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Extraction of microplastics from marine sediment in Burgas Bay, Black Sea

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The present study aims a comparative analysis of the effectiveness of three salt solutions for the extraction of microplastic pollution in marine sediment samples from the Burgas Bay, Black Sea. For this purpose, pre-purified sediments were artificially contaminated with known amounts of five different types of microplastics (high density polyethylene, polyamide, polyethylene terephthalate, polymethyl methacrylate, and polyvinyl chloride). Based on the density differences between sediment and microplastics, the extraction was carried out using salt solutions of sodium chloride (1.20 g/cm³), magnesium chloride (1.26 g/cm³), and calcium chloride (1.30 g/cm³). To identify and quantify micropolymer particles in the sediments, the samples were analyzed by μ-FT-IR and processed with the Purency Microplastics Finder software.

Keywords: extraction, microplastics, marine sediment, Burgas Bay, Black Sea, μ-FT-IR

INTRODUCTION

The problem of microplastics (MPs) contamination of soils, atmospheric air, drinking and seawater, sediment, biota, and food on a regional and global scale has been widely reported in the literature, and their presence in these environments increases the risk to various organisms and human health. The presence of MPs in water, sediment, and biota is used as an indicator for assessing the quality of the marine environment and its components, according to the EU Marine Strategy Framework Directive and the Oslo Paris Convention. It is believed that, in the aquatic environment, MPs tend to deposit and accumulate in sediments, and Simon-Sánchez *et al.* [1] suggest that the seabed is a major sink of MPs pollutants.

To assess the degree of MPs contamination in sediment samples and to ensure the comparability, repeatability, and reproducibility of the obtained data, it is necessary to create a standardized methodology that follows uniform protocols for sampling and methods for preparation, extraction, and analysis of MPs. However, such unified protocols and standard methods have not been developed to date, which makes it impossible to draw valid conclusions about the amount and nature of MPs contamination in sediments.

To date, various methods have been proposed for the extraction of polymer particles, ranging from methods based on density separation, methods based on density separation in combination with other techniques, extraction with solvent or petroleum, as well as complex methods based on oleophilic, electrophilic, or magnetic properties [2–6]. All of them differ in the chemicals used, operating time and efficiency, and the latter methods have limited application due to the expensive equipment required.

The technique that relies on the variations in densities between microplastics and the sediment matrix is the most commonly employed method and serves as the primary approach for isolating MPs in the majority of studies conducted thus far. The separation between sediments and MPs is achieved by preparing a salt solution with a fixed density, mixing them in an appropriate ratio, and allowing the mixture to stand for a certain period. In this case, the heavier particles of the sediments settle (their average density is 2.65 g/cm^3 [7]), while the lighter particles of synthetic polymers (their maximum density is 1.58 g/cm^3 [6]) float to the surface of the solution. The MPs can then be separated (filtered) and analyzed. In this process, the choice of the density of the extraction solution in relation to that of the polymer type is crucial [8].

Although density separation approaches are considered easy to apply, to achieve a good degree of extraction of MPs, it is necessary to use salt solutions with appropriate densities depending on the type of polymers [8]. Furthermore, the extraction of MPs from real samples can be affected by a number of factors such as matrix complexity, sample

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volume and/or mass, different types and chemical compositions of polymer particles, sometimes with overlapping densities, a wide range of sizes and shapes, different degrees of aging, surface charge, hydrophobicity, presence of additives, contaminants, and residues of organic matter.

The aim of this study was to evaluate the efficiency of extracting MPs from marine sediment in Burgas Bay, Black Sea, by a method based on density differences using three different salt solutions – sodium chloride (NaCl), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$). To identify and classify the quantitative presence of MPs contamination in the sediment samples, the samples were analyzed by μ-FT-IR.

EXPERIMENTAL

Preparation of marine sediments and their contamination with microplastics

Marine sediments from the Burgas Bay, Black Sea, were preliminarily purified by thermal treatment at 850°C for 60 min. The samples were subsequently contaminated with particles of high-density polyethylene (HDPE), polyamide (PA), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), and polyvinyl chloride (PVC) in sizes 100 – 200 μm.

