23.81$  items/kg. The MPs abundance in salts bought at the urban markets of Ho-Chi-Minh city varies in a small range  $(48.93 \pm 25.14 \text{ to } 119.07)$  $\pm$  71.80 items/kg). Nevertheless, there was a considerably high number of MPs in salts purchased at a suburban district, up to  $402.52 \pm 168.43$  items/kg (MM4). Indeed, the Turkey's test shows that only MM4 was statistically different from other non-branded samples (Sig. values < 0.014, Table S2c). Figure 7a shows that PET accounted for highest percentage (33.63%), followed by PE (24.78%), PS (20.35%) and PVC (8.85%). In contrast, PP and acrylic glass were not dominant. Almost nonfibrous items were fragments smaller than 50 µm, and their minimal size was 20 µm. Nevertheless, for fibrous structures, long fibers (  $>100 \ \mu m$ ) were predominant, and only there were few of fibers longer than 500 µm.

The plastic composition in brands was different from non-labeled salts. The period of use of branded salts is one to two years from the production date. The samples were analyzed within 5 months from the production date. Hence, the possibility of transferring PP micro plastics from polypropylene packages to the salts inside was fairly low. PET was again dominant (51.74%), followed by PE (23.7%) (Figure 7b). The findings are similar to Spanish (Iñiguez *et al.*, 2017) and Chinese salts (Yang *et al.*, 2015). It is suggested heavy PET debris tended to move to the bottom and remain with the salt during the salt crystallization because of high density (1.3 g/cm<sup>3</sup>) compared with PE (0.94 g/cm<sup>3</sup>) and PP (0.90 g/cm<sup>3</sup>) (Yang *et al.*, 2015).

On the other hands, MPs amounts did not demonstrate statistically significant differences between brands (n = 14, Sig. = 0.473 - ANOVA in Table S3b. In addition, the Turkey's test shows that there were no differences among branded products with the minimum Sig. value of 0.483 (Table S3c – Supplementary data). The salts had similar quality because the process and quality were controlled to meet Vietnam's food safety regulations. Nevertheless, in this group, the presence of MPs varies from pellet to refined salts, which reveals the effects of salt processing on MPs accumulation in salts to some extent. In refined salts, the amount of insoluble impurities was lower, thereby requiring simpler sample treatment.



Figure 6. The partition of micro debris in the saline mixture



Figure 7. Anthropogenic pieces in non-branded (a) and branded salts (b).

MPs might be removed along with the impurities. This is why refined type contained 140 plastic items slightly lower than the pellet type (178 items/kg) in brand A. In contrast, salts could be contaminated with MPs from the environment during the grinding and refinery, leading to the higher number of MPs in the refined salts (201 items) compared with 144 items/kg in salt pelelts of brand B. PVC fibers was most predominant in fine salts though PVC is not a common plastic in the sea water. Hence, PVC from water pine could probably intrude into the salt during the refinery. In Figure 8, PMMA did not appear in salt pellets, whilst small particles were present in refined salts. PE fibers dominated while PA-6 fibers were not found in the coarse salts. In contrast long fibers were found in fine salts that could come from the clothing of workers at the factory. PE, PVC and PMMA pieces were commonly in transparent and light to mid colors (white, blue), while PET and PS pieces were in mid to dark colors (blue, orange, purple). There was no presence of green and white-striped black items in all salts.

No MPs or impurities were seen in water control. Several black particles were on air controls, however, they were dust. MPs frequently appeared when the laboratory was so crowded, but in a small amount, only  $5 \pm 2$  particles/filter. It is strongly believed contamination from the laboratory coat did not occur because of no cotton fibers found

# *3.4. The enhanced toxicity of MPs-contaminated salt consumption*

The daily salt intake has increased by 50 times in the civilized societies, average from 9 to 12 g/day today. WHO recommends a healthy level of 5.1 g salt because an inappropriate salt/sodium intake has resulted in kidney diseases, hypertension, and mortality (Partearroyo *et al.*, 2019). The daily salt intake varies from genders, ages to countries, and is not fully conscious during cooking or at the table (Partearroyo *et al.*, 2019). Therefore, the exact amount of daily digested plastic items via salts cannot well established.