Extraction of micropolymers

The extraction of the MPs from the matrix was carried out by a method based on differences in density. Three salts with different densities of their saturated solutions were used: NaCl (ρ = 1.20 g/cm^3), $MgCl_2$ (ρ = 1.26 g/cm^3), and $CaCl_2$ (ρ = 1.30 g/cm^3). Saturated solutions were prepared and filtered. The extraction was carried out by the flotation method, in which the sediments were mixed with the selected salt solution and barbotated for one h at room temperature. The samples were left for 24 h until the sediment completely settled. This was followed by decantation of the solutions and additional treatment with 30% H_2O_2 on stirring at 400 rpm.

Filtration and drying

The floating fraction of the purified sediment samples contaminated with micropolymer particles was filtered using a vacuum pump and filtration system through an Anodisk (Al_2O_3) filter with a pore size of 0.2 μm. The filters were then washed with distilled H_2O and dried at 40°C. For comparison, a control sample of purified sediment and ultrapure water was made.

Identification of microplastics

The filters were analyzed by μ-FT-IR imaging microscope (LUMOS II, Bruker), a focal plane detector with dimensions of 32×32 and a spatial resolution of 5 μm was used. The scan was performed in the range of 4000 to 1000 cm^{-1} with a resolution of 5 cm^{-1}. The results of μ-FT-IR were processed with Purency Microplastics Finder software, which provides statistical information on the number, size, and type of all microplastic particles trapped on the filter – Fig. 1.

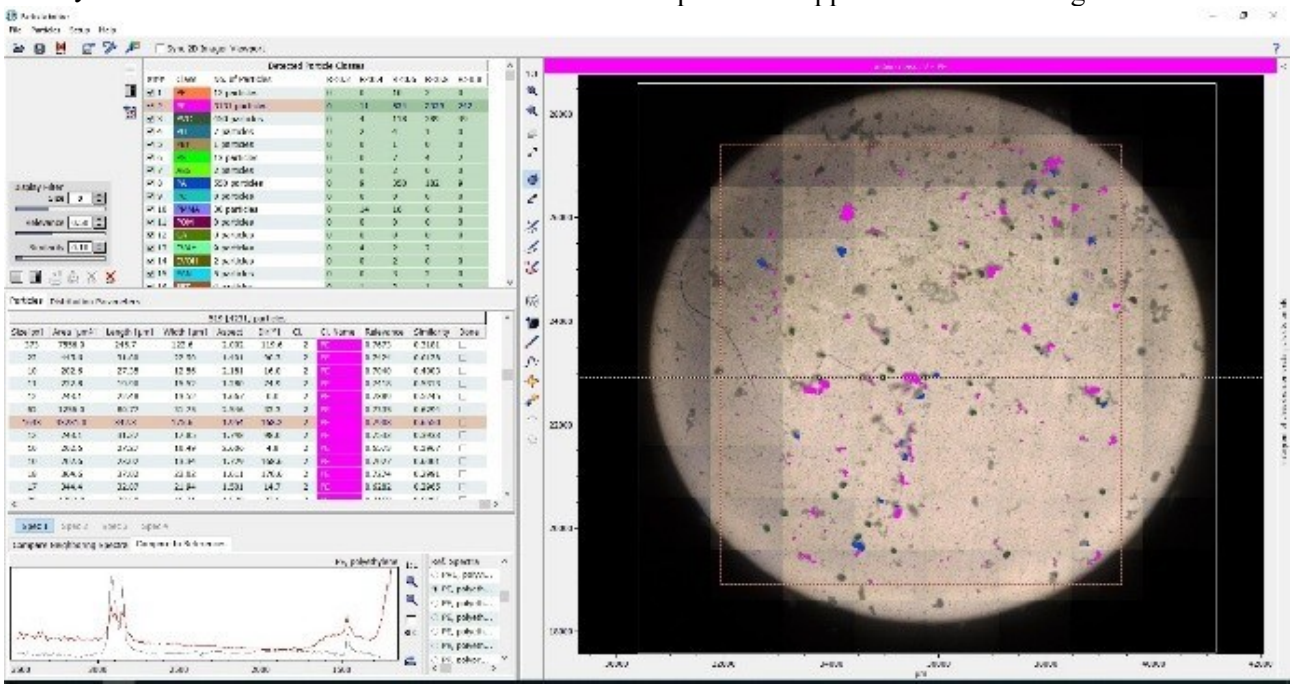

Fig. 1. Visualization of the μ-FT-IR results

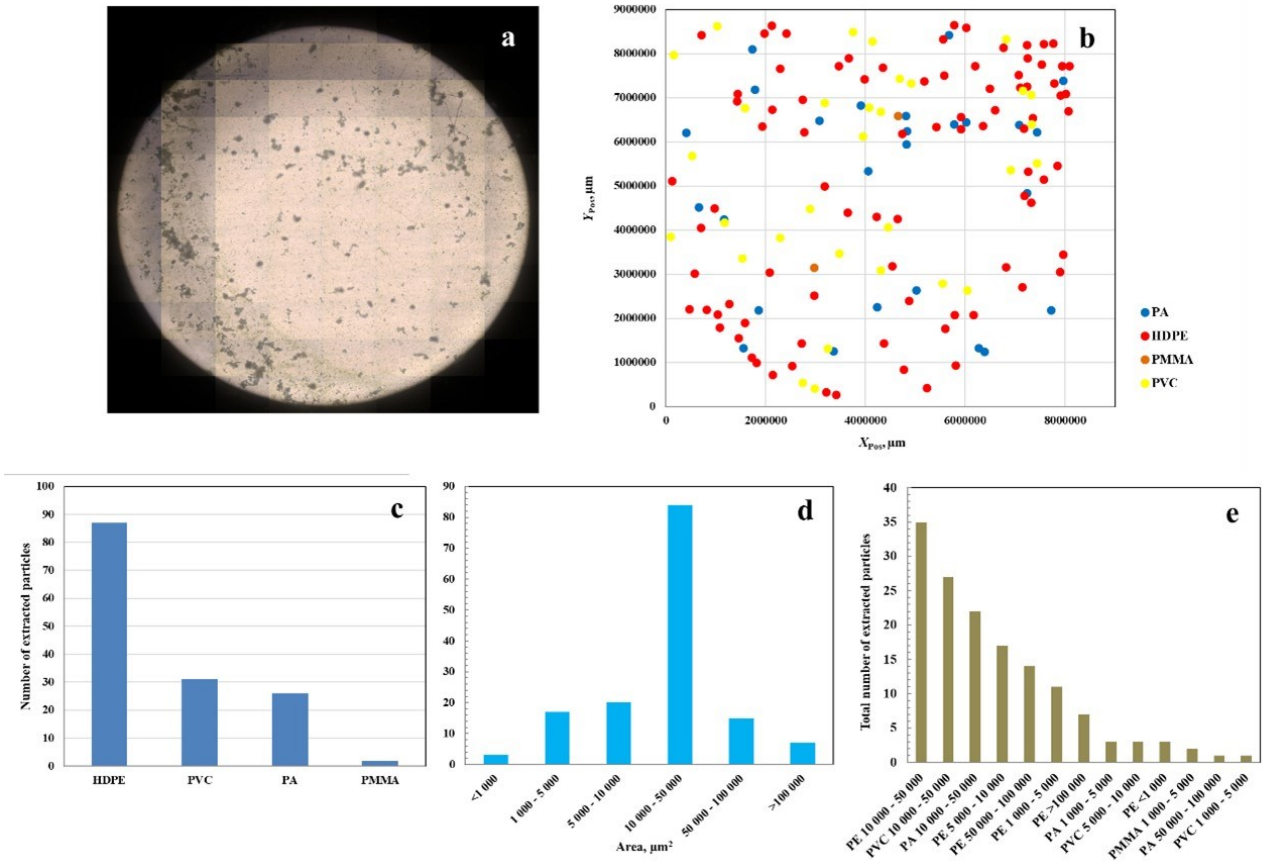

Fig. 2. Characteristics of the microplastic particles after extraction with NaCl: (a) microscopic image of the filter; (b) spatial distribution by polymer class; (c) distribution by polymer type; (d) distribution by area; (e) combined distribution by area and polymer type

The filters with the extracted MPs from the $CaCl_2$ extraction solution were further treated with ethyl alcohol due to the formation of a film on them, which makes it difficult to analyze with µ-FT-IR. The control sample, containing only purified sediment and ultrapure water, did not contain microplastics, confirming the effectiveness of the methodology in terms of avoiding external contamination.