The annual MPs intake was calculated on daily salt consumption of 10 g/day (Table 3). Though MPs in Turkish sea salts were relatively low, the salt consumption was very high, up to 18.5 g per day, leading to a high number of 554.95 MPs consumed per year. In almost European countries, the daily salt consumption is 7 to 13 g/day in adults (Partearroyo et al., 2019), and an estimated of over 1000 plastic pieces could be digested via Spanish salts (Iñiguez et al., 2017). The MPs contents in Vietnamese, Indonesian and Korean commercial salts were similar (Kim et al., 2018), much higher than Taiwanese (9.77 particles/kg) (Lee et al., 2019). The amount of MPs in Chinese sea salts was highest, up to 718 particles/ kg (Yang et al., 2015) relevant to 2620 MPs ingested annually as effects of heavy plastic pollution in 81% of coastal regions according to the Chinese State Oceanic Administration (SOA) (Wang et al., 2019).



Figure 8. Composition and size of microplastics in branded coarse salts (a) and refined salts (b)

| Countries | No.<br>brand | Most common<br>shape and<br>sizes (µm)                               | Method         | Plastic types<br>(in decreasing<br>percentage)  | MPs<br>abundance<br>(items/kg)              | Annual<br>MPs intake<br>(items/<br>year) | Ref.                                    |
|-----------|--------------|--|----------------|---|---|--|---|
| Vietnam   | 2            | Fragments, fibers<br>100–5000  | FT-IR          | PE, PET, PP   | 100 - 200                                   | 365 - 730                                | Kim et<br>al.,<br>2018                  |
|           | 20           | $20 \le \text{fragments} \le 50$<br>$100 \le \text{fibers} \le 1000$ | Raman<br>FT-IR | $\begin{array}{c} \text{PET, PE,} \\ \text{PP} \sim \text{PA-6,} \\ \text{PS, PVC,} \\ \text{PMMA} \end{array}$ | 34.33 -<br>402.67<br>(133.62<br>in average) | 125 – 1469                               | This<br>study<br>(data<br>2020)         |
| China     | 15           | < 200  | FT-IR          | PET, PE, PP, cellophane   | 550 - 681                                   | 2007 -<br>2486                           | Yang et<br>al.,<br>2015                 |
| Turkey    | 5            | Fibers <100 - 1000   | Raman          | PU, PET~PP,<br>PE~PA-<br>6~PVC  | 16 - 84                                     | 58-307                                   | Gündo<br>ğdu,<br>2018                   |
| India     | 8            | Fragments < 500<br>Fibers < 2000                                     | FT-IR          | Polyesters,<br>PET, PA, PE,<br>PS   | 56 - 103                                    | 204 - 376                                | Seth<br>and<br>Shriwa<br>stav<br>(2018) |
| Spain     | 16           | Fibers<br>30 – 3500  | FT-IR          | PET, PE, PP   | 50 - 280                                    | 183 - 1022                               | Iñiguez<br><i>et al.</i><br>2017        |

 Table 3. Summarized information of in commercial sea salts and their annual intake via salt consumption route in the world

Humans are ingesting hundreds of MPs from salt alone. Nonetheless, the particular harmful effects of MPs on human health are still being investigated. MPs of 0.2 to 150 µm were believed to translocate across living cells to intestinal lymphatic and circulatory systems of human body (Hussain et al., 2001), but their mechanisms has not fully understood yet. A study reported the human's excretory system could itself eliminate over 90% of micro to nano - plastic debris via feces (Peixoto et al., 2019) while the 10% were absorbed into the blood steam and actually their behaviours in our body are well unknown. Bioaccumulation of non-degradable plastics is dangerous because they can be a home for microorganisms, hydrophobic organic substances and heavy metals.

### 4. Conclusions

Although there were differences in the number of MPs in brands and no - name salts, the average of MPs was finally similar (133.62 items/kg), which shows MPs contamination in seawater of coastal salt production locations was similar in Vietnam. PET was dominant followed by PE and PP, whilst PMMA was the least common. Vietnamese people are suffering from combination harmful effects of inappropriate salt consumption and hundreds of plastic particles via salt digestion every year.

The success of salt reduction depends on eating habits of consumers and objective factors such as availability, food accessibility and salt costs on the market. On Vietnamese salt market, in no-name bags varied noticeably, up to 402.67 items/kg, compared with maximum of 276.25 items/kg in branded salts. Hence, it is so necessary to add MPs parameter in food safety standards, control more strictly the quality and price of non - labeled salts as well.

## Supplementary data

Supplementary data are available at EnvironmentAsia Journal website.

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