RESULTS AND DISCUSSION

In this study, a detailed analysis of microplastic particles (HDPE, PVC, PA, PET and PMMA) extracted from pre-purified sediment using three salt solutions of different density (NaCl, $MgCl_2$, and $CaCl_2$) was performed. The µFT-IR data were analyzed in terms of the distribution of the total number of extracted particles from the respective extraction solution by the parameters: area, including polymer type and their combination (distribution of the total number of extracted particles by polymer type and area).

Analysis of microplastic particles extracted from NaCl solution

Figures 2 *a* and *b* present a microscopic image of the filter and the corresponding two-dimensional spatial distribution of the particles identified in the XY plane (in µm). Each point corresponds to the coordinates of a micropolymer particle on the filter, and the coloring shows the distribution of micropolymers by type. The type of all microplastic particles extracted from the sediment, based on the differences in their density and the NaCl solution, was determined with a µ-FT-IR imaging microscope and processed with Purency Microplastics Finder software. Possible areas with an increased concentration of the studied micropolymers were observed – Fig. 2 *b*. Spatially distributed throughout the filter are HDPE particles, followed by PVC and PA (evenly distributed, but fewer particles) and two single points of PMMA micropolymer particles.

After extraction of micropolymers from marine sediments from the Burgas Bay, Black Sea with NaCl solution, it was found that predominantly HDPE particles were extracted (87 pieces) – Fig. 2 *c*. This represents over half of all particles – 57%. PVC and PA particles were also successfully extracted, but in significantly smaller quantities – 31 and 26 pieces, respectively. PMMA – only 2 particles, and not a single extracted PET particle. The probable reason for the inability to effectively separate all types of plastic particles from NaCl

solution is its low density [9]. The insufficient density of the solution allows the extraction of polymers with low density (below 1.2 g/cm^3) [10], and denser polymers, in our case PET, are not extracted and/or are extracted poorly. This is in full accordance with the studies reported in the scientific literature, which determine NaCl as less effective compared to other salts [11–13].

The largest number of particles (about 85) falls in the range of 10 000 – 50 000 μm^2 – Fig. 2 *d*. There is a moderate amount of micropolymer particles from HDPE, PA, PMMA and PVC in the intervals of 1 000 – 10 000 and 50 000 – 100 000 μm^2. The smallest are particles with an area of <1 000 μm^2 and >100 000 μm^2. This means that the NaCl extraction process captures mostly medium-sized particles, which may be a limitation of the method. The absence of small-sized particles (below 1 000 μm^2) may lead to an incorrect determination of the total amount of microplastics in the sample, i.e., the method shows difficulty in extracting/identifying these particles and is probably not sensitive to them.

The combined plot of the total number of extracted NaCl particles by polymer type and area (Fig. 2 *e*) shows that HDPE particles with an area of 10 000 – 50 000 μm^2 (35 particles) predominate. The other significant groups are: PVC particles with an area of 10 000 – 50 000 μm^2 (27 particles) and PA with an area in the range of 10 000 – 50 000 μm^2 (22 particles). The smaller sizes, 1 000 – 5 000 μm^2 (PVC and PMMA), and the larger PA (50 000 – 100 000 μm^2) show a low presence of extracted particles, below 3 particles.

Analysis of microplastic particles extracted from $MgCl_2$ solution

Fig. 3 *b* shows the distribution of particles classified by chemical composition in the coordinate system (X_{Pos} – Y_{Pos}, μm). Analysis of the distribution of the polymers used in this study reveals areas of accumulation of microparticles with increased concentration (see Fig. 3 *a*) when a $MgCl_2$ solution is used to separate them from sediment samples.

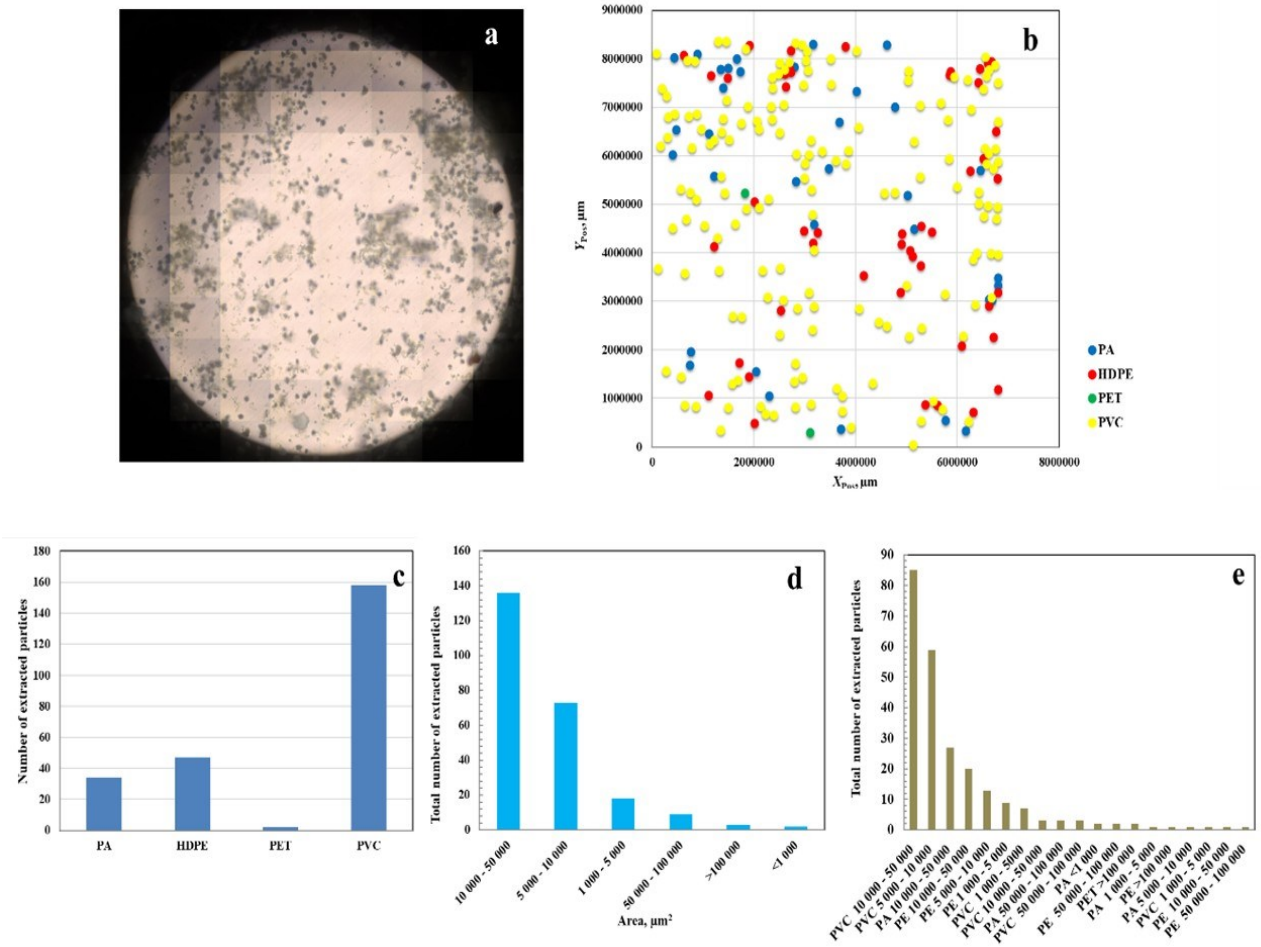

Fig. 3. Characteristics of the microplastic particles after extraction with $MgCl_2$: (a) microscopic image of the filter; (b) spatial distribution by polymer class; (c) distribution by polymer type; (d) distribution by area; (e) combined distribution by area and polymer type

When using a $MgCl_2$ solution to extract the added HDPE, PA, PET, PMMA and PVC particles to pre-purified sediment, 241 microplastic particles were identified. After classifying them by polymer type, it was found that the largest number of extracted PVC particles were: 158 particles, followed by HDPE - 47, PA - 34, PET only 2 particles and PMMA - 0 particles – Fig. 3 *c*. The dominance of PVC particles, which are denser than the others, suggests the effectiveness of the $MgCl_2$ solution. Since the density of the NaCl solution is lower than that of $MgCl_2$ [14], the latter proves to be suitable for density separation of microplastic particles from samples with a composition of HDPE, PA, PET and PVC, and could be a good alternative to more expensive and/or hazardous salts.

The following Fig. 3 *d* shows the distribution of the total number of extracted particles from the $MgCl_2$ solution by area. As can be seen, the majority of the total number of microplastic particles falls in the area range of 10 000 – 50 000 μm^2 (136 particles). The remaining particles can be grouped into the following categories: 5 000 – 10 000 μm^2 (73 particles), 1 000 – 5 000 μm^2 (18 particles), 50 000 – 100 000 μm^2 (9 particles), and >100 000 μm^2 (3 particles).

The combined plot of the distribution of the total number of extracted $MgCl_2$ particles by polymer type and area (Fig. 3 *e*) shows that PVC particles with an area of 10 000 – 50 000 μm^2 (85 particles) predominate. The other significant groups are: PVC particles with an area of 5 000 – 10 000 μm^2 (59 particles), PA with an area in the range of 10 000 – 50 000 μm^2 (27 particles) and HDPE, 10 000 – 50 000 μm^2 (20 particles).

Analysis of microplastic particles extracted from $CaCl_2$ solution

116 particles extracted from $CaCl_2$ solution were studied. The spatial distribution of the identified particles on the filter is shown in Fig. 4 *b*, and the image of the filter itself in Fig. 4 *a*. It can be seen that the distribution of the five types of polymers from the sediment samples contaminated with them is relatively more uniform and with fewer areas with accumulations of microparticles.

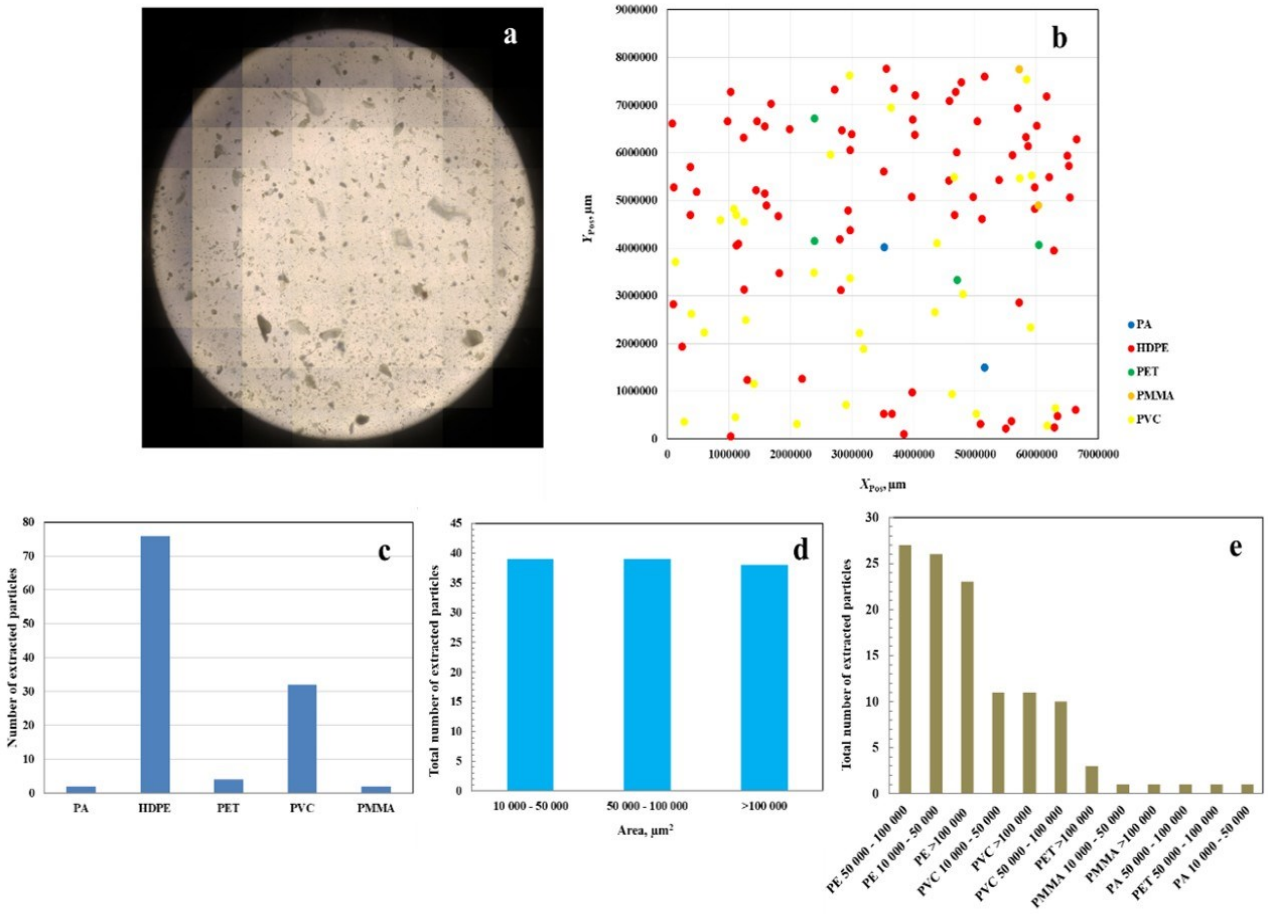

Fig. 4. Characteristics of the microplastic particles after extraction with $CaCl_2$: (a) microscopic image of the filter; (b) spatial distribution by polymer class; (c) distribution by polymer type; (d) distribution by area; (e) combined distribution by area and polymer type

Based on the summarized analysis of the particles, they are classified by polymer class (PA, HDPE, PET, PMMA, PVC), as well as by area. In the sediment sample extracted with $CaCl_2$, HDPE predominates with 76 particles (a little over 60%). This is followed by PVC with 32 particles (about 26), PET - 4 particles and with the lowest amount are PA and PMMA - only 2 particles of each were identified – Fig. 4 *c*.

From the dependence shown in Fig. 4 *d* it can be seen that the calcium chloride solution extracts micropolymer particles in three ranges of surface area: 10 000 – 50 000 μm^2, 50 000 – 100 000 μm^2 and >100 000 μm^2, with the number of particles being approximately the same. This is probably due to the additional treatment of the filter with ethyl alcohol, due to the formation of a film on it. Although the use of $CaCl_2$ for density separation has been reported [15, 16], since the density of the solution is 1.3 g/cm^3 [5], i.e. not much higher than that when using NaCl and/or $MgCl_2$, we believe that the extraction with $CaCl_2$ has limitations in this particular case.

The dependence in Fig. 4 *e* shows that the size distribution is relatively uniform, suggesting equal extraction efficiency and similar particle sizes. It was found that micropolymer particles of HDPE predominated in all sizes: 10 000 – 50 000 μm^2 (26 pieces), 50 000 – 100 000 μm^2 (27 pieces), and > 100 000 μm^2 (23 pieces). PVC particles were distributed in the above-mentioned area ranges as follows: 11, 10 and 11 pieces. The remaining polymers (PET, PMMA, PA) were present with 1 to 3 particles in each group.

CONCLUSIONS

The present study clearly demonstrates the influence of the extraction solution on the amount and type of extracted particles. The results obtained show that, due to its higher density, $MgCl_2$ is the most effective extraction solution for both a larger number and a variety of microplastic particles with different densities, including denser ones such as PVC and PET in a wide range of sizes, including fractions with an area below 10 000 μm^2. On the other hand, NaCl shows limited sensitivity to smaller particles and is ineffective for denser polymers (no PET particles were extracted). $CaCl_2$ demonstrates a uniform particle size distribution but significantly limited particle extraction in number, which suggests some selectivity in order to draw definitive conclusions about its effectiveness. Particles of polyethylene, polyvinyl chloride and polyamide are extracted to the greatest extent, with prevailing particles with an area in the range of 10 000 – 50 000 μm^2. The presence of particles with an area below 1 000 μm^2 is weakly expressed in extraction with all the mentioned salt solutions, suggesting a possible underestimation of the total level of microplastic pollution, especially when using NaCl and $CaCl_2$. The obtained data highlight the need to use combined or improved extraction methods that ensure the capture of a wide range of sizes and types of microplastics. The use of $MgCl_2$ shows potential as a more reliable approach for quantitative and qualitative analysis of microplastic pollution in marine sediment.

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